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Edited by Novikov D.K.

HANDBOOK OF CLINICAL IMMUNOLOGY

Textbook for students of high medical educational establishments

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The content of «Handbook of clinical Immunology» is update, believed to be reliable when checked with sources and is in accordance with simple language and in step wise manner, which coil create interest – and enthusiasm to learn and develop the concept in clinical Immunology.

This book gives a new orientation to the subject of Immunology so that the student appreciate the great importance and significance of application of immunology to medicine.

Authors welcome comments and suggestion from faculty students and other readers to make improvement in the next edition.

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Symbols and abbreviations

ADA adenosine deaminase
AIDS acquired immune deficiency syndrome
AIRE autoimmune regulator
ALL acute lymphoblastic leukaemia
ALPS autoimmune lymphoproliferative syndrome
AML acute myeloid leukaemia
ANA anti-nuclear antibody
ANCA anti-neutrophil cytoplasmic antibody
APC antigen-presenting cell
APECED autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia
APGS autoimmune polyglandular syndrome
APS anti-phospholipid syndrome
APP amyloid-precursor protein
APTT activated partial thromboplastin time
ARA American Rheumatism Association
ARDS adult respiratory distress syndrome
AS ankylosing spondylitis
ASCA anti-Saccharomyces *cerevisiae* antibodies
ASOT antistreptolysin O titre
AT ataxia telangiectasia
ATG anti-thymocyte globulin
ATM ataxia telangiectasia mutated protein
AZT azidothymidine (zidovudine)
BACNS benign angiitis of the CNS
BAL bronchoalveolar lavage
BCG bacille Calmette-Guerin
BCOADC branch-chain 2-oxo-acid dehydrogenase complex
bd twice a day
BIS bias index score
BM bone marrow
BMS biomedical scientist
BMT bone marrow transplantation
BSACI British Society for Allergy and Clinical Immunology
BSI British Society for Immunology
CAH chronic active hepatitis
CALLA common acute lymphoblastic leukaemia antigen
cAMP cyclic adenosine monophosphate
C-ANCA cytoplasmic ANCA
CAR cancer-associated retinopathy
CATCH-22 cardiac abnormalities, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcaemia, associated with 22q11 deletions
C4BP G4 binding protein
CCP cyclic citrullinated peptide
CCV chosen coefficient of variance
2CDA 2-chloro-deoxyadenosine
CE Conformite Europeenne
CF cystic fibrosis
CFS chronic fatigue syndrome
ICGD chronic granulomatous disease
CHAD cold haemolytic disease
CHARGE colobomata, heart disease, atresia of choanae, retarded growth, genital hypoplasia, ear anomalies
CHH cartilage-hair hypoplasia
CHS Chediak-Higashi syndrome
CID combined immunodeficiency
CIDP chronic inflammatory demyelinating polyneuropathy
CJD Creutzfeldt-Jakob disease
CK creatine kinase
CLL chronic lymphocytic leukaemia
CMC chronic mucocutaneous candidiasis
CME continuing medical education
CMI cell-mediated immunity
CML chronic myeloid leukaemia
CMV cytomegalovirus
CNS central nervous system
ConA concanavalin A
COPD chronic obstructive pulmonary disease
COSHH control of substances hazardous to health
CPA Clinical Pathology Accreditation
CPD continuing professional development
ICPSM Council for Professions Supplementary to Medicine
Cr & E creatinine and electolytes
CREST calcinosis-Raynaud's-oesophageal dysmotility-sclerodactyly-telangiectasia
CRF chronic renal failure
CRP C-reactive protein
CSF cerebrospinal fluid
CSF-1 colony-stimulating factor-1
CSS Churg-Strauss syndrome
CT computed tomography
CTD connective tissue disease
CTL cytotoxic T lymphocyte
CTLp cytotoxic T-lymphocyte precursor
CV coefficient of variation
CVA cerebrovascular accident
CVID common variable immunodeficiency
CyA cyclosporin A
DAF decay accelerating factor
dATP deoxyadenosine triphosphate
DBPC double-blind, placebo-controlled
DCT direct Coombs test
dGTP deoxyguanosine triphosphate
DH dermatitis herpetiformis *or* Department of Health
DIC disseminated intravascular coagulation
DIF direct immunofluorescence
DM dermatomyositis

DMARDs	disease-modifying drugs anti-rheumatic drugs
DNA	deoxyribonucleic acid
DNA-PKcs	DNA protein kinase catalytic subunit
DPT	diphtheria-pertussis-tetanus
DRL	drug-related lupus
dRWT	dilute Russell's viper venom test
dsDNA	double-stranded DNA
DTH	delayed-type hypersensitivity
DV	designated value
DVT	deep vein thrombosis
EA	early antigen
EAA	extrinsic allergic alveolitis
EBNA	EBV nuclear antigen
EBV	Epstein-Barr virus
ECG	electrocardiogram
ECP	eosinophil cationic protein
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EIA	enzyme-linked immunoassay
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EMA	endomysial antibodies
EMG	electromyogram
ENA	extractable nuclear antigen
ENT	ear, nose, and throat
EPD	enzyme-potentiated desensitization
EPO	erythropoietin
EQA	external quality assessment
ESR	erythrocyte sedimentation rate
FAST	fluorescent allergosorbent test
FBC	full blood count
FCAS	familial cold autoinflammatory syndrome
FcR	Fc receptor
FCS	fetal calf serum
FEV1	forced expired volume (one second)
FFA	free fatty acid
FFP	fresh frozen plasma
FIA	fluorescent immunoassay
FISH	fluorescent *in situ* hybridization
FITC	fluorescein isothiocyanate
FLH	familial lymphohistiocytosis
FMF	familial Mediterranean fever
fMLP	N-formyl-methionyl-leucyl-phenylalanine
FSH	follicle-stimulating hormone
FT3	free triiodothyronine
FT4	free thyroxine
FVC	forced vital capacity
GABA	γ-aminobutyric acid
GACNS	granulomatous angiitis of the CNS
GAD	glutamic acid carboxylase
GBM	glomerular basement membrane
GBS	Guillain-Barre syndrome
GCA	giant-cell arteritis
G-CSF	granulocyte colony stimulating factor
GDP	guanosine diphosphate
GFD	gluten-free diet
GFR	glomerular filtration rate
GGT	gammaglutamyt transferase
GI	gastrointestinal
GLP	good laboratory practice
GM-CSF	granulocyte-macrophage colony stimulating factor
GN	glomerulonephritis
GP	glycoprotein
GPC	gastric parietal cell
G6PD	glucose 6-phosphate dehydrogenase
GPI	glucosylphosphatidylinositol
GS-ANA	granulocyte anti-nuclear antibody
GTN	glyceryl trinitrate
GvHD	graft-versus-host disease
GvL	graft-versus-leukaemia
GvT	graft-versus-tumour
HAART	highly active antiretroviral therapy
HAE	hereditary angioedema
H & E	haematoxylin and eosin
HAMA	human anti-mouse antibodies
HANE	hereditary angioneurotic oedema
Hb	haemoglobin
HBsAg	hepatitis B surface antigen
HCC	Healthcare Commission
HCL	hairy cell leukaemia
HCV	hepatitis C virus
HD	Hodgkin's disease
hdIVIg	high-dose intravenous immunoglobulin
HELLP	haemolysis-elevated liver enzymes-low platelets
HEP	histamine equivalent potency
HEp-2	human epithelial cell line
HGV	hepatitis G virus
HHV	human herpesvirus
Hib	*Haemophilus influenzae* type b
HIGE	hyper-IgE syndrome
HIGM	hyper-IgM
HIT	heparin-induced thrombocytopenia
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HR-CT	high-resolution CT
HRF	homologous restriction factor
HRT	hormone replacement therapy
HSCT	haematopoietic stem cell transplantation
HSE	Health and Safety Executive
Hsp	heat-shock protein
HSP	Henoch-Schonlein purpura
HSV	herpes simplex virus
5-HT	5-hydroxytryptamine (serotonin)
HTLp	helper-T lymphocyte precursor
HTLV-1	human T-cell leukaemia virus-1
HUS	haemolytic-uraemic syndrome
HUV	hypocomplementaemic urticarial vasculitis
ICA	islet cell antibodies
ICAM	intercellular adhesion molecule
ICF	immunodeficiency-centromeric instability-abnormal fades
ICOS	inducible co-stimulator
ID	intradermal
IDDM	insulin-dependent diabetes mellitus
IDT	intradermal test
IEF	Immunoelectrophoresis

IF	intrinsic factor
IFN	interferon
Ig	immunoglobulin
IGF1-R	insulin-like growth factor-1 receptor
IIF	indirect immunofluorescence
IL	interleukin
IM	intramuscular(ly)
IMIg	intramuscular immunoglobulin
INR	international normalized ratio
IPEX	X-linked immune dysregulation with polyendocrinopathy
IPF	idiopathic pulmonary fibrosis
IRAK-4	interleukin-1 receptor-associated kinase 4
ISCOMS	immunostimulatory complexes
ITP	immune thrombocytopenia
ITU	intensive care unit
IU	international units
IV	intravenous(ly)
IVIg	intravenous immunoglobulin
JCA	juvenile chronic arthritis
JDF	Juvenile Diabetes Foundation
KIR	killer-cell Ig-like receptors
LAC	lupus anticoagulant
LAD	leucocyte adhesion defect
LAK	lymphokine-activated killer cell
LC	liver cytosol
LDH	lactate dehydrogenase
LE	lupus erythematosus
LEMS	Lambert-Eaton myasthenic syndrome
LFA	lymphocyte function antigen
LFT	liver function test
LG	lymphomatoid granulomatosis
LGL	large granular lymphocyte
LIF	leukaemia inhibitory factor
LKM	liver-kidney microsomal antibodies
LM	liver microsome
LP	lumbar puncture
LPS	lipopolysaccharide
LYDMA	lymphocyte-determined membrane antigen
mAbs	monoclonal antibodies
MAG	myelin-associated glycoprotein
MAOI	monoamine oxidase inhibitor
MAST	multiple allergosorbent tests
MBL	mannan-binding lectin
MBP	mannan-binding protein
MCP	macrophage chemotactic peptide
MCTD	mixed connective tissue disease
MCV	mean corpuscular volume
MDP	muramyl dipeptide
MDS	myelodysplastic syndrome
'ME'	'myalgic encephalomyelitis'
MFE	materno-fetal engraftment
a_2MG	$\alpha_2$macroglobulin
β_2MG	β_2-microglobulin
MG	myasthenia gravis
MGUS	monoclonal gammopathy of unknown significance
MHC	major histocompatibility complex
MI	myocardial infarction
MIS	misclassification score
MLA	medical laboratory assistant
MLR	mixed lymphocyte reaction
MMF	mycophenolate mofetil
MMR	measles, mumps, and rubella virus
MND	multiple nuclear dots
MOG	myelin oligodendrocyte glycoprotein
MPA	microscopic polyarteritis
MPGN	membranoproliferative glomerulonephritis
MPO	myeloperoxidase
MRA	magnetic resonance angiography
MRBIS	mean running bias index score
MRI	magnetic resonance imaging
MRVIS	mean running variance index score
MS	multiple sclerosis
MSA	mitotic spindle antigens
mTOR	mammalian target of rapamycin
MTX	methotrexate
MUD	matched unrelated donor
MuSK	muscle-specific tyrosine kinase
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NAP	neutrophil alkaline phosphatase
NARES	non-allergic rhinitis with eosinophilia
NB	*nota bene*
NBT	nitroblue tetrazolium test
NCAM	neuronal cell adhesion molecule
NF-AT	nuclear factor of activated T cells
NHL	non-Hodgkin's lymphoma
NIDDM	non-insulin-dependent diabetes mellitus
NK	natural killer
NMR	nuclear magnetic resonance
NOMID	neonatal-onset multisystem inflammatory disease
NRL	natural rubber latex
NSAIDs	nonsteroidal anti-inflammatory drugs
NuMA	nuclear mitotic apparatus protein
OCP	oral contraceptive pill
od	once a day
OGDC	oxoglutarate dehydrogenase complex
OMIS	overall misclassification score
OMPC	outer membrane porin C
P450 sec	P450 side-chain cleavage enzyme
PA	pernicious anaemia
PACNS	primary angiitis of the CNS
PAF	platelet-activating factor
PAN	polyarteritis nodosa
P-ANCA	perinuclear ANCA
PANDAS	paediatric autoimmune neuropsychiatric disorders associated with *Streptococcus*
PBC	primary biliary cirrhosis
PCNA	proliferating cell nuclear antigen
PCP	*Pneumocystis carinii* pneumonia
PCR	polymerase chain reaction
PDC	pyruvate dehydrogenase complex
PDGF	platelet-derived growth factor
PE	pulmonary embolism or phycoerythrin
PEFR	peak expiratory flow rate
PEG	percutaneous endoscopic gastrostomy or polyethylene glycol
PFAPA	periodic fever with aphthous ulcers, pharyngitis, and cervical adenopathy
PGE2	prostaglandin E2

PHA phytohaemagglutinin
PID primary immunodeficiency
PLL prolymphocyte leukaemia
PM polymyositis
PMA phorbol myristate acetate
PMR polymyalgia rheumatica
PNH paroxysmal nocturnal haemoglobinuria
PNP purine nucleoside phosphorylase
PNU protein nitrogen units
POEMS polyneuropathy-organomegaly-endocrine abnormalities-monoclonal gammopathy-skin changes
POTS postural orthostatic tachycardia syndrome
PPD purified protein derivative (of tuberculin)
PPV positive predictive value
Pr3 proteinase 3
PrP prion protein
PRU Protein Reference Unit
PSC primary sclerosing cholangitis
PSS progressive systemic sclerosis
PTH parathyroid hormone
PTLD post-transplant lymphoproliferative disease
PUO pyrexia of unknown origin
PUPP pruritic urticaria and plaques of pregnancy
PUVA photochemotherapy using psoralens with ultraviolet A irradiation
PV pemphigus vulgaris
PWM pokeweed mitogen
QA quality assurance
QC quality control
QMS quality management system
RANA rheumatoid-associated nuclear antibodies
RAST radioallergosorbent test
RBCs red blood cells
RFLP restriction fragment length polymorphism
RFT respiratory function tests
RhA rheumatoid arthritis
RhF rheumatoid factor
RIA radioimmunoassay
RID radial immunodiffusion
RNA ribonucleic acid
RNP ribonucleoprotein
rRNP ribosomal ribonucleoprotein
RS Reed-Sternberg
RSCA reference-strand-mediated conformation analysis
RSV respiratory syncytial virus
SAA serum amyloid A
SAC *Staphylococcus* strain A Cowan
SAP serum amyloid P
SAPHO synovitis-acne-pustulosis-hyperostosis-osteitis
SBE subacute bacterial endocarditis
SC subcutaneous(ly)
SCAT sheep-cell agglutination test
SCID severe combined immunodeficiency
SCIg subcutaneous immunoglobulin
Scl scleroderma
SCLC small-cell lung carcinoma
SCT stem cell transplantation
SD standard deviation
SERPIN serine protease inhibitor
SIFTR service increment for teaching and research
SIRS systemic inflammatory response syndrome
SLA soluble liver antigens
SLE systemic lupus erythematosus
SLVL splenic lymphoma with circulating villous lymphocytes
Sm Smith antibodies
SMA smooth muscle antibodies
SOP standard operating procedure
SPET single-photon emission tomography
SPS stiff person syndrome
SPT skin-prick test
SRP signal recognition particle
SRSV small round structured virus
SS Sjogren's syndrome
SSc systemic sclerosis
ssDNA single-stranded DNA
SSOP sequence-specific oligonucleotide probes
SSP-PCR sequence-specific primer PCR
SV40 simian virus 40
T3 triiodothyronine
T4 thyroxine
TAME tosyl-L-arginine methyl ester
TB tuberculosis
TC transcobalamin
Tcr T-cell receptor
TdT terminal deoxytransferase
TFT thyroid function tests
TGF T-cell growth factor
TGSI thyroid growth stimulating antibody
Th1 T helper-1
Th2 T helper-2
THI transient hypogammaglobulinaemia of infancy
TIA transient ischaemic attack
TIL tumour-invading lymphocytes
TLI total lymphoid irradiation
TNF tumour necrosis factor
TPMT thiopurine methyltransferase
TPN total parenteral nutrition
TPO thyroid peroxidase
TRAB thyrotropin receptor antibody
TRAPS TNF-receptor-associated periodic syndrome
TREC T-cell receptor excision circle
TSH thyroid stimulating hormone
TSH-R thyroid stimulating hormone receptor
TSI thyroid stimulating antibody
tTG tissue transglutaminase
TTP thrombotic thrombocytopenic purpura
UC ulcerative colitis
UNG uracil-DNA gtycosylase
USS ultrasound scan
UV ultraviolet
VCA viral capsid antigen

VCAM	vascular celt adhesion molecule
VCF	velocardiofacial syndrome
VDRL	Venereal Disease Research Laboratory
VEGF	vascular endothelial growth factor
VGCC	voltage-gated calcium channel
VGKC	voltage-gated potassium channel
VI	variance index
VIP	vasoactive intestinal polypeptide
VIS	variance index score
VKH	Vogt-Koyanagi-Harada syndrome
VLA	very late antigen
VNTR	variable N-terminal repeat analysis
vWF	von Willebrand factor
VZV	varicella zoster virus
WAS	Wiskott-Aldrich syndrome
WASP	Wiskott-Aldrich-associated protein
WG	Wegener's granulomatosis
WHIM	warts, hypogammaglobulinaemia, infection, myelokathexis (syndrome)
XLA	X-linked agammaglobulinaemia
XLPS	X-linked lymphoproliferative syndrome
XLT	X-linked thrombocytopenia
ZAP	zeta-associated protein

INTRODUCTION

The immune system

The immune system is a remarkable defense mechanism. It provides the means to make rapid, highly specific, and often very protective responses against the myriad potentially pathogenic microorganisms that inhabit the world in which we live. The mechanisms of immunity act in a wide range of clinical conditions, including recovery from infectious diseases, rejection of tumors, transplantation of tissue and organs, autoimmune and other immunopathologic conditions, and allergy.

Many of the functions of cells of the immune system are mediated through the production of a set of small proteins referred to as cytokines. Many of the cytokines are T-cell products; their production represents one of the means through which the wide variety of functions of T-cells are effected. Most cytokines are not constitutive products of the T-cell. Rather, they are produced in response to T-cell activation, usually resulting from presentation of antigen to T cells by APCs in concert with the action of a costimulatory molecule, such as the interaction of CD80/86 with CD28. Although cytokines are produced in small quantities, they are very potent, binding to their receptors with equilibrium constants of approximately 10^{10} M^{-1}. In some instances, cytokines are directionally secreted into the limited space between the T cells and the APCs, so that they act in a paracrine manner and have limited action at a distance from the cell that produced them. This appears to be particularly true of many of the type I cytokines. However, other cytokines act by diffusion through extracellular fluids and blood to target cells that are distant from the producers. Among these are cytokines that have proinflammatory effects, such as IL-1, IL-6, and TNF.

Hematopoiesis and Immunopoiesis

Hematopoiesis is the dynamic and complex developmental process of the formation of new blood cells, which includes red blood cells (erythrocytes), white blood cells (leukocytes), and platelets. Per day, hematopoiesis yields approximately 175 billion red cells, 70 billion granulocytes (neutrophils, eosinophils, basophils), and 175 billion platelets. If needed, production can be increased 5-10 fold.

Hematopoiesis taking place prior to the development of the fetal liver is referred to as primitive hematopoiesis, which is transient and restricted to the production of erythrocytes and megakaryocytes. The fetal liver is the site of definitive hematopoiesis early during embryonal development. The term definitive hematopoiesis is used to describe blood formation after the formation of the fetal liver. It takes place in spleen and lymph nodes and, from fetal week 20 up, in bone marrow. The bone marrow with its intersinusoidal spaces is also the site responsible for the generation of blood cells in the post-natal phase. Red marrow (hematopoietically active marrow) usually becomes restricted to proximal ends of long bones, and flat bones (ileum, sternum, vertebrae, ribs). During growth, the red marrow is gradually replaced by yellow marrow, which is mostly fat.

The sinusoids (venous channels) feed into the marrow venous drainage system. The sinusoids are lined with specialized fenestrated endothelial cells. They produce growth factors and cytokines, which influence proliferation and differentiation of hematopoietic cells and thus play an important regulatory role. Mature blood cells enter the blood stream by passing through the sinusoidal wall to get into the sinuses. In order to do this, maturing cells become more deformable and no longer express adherence receptor.

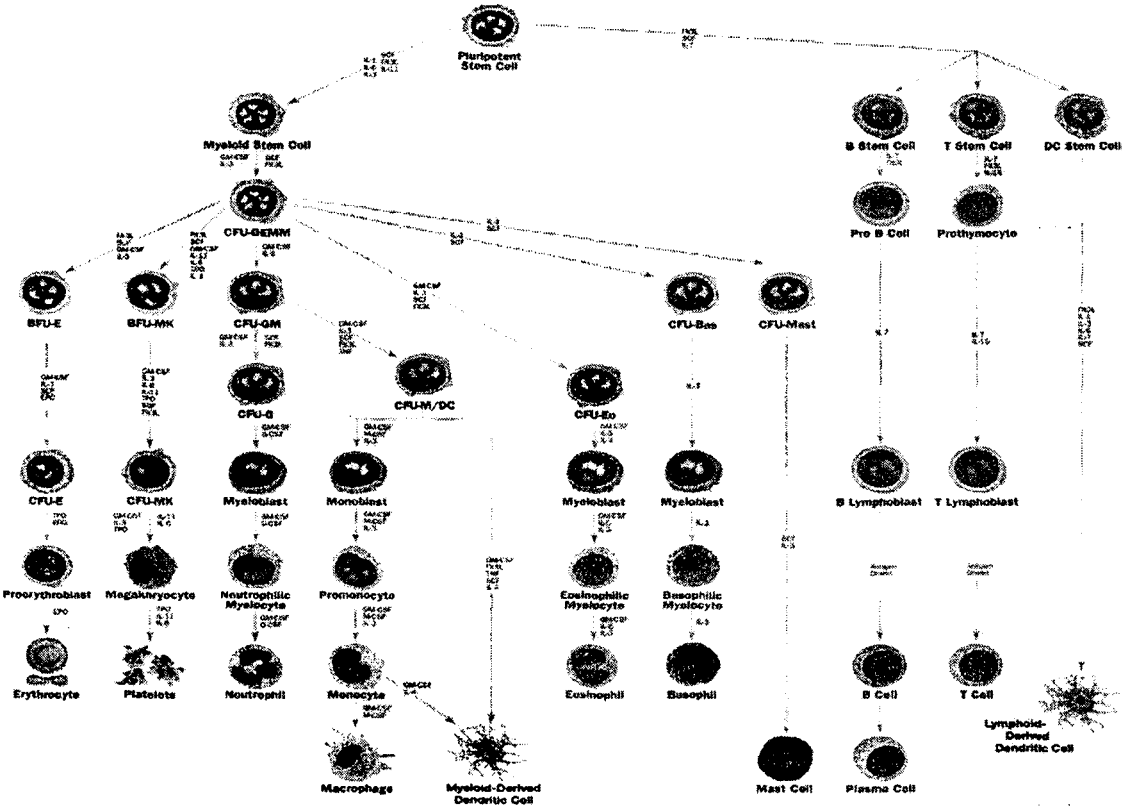
Immature hematopoietic cells bind to the stromal matrix and to receptors on the stromal cells by expressing special receptors that recognize proteoglycans on the target cells. This is also the mechanism underlying the homing of bone marrow cells that have been injected intravenously. The nature of hematopoietically active structures is determined predominantly by a network of stromal cells. The microenvironment of a cell plays an important role in the differentiation of individual bone marrow cells. Further differentiation of cells into one of several lineages criti-

cally depends on the nature of factors acting on these cells at a particular time, at a particular concentration, and/or in a particular sequence.
The bone marrow stroma contains many different cell types, including macrophages, fibroblasts, endothelial cells, smooth muscle cells, T-lymphocytes, monocytes etc. These cells, in combination with components of the extracellular matrix and basement membranes as well as a plethora of soluble and membrane-bound cytokines and growth factor, form the so-called hematopoietic inductive microenvironment, which maintains the functional integrity of this complex system of resident and circulating cells. Cells of the hematopoietic microenvironment show low or no detectable cell growth and are believed to be in the G0 phase of the cell cycle. Without bone marrow stromal cells, hematopoietic stem cells cannot be maintained in vitro, even when they are cultured with a cocktail of growth factors and cytokines.
All different types of blood cells are derived from a small common pool of totipotent cells, called hematopoietic stem cells, laid down in hematopoietic organs early during embryogenesis. These totipotent stem cells are referred to also as HSC (hematopoietic stem cells), PHSC (primitive (or pluripotent) hematopoietic stem cells), PLSC (pluripotent lymphoid stem cells), PPSC (pluripotent stem cells, or PSC), and THSC (totipotent hematopoietic stem cells).
These cells have the unique property of self-renewal (abbr. SRP for Self-renewal potential), i.e., they give rise to progeny identical in appearance and differentiation potential. These cells persist throughout adult life and are therefore responsible for the maintenance of hematopoiesis. This process is called also Steady-state hematopoiesis or Constitutive hematopoiesis.
Pluripotent stem cells are quiescent cells. This is shown by their resistance to treatment with Fluorouracil or 4-HC (4-hydroperoxycyclophosphamide), which spare them and eliminates dividing cells without adversely affecting the long-term repopulating ability of bone marrow.
These cells are of interest not only because of their developmental capacity but also because of their potential usefulness as a source of autologous bone marrow cells for the treatment of hematological disorders and as vectors for gene therapy. This type of cell is found not only in bone marrow but also in peripheral blood (PBPC, peripheral blood progenitor cells). Treatment of patients with colony stimulating factors such as G-CSF and GM-CSF induce an impressive rise of up to 100-fold in levels of progenitor cells of all lineages in the peripheral blood. Stem cells are elevated in this response, with the blood populations being capable of initiating long-term repopulation of irradiated recipients. The mechanisms responsible for the release of progenitor and stem cells from the marrow probably involve changes in adhesion to marrow stromal elements and also CSF induced changes induced in the cells destined to leave the marrow.
The totipotent hematopoietic stem cells give rise to transit populations with restricted differentiation capacity. Differentiation of stem cells in vivo has long thought to be a stochastic process but it appears that hematopoiesis progresses through an ordered restriction process rather than random processes with a hierarchy in the lineage restriction process. The Progenitor cells arising as the result of stem cell differentiation are called Committed cells, or, for historical reasons, colony-forming units because experimentally they were detected by their ability to form colonies in soft agar in a colony formation assay (BFU-E, CFU-E, CFU-Eo, CFU-G, CFU-GEMM, CFU-GM, CFU-M, CFU-MEG).
Committed cells, which can be identified by expression of specific lineage markers, comprise multipotent (MPSC), bipotent (BPSC), and unipotent (UPSC) cell types which are determined to differentiate into any of the hematopoietic lineages, i.e., lymphoid cells that ultimately give rise to B-cells and T-cells, and myeloid cells that eventually give rise to monocytes, platelets, granulocytes/monocytes (neutrophils, eosinophils, basophils), and erythrocytes.
These lineages are defined by the nature of the fully differentiated cells eventually evolving from these precursor cells. The developmental potential of these cells is generally limited to only one or two of the hematopoietic lineages, and these cells progressively display the antigenic, biochemical, and morphological features characteristic of the mature cells of the appropriate lineages and lose their capacity for self-renewal. Their proliferation is normally tightly controlled and coupled to development. Cells leaving the bone marrow usually possess little or no prolifera-

tive potential; erythrocytes and platelets do not contain genetic material, and neutrophils have condensed DNA and cannot undergo replication. Some hematopoietic cells, including pre-T-cells, mast cells, and monocytes can undergo further replication and development in various tissues.

Pluripotent stem cells are still difficult to characterize morphologically. The pool of determined stem cells can usually be differentiated in functional assays. Most of the distinct intermediate forms can be distinguished from each other by the stage-specific expression of cell surface markers and by their dependence on the presence of one or several growth factors or cytokines that are absolutely required for their survival and proliferation. All members of a particular lineage that are still capable of proliferation can give rise to malignant variants at all stages of differentiation.

Simplified diagram of hematopoiesis cytokines involved in its regulation:



Immune responses are initiated by the encounter of an individual with a foreign antigenic substance, generally an infectious microorganism. The infected person rapidly responds with the production of antibody molecules specific for the antigenic determinants of the immunogen and with the expansion and differentiation of antigen-specific regulatory and effector T-lymphocytes. The latter include both cells that produce cytokines and killer T-cells, capable of lysing infected cells. Generally, the initial immune response is sufficient to control and eradicate the microbe. Indeed, the most effective function of the immune system is to mount a response that eliminates the infectious agent from the body.

Immunoglobulin class

In the human there are five main classes of immunoglobulin heavy chains have been demonstrated: IgM, IgD, IgG, IgA, and IgE. In addition, four subclasses of IgG are produced IgG_1, IgG_2, IgG_3, and IgG_4. In the human, there are also two classes of IgA (IgA_1 and IgA_2). In addition to differences in size and valency, differences in the structures of the heavy chains of the different isotypes result in differences in in vivo half-life, binding to cellular receptors, complement fixation, sensitivity to digestion by proteolytic enzymes, and the tendency to form aggregates.

General functions of immunoglobulins

Ag binding - Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host.

• Valency - the valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

Effector Functions - Often the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions.

• Fixation of complement - lysis of cells, release of biologically active molecules.

• Binding to various cell types - phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins and the binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts. The binding results in transfer of the immunoglobulin across the placenta and the transferred maternal antibodies provide immunity to the fetus and newborn

The mucosal immune system

The human has evolved organized secondary lymphoid tissues in the upper respiratory and GI tract regions that facilitate antigen uptake, processing, and presentation for induction of mucosal immune responses. Collectively, these tissues are known as inductive sites. Although the gut-associated lymphoreticular tissues (GALT), e.g., Peyer's patches, are major inductive sites in all of the most common experimental mammalian systems, the degree of bronchus-associated lymphoreticular tissue (BALT) developed at airway branches for defense against intranasal/inhaled antigens differs considerably among species. In rabbits, rats, and guinea pigs, such BALT development is significant, whereas in humans and mice it is negligible unless chronic inflammation, such as panbronchiolitis or rheumatoid arthritis, occurs. Instead, the major inductive tissues for intranasal/inhaled antigens in humans appear to be the palatine tonsils and adenoids (nasopharyngeal tonsils), which together form a physical barrier of lymphoid tissues termed the Waldeyer's ring, now more frequently referred to as a nasopharyngeal-associated lymphoreticular tissue (NALT). To summarize, then, NALT and GALT in humans and mice and NALT, BALT, and GALT in other human comprise a mucosa-associated lymphoreticular tissue.

(MALT). Two major features distinguish MALT from the other systemic lymphoid tissues. First, the epithelium, which separates the tissue from the lumen, contains a specialized cell type termed M cells that are closely associated with lymphoreticular cells. Together this epithelial cell network is called the follicle-associated epithelium (FAE). Second, MALT contains organized regions that include a subepithelial area (dome), B-cell zones with germinal centers containing IgA-committed B cells (surface IgA^+ B-cells) and adjacent T-cell regions with APCs and high endothelial venules (HEVs). Naive, recirculating B and T lymphocytes enter MALT through HEVs. Antigen-activated and memory B- and T-cell populations then emigrate from the inductive environment via lymphatic drainage, circulate through the blood stream, and home to mucosal effector sites. These effector sites include more diffuse tissues, where antigen-specific T- and B-lymphocytes ultimately reside and perform their respective functions (cytotoxic T-lymphocyte, and regulatory functions or antibody synthesis, respectively) to protect mucosal surfaces.

Normal ranges (adults)

Serum immunoglobulins

IgG	6.8-15.4 g/l
IgA	0.84-2.97 g/l
IgM (males)	0.54-1.90 g/l
IgM (females)	0.81-2.30 g/l
IgG subclasses	
IgG_1	4.2-10.8 g/l
IgG_2	1.5-6.0 g/l
IgG_3	0.5-1.9 g/l
IgG_4	0.2-1.4 g/l
IgA subclasses	
IgA_1	0.7-2.4 g/l
IgA_2	0.2-0.9g/l
Total IgE	<100 kU/l or <100 IU/ml
IgD	0.01-0.04 g/l

Antibacterial antibodies (protective level)

Diphtheria >0.1 IU/ml	
Pneumococcal (Pneumovax)	>20 u/l
Tetanus	>0.1 IU/ml
Haemophilus influenzae B	>1 µg/ml

Complement

C1q	50-250 mg/l
C3	0.68-1.80 g/l
C3a	10-570 µg/l
C4	0.18-0.60 g/l
C4a	102-212 µg/l
C4BP	140-220 µg/l
C5a	< 10 µg/l
Cl-esterase inhibitor	0.18-0.54 g/l
Factor H	430-610 mg/l
Factor I	38-58 mg/l

Lymphocyte subsets

Total T cells ($CD3^+$)	800-2540 cells/mm^3 - |50-70%|
T helper cells ($CD3^+CD4^+$)	570-1590 cells/mm^3 - |35-45%|
T cytotoxic cells ($CD3^+CD8^+$)	400-1140 cells/mm^3 - |27-35%|
Total B cell ($CD19^+$ or $CD20^+$)	190-660 cells/mm^3 - |22-30%|
NK cells ($CD16^+CD56^+CD72$)	170-590 cells/mm^3 - |14-24%|

Classification of immunodeficiency

Genetic

- Autosomal recessive
- Autosomal dominant
- X-linked
- Gene deletions, rearrangements.

Biochemical and metabolic

- Adenosine deaminase deficiency
- Purine nucleoside phosphorylase (PNP) deficiency
- Biotin-dependent multiple carboxylase deficiency
- Deficient membrane glycoproteins.

Vitamin or mineral deficiency

- Zinc deficiency
- B_{12} deficiency
- Biotin.

Undefined primary

- Common variable immunodeficiency
- Specific antibody deficiency
- IgG subclass deficiency
- IgA deficiency.

Maturational

- Transient hypogammaglobulinaemia of infancy.

Secondary

- Viral infections (HIV, CMV, EBV, rubella)
- Chronic infections (TB, leishmania)
- Malignancy
- Lymphoma/leukaemia
- Extremes of age
- Transfusion therapy
- Drugs
- Plasmapheresis
- Radiation
- Nutrition
- Chronic renal disease (including dialysis)
- Toxins (including alcohol, cigarettes)
- Splenectomy
- Tonsilectomy
- Burns.

Clinical features of immunodeficiency

Recurrent infections

There is no universally accepted definition of what constitutes 'recurrent infection' and therefore it is difficult to be categorical about who should be investigated for immunodeficiency. The following should be used as guidance.

- Two major or one major and recurrent minor in 1 year.
- Unusual organisms (Aspergillus, Pneumocystis).
- Unusual sites (liver abscess, osteomyelitis).
- Chronic infections (sinusitis).
- Structural damage (bronchiectasis).
- Other suspicious features.

Other features raising suspicion of an underlying immunodeficiency

• Skin rash (atypical eczema): Wiskott-Aldrich syndrome, hyper-IgE syndrome, Omenn's syndrome.
• Chronic diarrhoea: SCID, antibody deficiencies.
• A major infection is severe infection, usually requiring parenteral treatment in hospital, with objective evidence of infection (elevated CRP, positive culture). Minor infection is less severe, usually treatable in the community with oral therapy, but with objective evidence of infection.
• Failure to thrive: any immune deficiency in childhood.
• Hepatosplenomegaly: common variable immunodefciency (CVID), Omenn's syndrome.
• Chronic osteomyelitis or deep-seated abscesses: chronic granulomatous disease.
• Mouth ulceration (cyclical): neutropenia.
• Autoimmunity: CVID, hyper-IgM syndrome.
• Family history.

Features associated with specific immunodeficiencies

Some features are diagnostic of particular immunodeficiencies.
• Ataxia: ataxia telangiectasia, PNP deficiency.
• Telangiectasia: ataxia telangiectasia.
• Short-limbed dwarfism: X-linked immunodeficiency.
• Skeletal abnormalities: ribs in ADA deficiency.
• Cartilage-hair hypoplasia.
• Ectodermal dysplasia.
• Endocrinopathy (particularly with hypocalcaemia): chronic mucocutaneous candidiasis.
• Partial albinism: Chediak-Higashi disease; Griscelli syndrome.
• Thrombocytopenia (particularly with small platelets): X-linked thrombocytopenia; Wiskott-Aldrich syndrome.
• Eczema: Wiskott-Aldrich syndrome; hyper-IgE syndrome; Omenn's syndrome.
• Neonatal tetany: 22q11 deletion syndromes (DiGeorge).
• Abnormal facies (leonine; fish-shaped mouth, low-set ears): hyper-IgE (leonine); 22q11 deletion syndrome (fish-shaped mouth, low-set ears); ICF syndrome.
• Mental retardation: 22q11 deletion syndromes, PNP deficiency; other genetic immunodeficiencies.

Investigation of immunodeficiency

History

• History of all infections: site, severity, need for antibiotics, hospitalizations.
• Operations (lobectomies, etc.).
• Immunization history.
• Family history, especially for serious infections, unexplained sudden deaths, diagnosed immunodeficiencies, and autoimmune diseases.

Examination

• Weight and height (failure to thrive).
• Structural damage from infections (ears, sinuses, lungs).
• Autoimmune features: vitiligo; alopecia; goitre.
• Other suspicious/diagnostic features, as above.

Formulate differential diagnoses in rank order.

Laboratory investigation

• Target investigations to differential diagnosis.
• Do not blanket screen.
• Ensure basic tests are done before exotic tests.
• Think whether tests will contribute to diagnosis or management: if they will not then do not do them.

B-cell function

• Serum immunoglobulins (be sure to use low-level detection system for IgA to confirm absence).

• Serum and urine electrophoresis (evidence for bands and urinary loss).
• IgG subclasses.
• IgE.
• Antibacterial, antiviral antibodies (appropriate to immunization and exposure history).
• Immunization responses (protein and polysaccharide antigens).
• Isohaemagglutinins (IgM, dependent on blood group).
• B lymphocyte numbers; class-switch memory B cells ($CD27^+/IgD^-/IgM^-$).
• Pokeweed mitogen (PWM) and antigen-stimulated antibody production *in vitro* (clinical indications limited).

T-cell function

Use absolute T-cell counts, not percentages.
• T-cell numbers and surface phenotype.
• CD2, CD3, CD4, CD8, CD7, TCR (αβ, γδ), CD40 and CD40L, MHC class II; CD45RA and RO, CD27.
• CD40-ligand expression on activated T cells.
• T-cell proliferation to antigens, mitogens (OKT3, PHA, phorbol myristate acetate (PMA), calcium ionophore, cytokines).
• T-cell cytokine production *in vitro*.
• *In vivo* skin (delayed-type hypersensitivity, DTH) testing: the Multitest CMI is a convenient tool, but responses will depend on prior exposure.

Neutrophil function Neutrophil function tests are not widely available, so if there is suspicion of a neutrophil defect, specialist help should be sought. Interpretation is difficult and tests may be influenced by intercurrent infection and drug therapy.
• Neutrophil markers (CD11a, CD11b, CD11c, CD18, CD15).
• Upregulation of neutrophil markers (PMA, fMLP).
• Oxidative metabolism (quantitative and qualitative nitroblue tetra-zolium reduction (NBT test); flow cytometric determination of oxidative burst).
• Phagocytosis.
• Bacterial or fungal killing (relevant organisms should be selected).
• Chemotaxis (difficult to standardize, with wide normal range).

NK-cell function

This is of research use only at present.
• NK-cell numbers by flow cytometry (absolute counts).
• K562 killing assay (radioimmunoassay, flow cytometry).
• Cytokine-stimulated killing (lymphokine-activated killer cell (LAK) assay).

Complement assays

• Measurement of specific components.
• Functional assays (haemolytic assays).

Genetic studies Genetic studies form an essential part of the investigation and management of primary immunodeficiencies.
• Cytogenetics (deletions, translocations).
• Ig and Tcr gene rearrangements (clonality).
• X-linked gene studies.
• 22q11 microdeletions (FISH-fluorescent *in situ* hybridization).
• Protein expression studies (SAP, btk).
• MHC studies.
• Prenatal diagnosis.

Other investigations

• Detection of autoimmunity:
  anti-red cell, platelet, neutrophil antibodies;
  anti-endocrine autoimmunity (thyroid autoantibodies).
• Exclusion of secondary causes:

renal disease, bowel disease (loss of immunoglobulins);
malignancy (lymph-node biopsy);
nutritional deficiencies (zinc, B_{12}, iron);
drugs (cytotoxics, anticonvulsants).

- Detection of nodular lymphoid hyperplasia (CT scanning, barium follow-through, endoscopy, and biopsy).
- Lung function (including FEV_1, FVC, transfer factor).
- Imaging studies (lungs, sinuses).
- Direct isolation of pathogens: bacteria, fungal, and viral (serology is usually unreliable—use PCR-based tests where culture is not possible).

Major B-lymphocyte disorders

- X-linked agammaglobulinaemia (Bruton's agammaglobulinaemia; XLA).
- Common variable immunodeficiency (acquired hypogammaglobulinaemia; CVID).
- Selective IgA deficiency.
- IgG subclass deficiency.
- Specific antibody deficiency with normal immunoglobulins.

Rare antibody deficiency syndromes

- X-linked hyper-IgM syndrome (HIGM-1); CD40-ligand deficiency.
- Autosomal hyper-IgM syndromes (HIGM-2-4):
- HIGM-2; autosomal recessive activation-induced cytidine deaminase deficiency;
- HIGM-3; autosomal recessive CD40 deficiency;
- HIGM-4; similar to HIGM-2, molecularly undefined;
- HIGM due to uracil-DNA glycosylase (UNG) deficiency.
- X-linked hypogammaglobulinaemia with growth hormone deficiency.
- Selective IgM deficiency.
- X-linked lymphoproliferative syndrome (Duncan's syndrome; XLPS).
- Hyper-IgE syndrome (Job's syndrome).
- Selective IgE deficiency.
- Transient hypogammaglobulinaemia of infancy.
- Mu-chain deficiency.
- Ig alpha or MB1 (CD79a) deficiency.
- BLNK deficiency.
- ICOS deficiency.
- λ5/14.1 (surrogate light chain) deficiency.
- SWAP-70 deficiency.
- κ and λ light chain deficiency.
- Thymoma with immunodeficiency (Good's syndrome).
- CD19 deficiency.

X-linked agammaglobulinaemia (Bruton's disease)

It has an incidence of 1 in 100 000-200 000 and a prevalence of 1 in 10 000.

Cause

- Genetic disorder due to mutations on the X chromosome affecting the btk gene.
- btk codes for a tyrosine kinase involved in B-cell maturation.
- Defects in the gene prevent B-cell maturation from pro-B-cetl to pre-B-cell.
- Gene is located at Xq21.3-22. Mutations include deletions and point mutations, either conservative or leading to premature termination.
- Phenotype correlates poorly with genotype.
- Mild phenotypes occur with some limited B-cell development.
- New mutations common, so a family history may be absent.

Presentation

- Usually early in childhood, after 6 months of age, when maternal antibody has largely disappeared.
- Recurrent infections of lungs and ears (children of this age don't have sinuses):
  Haemophilus influenzae and pneumococci (upper and lower respiratory tract, meningitis);
  meningococcus (meningitis);
  staphylococcus (septic arthritis);
  Giardia, Salmonella, and *Campylobacter* infections of the gut.

Diagnosis-key features

- Early onset bacterial infections in a male child with a family history.
- Family history often absent.
- Neutropenia very common at presentation but goes away with treatment and is probably due to chronic bacterial sepsis.
- Failure to thrive and chronic diarrhoea common.
- Distinction of milder forms, with some B cells and low but not absent IgG, from CVID is difficult and relies on the demonstration of abnormalities of the *btk* gene. Some patients previously classified as CVID will turn out to be XLA
- Specific antibody deficiency only reported with *btk* mutations.
- XLA may rarely be associated with growth hormone deficiency (and short stature).
- Occasional females will be identified with the immunological features of XLA.
- Differential diagnosis will include coeliac disease and cystic fibrosis, although the laboratory tests will rapidly identify antibody deficiency.

Immunology

In the complete forms the immunology is fairly distinctive.

- All immunoglobulins are absent or very low.
- B cells are low or absent.
- Btk protein absent (confirm with genetic studies where there is a good history, to exclude production of non-functional protein).
- Lymph nodes show no germinal centres; no tonsils; pre-B cells in bone marrow.
- T-cell numbers and function are normal.
- NK-cell numbers and function are normal.

Mild or incomplete variants may be difficult to distinguish from CVID.

- Variable numbers of B cells including normal.
- Variable immunoglobulins.
- Poor/absent specific antibodies to polysaccharide antigens.

Complications

The major complications relate to delay in diagnosis.

- Structural lung damage (bronchiectasis, sinusitis). Inadequate therapy will lead to progression of lung damage and the development of chronic sinus damage.
- Chronic meningoencephalitis due to echoviruses and coxsackieviruses may cause a progressive and fatal dementing illness; there is often muscle involvement, with a myositis and contractures. Diagnose by viral culture of CSF or by PCR-based techniques. Antiviral pleconaril is helpful (though not licensed by the FDA). This disease appears less often since the introduction of intravenous immunoglobulin (IVIg) therapy as standard, but has not disappeared completely.
- *Ureaplasma/Mycoplasma* septic arthritis. This is difficult to diagnose without special culture facilities. It is a highly destructive chronic infection and requires prolonged treatment (6 months) with tetracyclines and/or erythromycin.
- Haemophilus conjunctivitis.
- Crohn's-like disease of intestines. Possible increased risk of colonic cancer.

Treatment

- Iintravenous IgG should be started at the earliest opportunity; dose of 200-600mg/kg/month given at intervals of 2-3 weeks. Longer intervals do not give satisfactory replacement.

• Subcutaneous immunoglobulin given weekly (same total dose) is an alternative.
• Trough IgG levels should be monitored regularly, with the aim of maintaining a level well within the normal range (6-9 g/l). Early institution of IVIg and adequate trough levels preclude the development of bronchiectasis.
• Prompt antibiotic therapy (course of 10-14 days) for upper and lower respiratory tract infections together with physiotherapy and postural drainage if lung damage has already occurred. Ciprofloxacin is a valuable antibiotic (though not licensed for small children). Use cystic fibrosis approach of zero tolerance to cough.
• As children get older and can comply, perform regular lung function testing, including transfer factor.
• High resolution CT scanning (HR-CT) is useful for identifying subclinical bronchiectasis, but imposes a significant radiation burden and should not be overused.
• Do not give oral poliovaccine as patients often fail to clear it, which increases the risk of reversion to wild type, with consequent paralytic disease.
• Genetic counselling of the patient and family once genetic basis confirmed. Identify and counsel carriers.
• Long-term immunological follow-up (plus additional specialist input as required).

XLA with growth hormone deficiency
This is a very rare disorder with immunological features identical to XLA, in association with short stature (as opposed to failure to thrive). The disease maps the region of the X chromosome containing the *btk* gene, but not all cases appear to have mutations in the *btk* gene. Prognosis appears to be good. It is treated with IVIg and growth hormone.

Common variable immunodeficiency (CYID)
CVID is the commonest symptomatic antibody deficiency with an estimated incidence of 1 in 25 000-66 000.
Cause
• Cause of CVID is unknown: one hypothesis suggests that an environmental insult (virus infection) in a genetically susceptible individual triggers the disease. No conclusive viral trigger has been identified.
• Some evidence for a genetic background (linked to HLA A1 B8 C4 DR3QO and to polymorphisms in the TNF-α gene).
• May be a family history of other antibody deficiencies (especially IgA deficiency and IgG subclass deficiency) in up to 50% of cases, although other family members may be entirely asymptomatic.
• Disease is heterogeneous in phenotype and immunological findings.
• Diagnosis is now one of exclusion, once other genetic diseases have been ruled out.
Presentation
• May present at any age from childhood through to old age, although the peak of presentation is in early childhood and early adulthood.
• Usual presentation is with recurrent bacterial infections, as for XLA.
• May present with autoimmune problems, especially thrombocytopenia, haemotytic anaemia, and organ-specific autoimmunity (e.g. thyroid, diabetes, vitiligo, and alopecia); these are common and may precede the development of recurrent infections.
• Nodular lymphoid hyperplasia of bowel (polyclonal hyperplasia of Peyer's patches) is unique to CVID. The cause is unknown, but it is possibly premalignant. This has characteristic features on small-bowel radiology.
• Granulomatous disease with lymphadenopathy and (hepato-) splenomegaly, and often involving the lung, is common in the severe form of CVID (about 25% of cases). Disease resembles sarcoidosis, but is Kveim-test negative (although this test is now rarely used). Specifically associated with complete absence of class-switch memory B cells.

Diagnosis

• History gives the clues. Clues are usually missed by general physicians and an average diagnostic delay of over 7 years is typical, by which time structural lung and sinus damage is severe and irretrievable.

• Immunoglobulin levels are variably low: test immunization and exclusion of secondary loss (gut, urine) may be required.

• Lymphopenia affecting predominantly the $CD4^+$ T cells ($CD45RA^+$ naive cells in particular) and B cells is common.

• When splenomegaly is present it may be difficult to exclude lymphoma, without CT scanning, lymph node biopsy, and bone marrow examination.

Immunology

• Immunoglobulin levels are highly variable, and IgG may be only marginally reduced; specific antibodies are invariably low with poor/absent immunization responses.

• IgM may be normal, which contrasts with lymphoma, when the IgM is the first immunoglobulin to drop.

• B cells may be normal or low but some cases in males may be late-presenting XLA.

• $CD4^+$ T cells are low, with specific depletion of $CD45RA^+$ T cells. T-cell function *in vivo* and *in vitro* to antigens and mitogens is poor and there is poor NK-cell function, with reduced NK-cell numbers.

• Abnormalities of 5'-nucleotidase activity on the lymphocyte surface have been described but this is not a separate syndrome as is sometimes stated. The significance of the abnormality is not known.

Classification

Three groups are identified on the basis of B-cell responses to IL-2 and anti-IgM in *vitro*.

• Group A: severe disease, with granulomata (hepatosplenomegaly); no IgG or IgM production in *vitro*.

• Group B: rare; IgM production only *in vitro* (cryptic hyper-IgM).

• Group C: mild disease; IgG and IgM production *in vitro*.

This technique is unsuited to routine diagnosis; identification of class-switch memory B cells is more useful (group A patients lack class-switch memory B cells).

Complications

• Major complications of CVID relate to the delay in diagnosis with structural damage from infection: bronchiectasis and chronic sinusitis.

• Patients may also have unusual infections, such as Campylobacter cholangitis, and *Mycoplasma/Ureaplasma* arthritis. Rarely, opportunist infections such as *Pneumocystis* occur (but this should suggest hyper-IgM syndromes).

• Malabsorption may occur due to a coeliac-like enteropathy, with villous atrophy.

• Inflammatory bowel disease may occur with strictures.

• In group A patients with splenomegaly, hypersplenism may occur with marked thrombocytopenia: splenectomy may be required.

• 40-fold increase in the risk of lymphoma, including intestinal lymphoma; Any patient with lymphadenopathy should have a lymph node biopsy and BM examination to exclude the diagnosis. Lymphomas are often high-grade and respond poorly to treatment.

• Increase in gastric carcinoma, not related to *Helicobacter pylori* colonization.

• Autoimmune disease is common and patients should be monitored for the development of overt disease (hypothyroidism, pernicious anaemia, diabetes).

• Thymomas (benign or malignant) are also associated with CVID: this is Good's syndrome. These frequently give rise to myasthenia gravis and haematological problems such as aplastic anaemia and immune thrombocytopenia.

Treatment

• The earlier the diagnosis is made, the better the prognosis.

• Treatment is identical to that of XLA, with IVIg/SCIg, antibiotics, and physiotherapy for chest disease.
• Prophylactic antibiotics should be considered if there is an inadequate response to optimal immunoglobulin therapy.
• Patients with complete IgA deficiency may have a higher risk of developing anti-IgA antibodies to IVIg therapy, and a product low in IgA should be selected for them; most IVIg products now have reduced IgA content. The value of monitoring anti-IgA antibodies is questionable.
• Patients with splenomegaly may catabolize IgG faster and may require larger doses or more frequent doses (weekly).
• Regular lung function tests (volumes and transfer factor) and HR-CT scanning is required. Deteriorating lung function requires more aggressive therapy.
• Chronic sinus disease requires ENT review, with endoscopic inspection.
• Granulomatous disease responds well to steroids (alkaline phosphatase is a good marker); these are essential if there is interstitial lung disease (reduced transfer factor). They are not necessary for asymptomatic splenomegaly. Steroid use is associated with an increased risk of disseminated shingles. Splenectomy may be necessary for hypersplenism: such patients must have prophylactic penicillin, but immunizations are of little value. There is, however, an additional risk of infection splenectomy in CVID patients and caution is required.
• Treat inflammatory bowel disease as for Crohn's disease.
• Gluten-free diet may help if there is coeliac-like disease.
• Monitor for the development of malignant disease. Review frequency of cervical smears.
• PEG-IL-2 has been used successfully in a smalt cohort.
• Retinoic acid has been shown to benefit some patients.
• Cimetidine may also improve immunological function.

Selective IgA deficiency
Selective IgA deficiency is the most common primary immunodeficiency, but mostly passes unnoticed. Depending on the racial group, 1 in 400-800 individuals will be affected.
Cause
• Cause is unknown, although it forms part of the spectrum of disease with CVID and shares the HLA type (A1, B8, C4, DR3, QO). It occurs in relatives of patients with CVID in 50% of cases.
• Rarely due to a gene deletion, often including IgG2/IgG4.
• Defects of class switching have been identified in a few patients.
• Selective deficiencies of IgA1 or IgA2 have been reported (all due to gene deletions).
• May be associated with other chromosomal abnormalities, usually involving chromosome 18 (18q syndrome and ring chromosome 18). Also with variant Turner's syndrome (Xq), multibranched chromosomes, Klinefelter's syndrome, and a,antitrypsin deficiency.
• Associated with drug therapy, particularly with phenytoin and penicillamine, although in many reports it is not clear whether the defect was present before drug therapy was introduced.
• IgA-bearing B cells are present. IgA is synthesized but not secreted.
• In terms of mucosal protection, there is evidence that IgG and IgM may substitute as secretory immunoglobulins.
Presentation
• Most cases are asymptomatic.
• There is an increased incidence of allergic disease, including food allergies and intolerances.
• Connective tissue diseases (SLE, rheumatoid arthritis, and juvenile chronic arthritis), coeliac disease, pernicious anaemia, and other organ-specific autoimmune diseases.
• Infections are rarely a problem unless there are additional humoral defects present.
• Occasional cases will come to light as a result of adverse reactions to blood products. This is deemed to be very rare (incidence 1 in 15 million, transfusions).

Diagnosis

• Requires the demonstration of undetectable IgA, not just a low IgA. Automated analysers do not read low enough to ascertain this beyond doubt. Check with low-level radial immunodiffusion or Ouchterlony double-diffusion assays.

• Beware of the presence of anti-animal antibodies in IgA deficiency which give false readings in immunoassays using antisera derived from sheep, goats, horses.

• Patients should be screened for evidence of other humoral defects: IgG subclasses and specific antibodies if there is a history of infections. If there is doubt, then test immunization should be undertaken.

• IgA antibodies can be measured but current assays detect IgG and IgM antibodies and therefore do not identify risk of anaphylaxis. The significance of IgG and IgM anti-IgA antibodies is uncertain: high levels may be seen in the absence of reactions. There is very seldom detection of IgE anti-IgA antibodies.

Immunology

• The IgA will be undetectable (< 0.05 g/l), but total IgG and IgM will be normal. IgG subclasses may be reduced (G_2 and G_4). Secreted IgA will be absent (secretory piece deficiency is vanishingly rare), but testing for this is of little clinical value.

• T-cell function is normal (PHA and antigens).

• Autoantibodies may be present (anti-IgA antibodies). There will be an increased IgE in the presence of atopic disease.

• In the absence of IgA, IgM and IgG appear on mucosal surfaces.

Complications

• Major problem with IgA deficiency is the possibility of transfusion reactions thought to be due to anti-IgA antibodies. These are extremely rare (1 in 15 million).

• Malignancy may be increased, although this may depend on other diseases present in association with IgA deficiency, especially lymphoma and gastric adenocarcinoma.

• It is possible that there may be progression to more significant humoral immunodeficiency with time.

Treatment

• Treatment is directed at the presenting disease.

• Avoid IgA-containing products if possible: no longer routinely provides blood products from IgA-deficient donors. Use an IVIg/SCIg with a low IgA content.

• Where immunoglobulin replacement therapy is required for recurrent infections, this has been shown to be safe.

• In view of the low risk of reactions, the wearing or carrying of an alert card is now of doubtful value.

Selective IgA2 deficiency

Cases have been reported with selective IgA2 deficiency, with normal IgA1. This does not appear to be due to heavy chain gene deletions affecting the α2 gene in all cases.

IgG subclass deficiency

Cause

• Cause of IgG subclass deficiency is unknown, but it too forms part of the spectrum with CVID and IgA deficiency. It is possible that some cases represent CVID in evolution. Rarely, cases may be due to gene deletions, but these individuals may be entirely healthy.

• IgG subclass levels are related to allotypes of IgG; different racial groups may therefore have different normal ranges, depending on the prevalence of different allotypes. This should be taken into account when diagnosing IgG subclass deficiency.

Presentation

• Presentation, as for CVID, can be at any age. Recurrent infections may be a feature, particularly for IgG_2 and IgG_4 deficiency. IgG_4 deficiency occurring alone has also been associated with bronchiectasis.

• Conditions associated with subclass deficiency include: asthma (IgG_3 deficiency); sinusitis (IgG_3 deficiency); intractable epilepsy of childhood (though this may be due to anticonvulsants); and autoimmune disease (SLE).

Diagnosis

• Measurement of IgG subclasses on more than one occasion is required and it is important to check that appropriate age-specific normal ranges are used.

• Detection of low levels of IgG_4 may require more sensitive assays to detect true absence: earlier normal ranges using less-sensitive assays found a significant number of individuals with undetectable IgG_4. Most of these have detectable IgG_4 on sensitive assays.

• There is a poor correlation of specific anti-pathogen responses and IgG subclass levels. All patients should have specific antibodies measured and be test immunized.

• Detection of low or absent subclasses does not necessarily correlate with clinical disease.

Immunology

• A normal total IgG is entirely compatible with subclass deficiency, although low IgG1 usually reduces the total IgG (this also behaves like CVID for practical purposes). The IgA is normal or low; IgM is normal.

• B- and T-cell numbers are usually normal.

• Poor specific antibody responses to bacterial and viral antigens may be present in some patients.

Complications

Long-term progression to CVID is a possibility. Bronchiectasis may occur in IgG_4 deficiency.

Treatment

• If recurrent infections are a problem, then the first step might be to use continuous antibiotics, followed by IVIg if infections are not controlled.

• IVIg has been shown to be of benefit in asthma due to IgG_3 deficiency, and in chronic sinusitis.

• It is possible to bypass IgG_2 deficiency using protein-conjugated polysaccharide vaccines to generate a protective IgG_1 response.

Specific antibody deficiency with normal serum immunoglobulins

Normal serum immunoglobulins and IgG subclasses do not exclude humoral immune deficiency! This syndrome is probably much more common than hitherto realized.

Cause

• Cause is unknown. It is unrelated to IgG subclass deficiencies. There is usually a failure to respond to polysaccharide antigens (T-independent) and possibly other protein antigens (HBsAg).

• In small children, it may be due to a maturational delay that resolves spontaneously.

Presentation

• Recurrent bacterial infection of upper and lower respiratory tract *(Haemophilus, Pneumococcus, Moraxella)* is the usual presentation.

• In immunization programmes for hepatitis B, about 5% of individuals fail to respond to the standard three-dose schedule; a fourth dose still leaves 1-2% who fail to make a serological response. These patients clearly have some form of specific immune deficit.

Diagnosis

• There is a history of recurrent typical infections with normal immunoglobulins and IgG subclasses.

• Proof requires demonstration of failure to respond to specific antigens (test immunization).

Immunology

• Immunoglobulins and IgG subclasses are normal, but there are low specific antibodies, especially to capsulated organisms, and poor responses to test immunization, especially to polysaccharide antigens (*Pneumovax*).

• Children under the age of 2 years do not respond to *Pneumovax*. However the inability to respond to polysaccharide antigens in infants may be bypassed by conjugation of the polysaccharide to a protein, for example, the *Prevenar* (heptavalent pneumococcal polysaccharide vaccine).

• T- and B-lymphocyte numbers and T-cell function are normal.

Complications

Inevitable long delay in diagnosis leads to structural lung damage. The delay for such patients may be on the order of 15-20 years because clinicians fail to recognize immunodeficiency in the presence of normal total immunoglobulins.

Treatment

Treatment is still controversial. Continuous antibiotics are inadequate for patients with established lung disease: these should be managed on IVIg/SCIg. In small children, spontaneous improvement may occur, and continuous prophylactic antibiotics and close supervision may be sufficient.

X-linked hyper-IgM syndrome (HIGM 1)

Originally this was thought to be a B-cell disorder, but the demonstration in the X-linked form of a primary T-cell defect means that this form should be reclassified as a T-cell defect. The predominant effect, however, is of a humoral immune deficiency.

Cause

• X-linked form has now been shown to be due to a deficiency of the CD40-ligand (CD154) on T cells (gene located at Xq26-27), required for B-cell immunoglobulin class switch.

• CD40 is also expressed on monocyte-macrophages and the interaction with CD40L is integral to antigen presentation.

Presentation

• Presentation is with recurrent bacterial infections; this may include *Pneumocystis carinii* pneumonia.

• There is often neutropenia and thrombocytopenia. Autoimmune disease of all types is common.

Diagnosis

• There is usually an early onset; the diagnosis should always be considered when *Pneumocystis* pneumonia is the presenting illness. The differential diagnosis includes SCID and HIV infection.

• There will be a normal or high IgM, with low IgG and IgA.

• Most cases can be identified by failure to upregulate expression of CD154 on activation of T cells; this does not identify cases where point mutations permit expression of non-functional CD154.

• Genetic identification is possible.

Immunology

• IgM (and IgD) are normally raised with a low IgG and IgA. However, the IgM may be normal in the absence of infection. There will be high isohaemagglutinins. Specific IgM responses are present but may be short-lived. IgM^+ and IgD^+ B celts are present

• T-cell function may be normal or poor. Some patients have reduced celt-mediated immunity, as evidenced by the occurrence of *Pneumocystis* infection. The expression of CD40-ligand on activated T cells may be defective (use PMA and ionophore).

• Mild variants exist, compatible with minimal disease and survival into adult life.

Complications

• Complications include IgM^+ lymphomas (due to chronic overstimulation), opportunist pneumonias, autoimmune disease, and aplastic anaemia. There appears to be a particular risk of *Cryptosporidium* infection of the biliary tree, leading to a severe cholangitis and liver failure.

• Long-term prognosis without transplantation is poor (infections and liver disease).

Treatment

- IVIg/SCIg should be started at the earliest opportunity: the IgM returns to the normal range with adequate therapy. Doses should be in the range 0.4-0.6 g/kg every 2-3 weeks.
- Prompt antibiotics are required for infections, as for other antibody deficiencies.
- Consideration should be given to the use of PCP prophylaxis (co-trimoxazole 480 mg bd, 960 mg od, or 960 mg three times per week; azithromycin and atovaquone are alternatives where co-trimoxazole cannot be tolerated).
- All drinking water, even if bottled, should be boiled as domestic supplies cannot be guaranteed to be free of *Cryptosporidium*.
- Bone marrow/stem cell transplantation is the treatment of choice, where the diagnosis is made early in life and where compatible donors are available.
- Liver transplantation may be required for liver disease secondary to *Cryptosporidium* infection.

Autosomal hyper-IgM syndromes (HIGM 2-5)

Four rare types of autosomal hyper-IgM have now been identified.

HIGM-2

Caused by defects in the activation-induced cytidine deaminase gene (AID gene, 12p13). This causes an intrinsic B-cell defect with failure to class-switch. T-cell function is normal and thence opportunistic infections such as *Pneumocystis* do not occur. Presentation is with recurrent bacterial and gastrointestinal infections from early in childhood. Lymphoid histology is abnormal with hyperplasia and giant germinal centres. B cells express CD19, sIgM, and sigD. T-cell expression of CD154 is normal.

Treatment is with IVIg/SCIg.

HIGM-3

Caused by deficiency of CD40 expression on B cells, due to a genetic defect (20q12-13.2). Clinical features are identical to those of HIGM-1, and opportunist infections can occur. Class-switch memory B cells are markedly reduced *or* absent. Monocyte function is also defective (CD40 is expressed on monocytes). CD40 is also expressed on endothelial cells. Treatment is the same as for HIGM-1with IVIg/SCIg, prophylaxis against PCP, and consideration of BMT/SCT, although the latter will not completely restore CD40 expression in non-haematopoietic cell lineages.

HIGM-4

Clinically a mild variant of HIGM-2, but with normal AID levels. The molecular defect is unknown.

HIGM-5

Due to defects in uracil-DNA glycosylase (UNG) and clinically resembles HIGM-2.

From the limited clinical experience, IVIg/SCIg would appear to be the treatment of choice.

X-linked ectodermal dysplasia with HIGM (NEMO deficiency)

Cases have been reported of X-linked ectodermal dysplasia with HIGM due to loss-of-function mutations in the *NEMO* gene (Xq28), coding for IKK-γ, which block the release of NFκB on cellular activation. Gain of function mutations in IκBα, with which IKK-γ interacts, have been reported to produce an autosomal dominant ectodermal dysplasia similar to NEMO deficiency. Hypomorphic NEMO mutations have also been associated with ectodermal dysplasia associated with osteopetrosis and lym-phoedema, and incontinentia pigmenti.

Treatment with IVIg is helpful.

X-linked lymphoproliferative disease (Duncan's syndrome)

This is a very rare genetic disorder, leading to failure to handle EBV correctly.

Cause

- Genetic defect has been localized to Xq26, and the gene has now been cloned.

• Gene product, SAP (SLAM-associated protein) controls the activation of T and B cells via SLAM (signalling lymphocyte activation molecule), a surface protein. SLAM is involved in IFN-γ production and the switch fromTh2 toTh1.
• Reason for the failure to handle EBV appropriately is not yet known.

Presentation

• Patients are fit and well until EBV is encountered. Upon infection with EBV, five outcomes are possible:
  - Fulminant EBV infection (58%); mortality is 96%.
  - EBV^+ non-Hodgkin's lymphoma (30%); risk of lymphoma is 200-fold greater than the normal population.
  - Immunodeficiency, usually profound hypogammaglobulinaemia (30%).
  - Aplastic anaemia, often associated with hepatitis (3%).
  - Vasculitis, lymphomatoid granulomatosis (3%).
• Overall mortality is 85% by the age of 10 years.

Diagnosis

• Diagnosis is difficult, especially if there is fulminant EBV infection. There are no diagnostic immunological findings. Protein studies to confirm the absence of SAP and genetics may help.

Immunology

• Immunology is usually normal before infection, but it is rarely checked unless there is a family history. Carriers may have subtle immunological abnormalities, such as unusually high anti-EBV VCA antibodies.
• After infection, in those who survive, there are reduced immunoglobulins (all three classes). T-cell proliferation to mitogens and antigens is poor, and there is reduced IFN-γ production. NK-cell function is also poor. $CD8^+$ T cells may be persistently elevated.
• Antibodies to EBV may be poor or even absent

Treatment

• IVIG/SCIg should be used for the hypogammaglobulinaemia.
• Bone marrow transplantation may be an option, as part of the treatment of lymphoma.
• Prophylactic use of aciclovir in at-risk family members is of unproven value.
• During acute fulminant EBV, therapy directed against the abnormal $CD8^+$ T cells (*Campath*) and transformed B cells (*rituximab, anti-CD20*) may be valuable.

Transient hypogammaglobulinaemia of infancy (THI)

Cause

• THI is thought to be due to a delay in immune development, leading to a prolongation of the physiological trough of antibody after the age of 6 months, when maternal antibody has largely disappeared.
• Common in the families of patients with other antibody deficiencies.
• Diagnosis excludes hypogammaglobulinaemia by reasons of prematurity.

Presentation

• Usual presentation is with recurrent bacterial infections, typically of the upper and lower respiratory tract, occurring after 6 months of age. It may last up to 36 months before spontaneous recovery takes place.
• Transient neutropenia and thrombocytopenia have been reported.

Diagnosis

• There will be an early onset.
• Presence of normal B-cell numbers differentiates THI from XLA. IgM is frequently normal, and there may be evidence of specific antibody responsiveness.
• Check for *btk* expression, to exclude XLA.
• No specific diagnostic features and the diagnosis can only be made for certain after full recovery of immune function has taken place.

Immunology
• IgG and IgA are low for age; IgM is usually normal. B cells are present; T-cell numbers and function are normal. IgG must be reduced on more than one occasion. A reduction to < 2 SD below the lower end of the normal range has been suggested by some authorities.
• Vaccine responses may be normal or reduced.
Treatment
• Mild cases may be managed with prophylactic antibiotics.
• IVIg/SCIg treatment may be required in more severe cases, used for a fixed period, and be withdrawn at intervals to check for spontaneous recovery.
• By definition all recover! If there is no recovery, then the patient has an alternative diagnosis.

Hyper-IgE syndrome (Job's syndrome)
The underlying cause for this curious illness is unknown. It is frequently classified with neutrophil defects, but the neutrophil defects are secondary to the dysregulation of T- and B-cell function and the very raised IgE.
Cause
• Some families have shown autosomal dominant inheritance mapping to a locus on chromosome 4. In some cases there is incomplete penetrance.
• Autosomal recessive inheritance has also been described.
Presentation
• Patients present with atypical eczema and recurrent invasive bacterial infections. Staphylococci and Haemophilus are usual pathogens. Invasive candidiasis may occur.
• Pneumatocoeles due to staphylococcal infection are a diagnostic feature.
• Osteopenia, probably due to abnormal osteoclast function, is a feature and may lead to recurrent fractures.
• Coarse 'leonine' fades, but not all patients have red hair as originally described.
Diagnosis
• The clinical history is typical, especially the occurrence of pneumatocoeles.
• IgE levels are massively elevated and are usually much higher than in atopic eczema.
• The occurrence of invasive as opposed to cutaneous infections distinguish HIGE from atopic eczema.
Immunology
• IgE is massively elevated (>50 000 kU/l) and there may be IgG subclass and specific antibody deficiencies, with poor/absent immunization responses.
• Variable abnormalities of neutrophil function, affecting chemotaxis, phagocytosis, and microbicidal activity have been reported, but are likely to be due to inhibition by the high IgE.
• The underlying defect seems to involve an imbalance of cytokine production due to a Th2 predominance (IL-4, IL-5), and decreased Th1 cytokines (IFN-γ and TNFα).
• Absent CD45RO on T cells has been reported
Treatment
• IVIg/SCIg should be used for the antibody deficiency.
• Cimetidine (as an immunoregulatory agent) has been recommend, although the value appears to be limited. IFN-γ is a treatment in the light of proposed Th2 predominance, but has not yet been shown to be effective in small open trials. Cyclosporin A may be very helpful.
• Surgery for pneumatoceles and deep-seated abscesses may be required.
• Bone marrow transplantation has been tried in some cases, although with no clear benefit.

Rare antibody deficiency syndromes
Antibody deficiency, presenting as common variable immunodeficiency, has been associated with the following extremely rare genetically identified disorders.
• Kappa and lambda light chain deficiency.
• ICOS (inducible co-stimulator, 2q33) deficiency.

• Surrogate light chain deficiency (λ5/14.1).
• Mu chain deficiency.
• SWAP-70 deficiency.
• CD79a (Igα) deficiency.
• BLNK deficiency.
• CD19 deficiency.

Routine genetic tests for these are not yet available; screenings of large cohorts of CVID patients have shown that these disorders account for very few cases currently diagnosed as CVID.

Severe combined immunodeficiency (SCID)

Severe combined immunodeficiency involves both the T-cell arm and the B-cell arm. Often the major defect is on the T-cell side: B cells may be present, but, in the absence of T cells, fail to respond or develop appropriately. The diagnosis is frequently missed at first, which reduces the chance of a successful outcome from treatment. It is estimated that the incidence is 1 per 50 000 births. Almost all cases are now accounted for by identified genetic defects.

Successful management of SCID requires a multidisciplinary team used to dealing with very sick small infants. Management should be restricted to centres experienced in the diagnosis and care of SCID. It should not be undertaken in haematology-oncology bone marrow transplant units as the requirements are very different from those of leukaemic patients.

Presentation

Most cases of SCID present with common clinical features, no matter what the underlying defect.

• Typical features include:
  - early onset infections (bacterial, viral, fungal, opportunist);
  - persistent candidiasis;
  - chronic enteric virus excretion;
  - failure to clear vaccines (BCG, oral polio);
  - failure to thrive;
  - maternofetal engraftment, with GvHD, causing erythroderma (often with eosinophilia);
  - lymphopenia: an absolute lymphocyte count of $< 2.0 \cdot 10^9$ /L in a baby less than 6 months old is pathological and indicates SCID until proven otherwise. Looking at the differential white count is therefore mandatory in all babies with recurrent infections.

• If SCID is suspected investigation is urgent-seek advice at once from a paediatric immunologist.

Infections in SCID

There is onset of infections soon after birth.

• Recurrent bacterial infections (pneumonia, otitis media, sepsis).
• Persistent thrush.
• Persistent viral infections (RSV, enteroviruses, parainfluenza, CMV, other herpesviruses, rotavirus, small round structured virus (SRSV)).
• Opportunist infections (PCP; fungal infections, including aspergillus).
• There are very significant risks from the administration of live vaccines especially BCG and polio. BCG should not be given to babies where there is a family history of SCID.

Other features of SCID

• Failure to thrive.
• Diarrhoea (consider chronic enteroviral infection, rotavirus, SRSV).
• Skin rash (Omenn's syndrome, maternofetal engraftment).
• Bone abnormalities (flared ribs-ADA deficiency malabsorption-rickets).
• Short-limbed skeletal dysplasia.
• Hepatosplenomegaly (BCGosis, graft-versus-host disease (GvHD) from MFE or blood transfusions; Omenn's syndrome).

SCID: investigations in suspected cases

On suspicion, first-line investigations

- Lymphocyte subpopulations (T-, B-, NK-, and T-cell subpopulations, with absolute numbers).
- Immunoglobulins.
- If the results are suspicious, then baby should be referred at the earliest opportunity to a specialist centre with facilities for managing SCID.

Further investigations in the specialist referral centre

- T-cell proliferation.
- Cytokine assays.
- NK-cell function.
- Protein studies.
- Specific antibodies.
- Biochemical (exclusion of ADA deficiency) and genetic studies.
- HLA typing as part of work up for bone marrow transplantation.

Aims of the initial investigations

- To confirm the diagnosis of SCID.
- To identify unusual variants, as this may affect the conditioning protocol used prior to bone marrow transplantation.
- To provide evidence for subsequent genetic counselling of the family.
- To provide a baseline against which success of bone marrow therapy (BMT) can be measured.

Patterns of lymphocyte subpopulations in SCID and variants

- It cannot be stressed too often how important a low total lymphocyte count is as a marker of SCID: low lymphocyte counts should never be ignored.
- Typical pattern is of very low/absent T cells with normal or absent B cells; immature T cells may be present in low numbers ($CD3^+$, $CD2^+$, $CD4^-$, $CD8^-$).
- SCID with maternofetal engraftment (MFE): T cells are present but are usually $CD8^+$ and activated ($CD25^+$, DR^+); B cells are usually low or absent. Maternal origin of the cells may be confirmed by genetic studies.
- Omenn's syndrome (leaky SCID): T cells may be present, including CD4 T cells; clonal restriction with limited Tcr pi-chain repertoire (this requires genetic analysis).
- Bare lymphocyte syndrome (HLA class I deficiency (bare lymphocyte syndrome type I) and HLA class II deficiency (bare lymphocyte syndrome type II): absence or marked reduction of HLA class II antigen expression with variable HLA class I antigen expression; T cells are normal or low.
- ZAP-70 kinase deficiency and z-chain deficiency: marked reduction/absence of $CD8^+$ T cells; low normal $CD4^+$ T cells.
- Absence of CD3/TCR complexes (CD3 γ-, δ -, or ζ-chains) variable expression of CD3: T-cell numbers are usually normal.

Proliferation assays

- Functional studies are useful in cases when T cells are present.
- PHA response: invariably absent in all major forms of SCID, including Omenn's and SCID with MFE; may be low-normal in ZAP-70 kinase deficiency.
- Phorbol esters (PMA) and calcium ionophore: will be normal where there is a membrane defect (ZAP-70 kinase or CD3 complex) that can be bypassed (PMA acts intracellularly on protein kinase C).
- Anti-CD3 and IL-2: abnormal when the defect involves the CD3/TCR complex (CD3 deficiency, ZAP-70 kinase deficiency). The normal newborn anti-CD3 response is lower than in adults.
- Reconstitute of proliferation to mitogens with IL-2 may suggest a failure to produce this cytokine.
- Other stimuli used may include antigens, anti-CD2, anti-CD43 (Wiskott-Aldrich syndrome).

Cytokine assays are rarely used: IL-2 deficiency has been reported but is exceptionally rare.

NK-cell assays. The role of these assays in the management of SCID is experimental. It may be useful as a baseline marker for monitoring against graft failure.
Immunoglobulins. If the diagnosis is made within the first few weeks of life, maternal antibody will still be present Even if the child has B SCID, immunoglobulins and specific antibody are not produced. It may be worth checking for specific antibodies if there is late presentation and the child has been immunized.
Biochemistry and genetics
• Check for ADA deficiency: abnormal metabolites (dATP, S-adenosyl homocysteine) will be present.
• It is possible to test genetically for specific genetic abnormalities, e.g. X-linked SCID (common cytokine receptor γ-chain gene), Jak-3, IL-7Ra, RAG-1/RAG-2 HLA class II deficiency (CIITA or RFX-5 genes), ZAP-70 kinase deficiency, CD3 γ-, δ -, or ζ-chains deficiency.
• The genetics department will be able to assist in the distinction of Omenn's syndrome from SCID with MFE: skin biopsies are helpful. Cytogenetics can be used if it is a male baby; other wise molecular genetics are required.

SCID: treatment and outcomes
Treatment
The approach to treatment is also common to all types.
• Isolation to prevent further infection.
• Do not give any live vaccines.
• Irradiate all cellular blood products, to prevent engraftment of any donor lymphocytes.
• Ensure all blood products are from CMV-negative donors.
• Identify and treat all infections: use direct culture and PCR-based techniques.
• Transfer immediately diagnosis is suspected to nearest specialist paediatric immunodeficiency centre with facilities for definitive treatment.
• Do not initiate investigations for underlying cause. Delay in transfer significantly reduces the chances of a successful outcome (90% success rate for babies diagnosed and treated immediately after birth, compared to 40% where treatment is delayed).
Once in the tertiary centre, management will include the following.
• Isolation in laminar flow.
• Establish venous access and commence nutritional support (enteral or parenteral) if required.
• Initiation of diagnostic tests: T- and B-cell numbers; baseline T-cell function; serum immunoglobulins; screen for pathogens (nasal secretions, bronchial lavage, stool, urine, blood). Biopsies as required (lymph nodes, skin for GvHD).
• Undertake genetic testing to identify gene defect, if possible.
• Tissue typing of baby and family; initiation of search for matched unrelated donor on bone marrow registries.
• Counsel family about diagnosis and treatment.
• Initiation of IVIg replacement.
• Use PEG-ADA as a temporizing measure in ADA-deficient SCID.
• Initiate aggressive treatment for identified infections with antibacterials, antivirals, and antifungals as appropriate-good microbiological and virological control is essential.
• Cytoreductive conditioning of recipient (to prepare space for incoming stem cells).
• T-cell depletion of donor marrow (for mismatched grafts).
• Undertake BMT, SCT as appropriate; consider gene therapy (currently suspended due to development of leukaemias arising from the insertion of genes close to the proto-oncogene LMO-2).
BMT may be:
- sibling (identical);
- parent (haploidentical);
- matched unrelated donor (MUD);
- stem cells.

• Undertake post BMT/HSCT immunological monitoring, treating infection and GvHD as appropriate.

Outcomes

• Outcome is dependent on the promptness of the diagnosis and what infections are present at the time of diagnosis. Poor prognostic indicators are late diagnosis and chronic viral infections (especially parainfluenza pneumonitis).

• Meticulous care is required during the aplastic phase between conditioning and engraftment: this will require cell support (platelets, red cells), infection prophylaxis and treatment (fungi, viruses, and bacteria), management of the complications of conditioning (veno-occlusive disease of the liven pneumonitis) and of graft-versus-host disease (mismatched donors).

• Survival should be > 80% with early diagnosis, good matching of donors, and no pretransplant infections. This falls to < 40% with late diagnosis, chronic infections, and poorly matched donors. Regular monitoring post-BMT is required to follow engraftment and development of immune function. This should include the following.

• Lymphocyte subpopulations: the return of T- and B-cells, expression of activation markers in association with GvHD, effectiveness of immunosuppression.

• T-cell proliferation: this is only valuable when T-cells have returned to the circulation. The return of a PHA response defines probable safety for release from laminar flow confinement.

• NK-cell function and numbers may correlate with graft survival.

• Immunoglobulins: adequacy of replacement IVIg therapy, return of IgA and IgM synthesis, IgG subclass development (off IVIg).

• Specific antibodies: return of functional antibodies-isohaemagglutinins; immunization responses.

• Genetic studies: chimerism of lymphocytes (DNA studies on separated T- and B-cells).

• Biochemical reconstitution (ADA deficiency; enzyme deficiencies, e.g. ZAP-70 kinase).

SCID: RAG-1/RAG-2 deficiency

Cause

• Autosomal recessive. Mutations in RAG-1/RAG-2 genes, required for V(D)J recombination of the T- and B-cell antigen receptors. Located at 11p13.

Presentation

• Typical SCID.

Diagnosis

• Typical presentation with recurrent infections and lymphopenia on full blood count.

• Confirmed by immunological and genetic studies.

Immunology

• Low or absent serum immunoglobulins, severe lymphopenia (T^-, B^- SCID). NK cells are present and comprise the majority of circulating lymphocytes.

• Maternofetal engraftment is common.

SCID: Omenn's syndrome

Cause

• Autosomal recessive. Mutations in RAG-1/RAG-2 genes, required for V(D)J recombination of the T- and B-cell antigen receptors. Located at 11p13.

• Partial activity present, allowing the development of oligoclonal, peripherally expanded T-cell clones.

Presentation

• Presents with erythroderma rash, lymphadenopathy, and hepatosplenomegaly; must be distinguished from maternofetal engraftment. Cytogenetics of the peripheral blood cells may identify maternal cells.

Diagnosis

• Cytogenetics is required to exclude MFE.

• Immunological tests are variable.
• Oligoclonal T-cell pattern is distinctive.

Immunology

• Eosinophilia.
• Leucocytosis, variable $CD4^+$ and $CD8^+$ T-cell numbers; high level of activation marker expression (DR, CD25, CD45RO).
• B-cells absent; hypogammaglobulinaemia usual.
• Oligoclonal TCR usage.
• Abnormal lymph node histology: excess eosinophils, lymphocyte depletion.

SCID: Artemis deficiency

This defect causes a $T^-B^-NK^+$ radiation-sensitive phenotype of SCID, similar to RAG-1/RAG-2 deficiency.

Cause

• Mutation in the *Artemis* gene, located on chromosome 10p13, involved in DNA repair probably through interaction with DNA-PKcs.

Presentation

• As for SCID, but prone to oral and genital ulcers.

Diagnosis

• Should be considered in cases of $T^-B^-NK^+$ SCID, where RAG-1/RAG-2 mutations are not found.

Immunology

• Profound T- and B-cell lymphopenia with normal NK-cell numbers and function
• Maternofetal engraftment common.

SCID: Jak-3 kinase deficiency (autosomal recessive T^-B^+ SCID)

This form, in which T-cells are absent but B-cells are present in normal numbers, accounts for about 10% of cases.

Cause

• Mutations in the gene encoding Jak-3 kinase, which prevent signalling through the surface cytokine receptors containing the common gamma chain. Signals from IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 are blocked.
• T-cell but not B-cell differentiation is blocked Presentation Presents with typical SCID features.

Diagnosis

• Typical SCID presentation.
• Absent T-cells with normal or increased B- and decreased NK-cells; overall total lymphocyte count may be normal.
• Western blotting of lymphocyte lysates may identify the absence of Jak-3 protein and genetic studies will confirm the gene defect.

Immunology

• T cells are very low or absent; NK-cells are reduced, B-cells are normal or increased; absent mitogenic responses.
• Mild variants may exist with significant numbers of poorly functioning T-cells.
• Hypogammaglobulinaemia is usual.

SCID: Common gamma chain deficiency (X-linked T^-B^+ SCID)

This accounts for 50-60% of SCID.

Cause

• Mutations in the common cytokine receptor gamma chain; signals from IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 are blocked.
• Gene is located at Xq13.1-13.3.

• Missense mutations may lead to the expression of a mutant non-functional gamma chain.
Presentation
• Typical SCID features in male infant.
Diagnosis
• Pattern of infections.
• Low or absent T-cells, NK-cells; normal or increased B-cells.
• Demonstration of absent common gamma chain and genetic mutations. As levels of common γ-chain on lymphocytes are low, demonstration of absence is difficult, and may be confused if there is maternofetal engraftment.
Immunology
• Low serum immunoglobulins.
• Normal or increased B-cells, low or absent T-cells and NK-cells (dependent on IL-15).
• Poor mitogen responsivness. B-cells may make IgE *in vitro* via a common γ-chain independent IL-4 receptor.

SCID: Interleukin-7 receptor-α deficiency
Accounts for 1-2% of SCID patients.
Cause
• Autosomal recessive mutation in IL-7Rα.
• Gene is located at 5p13.
• Missense mutations may lead to the expression of a mutant nonfunctional protein that binds IL-7 poorly.
Presentation
• Typical SCID features in infant.
Diagnosis
• Pattern of infections.
• Low or absent T-cells, normal or increased B-cells, and normal NK-cell numbers.
• Demonstration of absent IL-7Rα and genetic mutations.
Immunology
• Low serum immunoglobulins.
• Normal or increased B-cells, low or absent T-cells, and normal NK-cells.
• Poor mitogen responsiveness.

SCID: ZAP-70 kinase deficiency
This is a rare cause of autosomal recessive SCID.
Cause
• Autosomal recessive mutation in CD3 zeta-associated protein, ZAP-70.
• Gene is located at 2q12.
• Mutations cluster in region of gene encoding the site for enzymatic activity.
• ZAP-70 is involved in signal transduction from the membrane CD3 complex to the internal cascade.
• ZAP-70 is crucial in thymic devlopment, especially in the development of CD8 T-cells from double-positive precursors.
Presentation
• Typical SCID features in infancy, with an early presentation.
• Lymph nodes and thymic shadow may be present.
Diagnosis
• Pattern of infections.
• Characteristic selective absence of circulating $CD8^+$ T-cells.
• Demonstration of absence of ZAP-70.
• Thymic biopsy may show double-positive cells in the cortex but only $CD4^+$ T-cells in the medulla.

Immunology
• Serum immunoglobulins may be normal.
• Normal or increased B-cells, absent $CD8^+$ T-cells, normal or low $CD4^+$ T-cells, and normal NK-cells.
• Failure of response to membrane acting mitogens, PHA and anti-CD3 monoclonal antibodies, with normal responses to phorbol esters and ionophores (acting at the level of protein kinase C).
Treatment
• Gene therapy may be possible.

SCID: purine metabolic disorders: Adenosine deaminase (ADA) deficiency
This is the first genetically identified cause of SCID. It accounts for 20% of cases of SCID.
Cause
• Mutations in the ADA gene on chromosome 20q13.11. This gene has now been cloned and sequenced
• Gene product is involved in purine metabolism.
• In the absence of ADA, there is a progressive reduction of T- and B-cells due to toxic effects of dATP and S-adenosyl homocysteine, with increased lymphocyte apoptosis.
Presentation
• Typical SCID features in infancy, with (usually) an early presentation.
• Liver enzymes are often raised.
• There is abnormal flaring of the rib ends, pelvic dysplasia, and neurological features, including cortical blindness.
• Presentation with mild form in adult life has been reported; presentation is with recurrent bacterial infections and opportunist infections such as PCP; evidence of immune dysregulation (autoimmunity and allergy).
• Relatives may show reduced levels of red cell ADA activity.
• Lymphocytes may be normal at birth (due to maternal ADA), but fall rapidly after delivery.
Diagnosis
• Pattern of infections (in early childhood); diagnosis of adults may be difficult.
• Lymphopenia.
• Bone changes are not specific, although suggestive in the context of the immunological abnormalities.
• Measurement of ADA in erythrocytes, together with serum levels of toxic metabolites is essential.
Immunology
• Serum immunoglobulins may be normal initially, but fall rapidly.
• Progressive severe lymphopenia, affecting all cell types.
• Poor or absent responses to all mitogens.
Treatment
• BMT or stem cell transplant is treatment of choice, as for other types of SCID.
• Red cell transfusions restore ADA levels and improve immune function; PEG-ADA is available and is highly effective in restoring immune function (although this makes BMT harder).
• Antibodies to PEG-ADA may occur.
• Gene therapy has been used successfully, but has been complicated in several cases by acute leukaemia, due to insertion of the retroviral vector into known oncogenes; gene therapy has therefore been suspended.

SCID: purine metabolic disorders: purine nucleoside phosphorylase (PNP) deficiency
This is a rare genetic deficiency (about 50 cases worldwide).
Cause
• Absence of PNP leads to a build-up of the toxic metabolite dGTP, which preferentially damages T-cells in the early stages, but later damages B-cells also.

• Disease is autosomal recessive; gene is located at 14q13.1.

Presentation

• Onset tends to be later than for other forms of SCID, and in this respect it behaves more as a combined immunodeficiency.
• Neurological signs (spasticity, tremor, ataxia, and mental retardation) occur early.
• Infections are common, especially disseminated varicella, but also other bacterial, viral, and opportunist pathogens.
• Haemolytic anaemia, immune thrombocytopenic purpura and thyroiditis are also common.

Diagnosis

• Combination of progressive neurological signs and infections should raise suspicion.
• A low serum urate is a useful marker.
• Diagnosis is made by enzymatic studies and by detection of elevated levels of the metabolite dGTP.

Immunology

• Immunoglobulins are normal or low, but some patients have elevated levels with monoclonal gammopathies.
• Poor specific antibody responses to immunization.
• Autoantibodies may be detected.
• B-cells are normal in numbers until late disease, but there is a progressive decrease in T-cell numbers with time.
• Mitogen responses are variably reduced.

Treatment

BMT or HSCT is probably best, although gene therapy is a future possibility.

SCID: HLA class I deficiency (bare lymphocyte syndrome type I)

This is a rare cause of combined immunodeficiency (about 20 cases worldwide).

Cause

• Deficiency of either TAP-1 or TAP-2 (transporter associated with antigen presentation).
• Causes unstable peptide/HLA class I/(β_2-microglobulin complex, which rapidly dissociates and cannot be expressed on the cell surface.

Presentation

• Recurrent bacterial sinopulmonary infections.
• Granulomatous skin lesions, with ulceration.
• Presentation at any age.
• May be asymptomatic.

Diagnosis

Symptoms plus failure of class I expression on cells.

Immunology

• Minor abnormalities of humoral immunity only.
• Lymphocyte subsets may be normal; abnormalities when present are minor and inconsistent.

Treatment

• Unsatisfactory; chest and sinus disease treated similarly to cystic fibrosis with aggressive antibiotics and physiotherapy.
• Skin disease is difficult to treat: immunosuppressive therapy and interferons may make the condition worse.
• Role of BMT/SCT undefined (but class I expression occurs on all nucleated cells, so this is not a pure stem cell disorder).

SCID: MHC class II deficiency (bare lymphocyte syndrome type II)

It is autosomal recessive, but genetically complex. This rare disease is found predominantly in North Africa and Mediterranean area (80 cases worldwide).

Cause
• CIITA, RFX-5, RFXAP, RFXANK, RFX-B transcription factor components are abnormal.
• Four genetic complementation groups.

Presentation
• Presentation is usually early (first 6 months of age) with severe diarrhoea, hypogammaglobulinaemia, and malabsorption.
• Recurrent bacterial and viral chest infections.
• Viral infections of the nervous system (HSV, enteroviruses).
• Haemolytic anaemia.
• Hepatitis, cholangitis.
• Rarely, it is asymptomatic.
Diagnosis
• Diagnosis is made on clinical suspicion (differential diagnosis includes X-linked immune dysregulation with polyendocrinopathy syndrome (IPEX) due to FOXP3 deficiency).
• Absence of MHC class II expression on lymphocytes.
Immunology
• Immunoglobulins are normal or low.
• B-cell numbers are normal; T-cell numbers may be normal but there may be low $CD4^+$ T-cells and increased $CD8^+$ T-cells, reversing the normal ratio.
• MHC class I expression is variable.
• MHC class II expression will be absent (DR, DP, DQ) but may be inducible with γ-IFN.
• Poor but variable mitogen responses.
Treatment
• BMT or HSCT is the treatment of choice, but is surprisingly difficult to do successfully.
• γ-IFN may have some beneficial effect.

SCID: reticular dysgenesis
This is a rare defect of the maturation of stem cells for both lymphoid and myeloid lineages. No genetic cause has yet been identified. There is marked granulocytopenia, lymphopenia, and thrombocytopenia (sometimes no cells at all). It presents with early overwhelming infections and often death before the diagnosis is made.
Treatment
Maternofetal engraftment is common. BMT/SCT is required.

SCID/CID: Nezelofs syndrome
Mainly a T-cell deficiency, with no identified molecular causes, presenting with recurrent sinopulmonary and gastrointestinal infections and malabsorption, warts, allergic disease autoimmunity (haemolytic anaemia, immune thrombocytopenic purpura, neutropenia, hepatitis). Unclear whether this is a discrete diagnostic entity or represents early onset CVID. T lymphopenia, variably reduced immunoglobulin levels, poor humoral function, autoantibodies.
Prognosis seems to be poor despite prophylactic antibiotics, antifungals, and IVIg: BMT/SCT suggested.

SCID/CID: CD3 ζ-chain deficiency
Similar to ZAP-70 kinase deficiency.

SCID/CID: CD3 deficiency
Deficiencies of CD3 γ-, δ-, and ε-chains have been described in small numbers of patients; there is variable expression of CD3.
Cases present as mild combined immunodeficiency, with later onset than SCID, recurrent sinopulmonary infections, and autoimmune phenomena. Reduced expression of the CD3 complex

on T-cells is suspicious; proliferative responses to mitogenic anti-CD3 monoclonal antibodies are absent but responses to phorbol esters and ionophore are normal.
BMT/SCT is probably the treatment of choice. IVIg is helpful in mild cases.

SCID/CID: CD25 (IL-2 receptor-α) deficiency
Single patient reported, presenting with severe T-lymphopenia and diffuse lymphocyte infiltrates associated with mutation in CD25 gene similar in presentation to autoimmune lymphoproliferative syndrome. Recurrent viral, bacterial, and opportunist infections.

SCID/CID: CD45 deficiency
Two cases presenting with SCID-like illness due to complete absence of surface CD45.

SCID/CID: Human nude phenotype (*whn* mutation) FOXN1 (17q11) nude human
One Italian family with complete alopecia and SCID-like syndrome reported, associated with winged-helix nude mutation, similar to nude mouse. Thymic function impaired.

SCID/CID: Other rare defects
- CD7 T-cell deficiency.
- Autosomal SCID with multiple intestinal atresias.
- Multiple cytokine deficiency.
- Lack of nuclear factor of activated T-cells (NF-AT).
- IL-2 deficiency: presented as SCID; no transcription of IL-2.
- Other signat-transduction defects, including T-cell calcium flux defects.

Disorders with predominant T-cell disorder
- DiGeorge syndrome (including all the variants).
- Wiskott-Aldrich syndrome (also includes X-linked thrombocytopenia and GATA-1 deficiency).
- Ataxia telangiectasia.
- Other chromosomal breakage syndromes:
    - Nijmegen breakage and Seemanova syndrome;
    - Bloom's syndrome;
    - Fanconi anaemia;
    - ICF syndrome;
    - Xeroderma pigmentosa.
- Chronic mucocutaneous candidiasis.
- AIRE gene deficiency.
- APECED.
- IPEX syndrome (including FOXP3 deficiency).
- Idiopathic CD4 lymphopenia.
- Autoimmune lymphoproliferative syndromes:
    - fas deficiency;
    - fas-ligand deficiency;
    - caspase 8 and 10 deficiencies.
- Cartilage-hair hypoplasia.
- WHIM syndrome.
- γ-Interferon/IL-12 pathway syndromes:
    - γ-IFNR1 deficiency;
    - γ-IFNR2 deficiency;
    - IL-12-Rβ1 deficiency;
    - IL-12p40 deficiency;
    - STAT1 deficiency.

DiGeorge (22q11 deletion complex) syndrome

DiGeorge originally described one phenotype of what is now realized to be a broad and complex array of developmental defects, probably due to more than one genetic lesion.

The range of defects is large and includes the CATCH-22 syndrome (cardiac abnormalities, abnormal fades, thymic hypoplasia, cleft palate, and hypocalcaemia, associated with 22q11 deletions), Shprintzen syndrome, velocardiofacial syndrome (VCF), cono-truncal face anomaly syndrome, Opitz-GBBB syndrome, CHARGE associations, Kallman syndrome, and arrhinencephaly/holoporencephaly. Similar features may also arise in the fetal alcohol syndrome, maternal diabetes, and retinoid embryopathy.

Cause

- Microdeletions at 22q11, possibly affecting a zinc-finger (DNA-binding) protein involved in early development, have been associated with many of the phenotypic variants.
- Candidate genes include TBX1, Crkol, and UFD1L
- Other mutations, including 10p deletions, may give a similar phenotype.
- Abnormal development of branchial arch-derived structures, including heart, thymus, and parathyroid glands.

Presentation

- Hypocalcaemic tetany, often occurring in first 48 hours after delivery, and due to parathyroid gland maldevelopment.
- Cardiac abnormalities, typically those of a truncus arteriosus type, interrupted aortic arch, or tetralogy of Fallot. Severity of the cardiac abnormalities often determines outcome.
- Dysmorphic face with cleft palate, low-set ears, and fish-shaped mouth.
- Highly variable immunodeficiency, associated with absence or reduction of thymic size; this often improves with age. Severe forms may present as SCID with absent T cells. Partial syndromes may occur without any immunological features (VCF and Shprintzen syndromes).
- Infections are mainly viral (adenovirus, CMV, rotavirus).
- GvHD from non-irradiated blood transfusions may occur if the diagnosis is not thought of during surgery for cardiac abnormalities.
- Increased risk of autoimmune disease and B-cell lymphomas.
- 10% have cleft palates.
- Development delay common, especially language.

Diagnosis

- Diagnosis is by clinical suspicion, based on the facial features, typical cardiac abnormalities, and abnormally low calcium in full-blown cases. Partial variants may be more difficult to identify.
- All children with relevant cardiac abnormalities should be screened for 22q11 deletions by FISH and for other cytogenetic abnormalities. If positive, screen parents and refer for genetic counselling (50% of subsequent pregnancies will be affected).
- Patients with an identified 22q11 deletion should be screened for immunological defects, both humoral and cellular (immunoglobulins, IgG subclasses, specific antibodies, lymphocyte surface markers, and proliferation assays).

Immunology

- Immunology is highly variable and tends to improve with age; extra-thymic development of T-cells may occur.
- SCID-like immunology is possible.
- Variable reduction in T-cells, with normal or low T-cell proliferation to mitogens.
- Immunoglobulins may be normal or reduced and specific antibody production may be poor, with low/absent immunization responses.
- Deficiency of IgA increased, and associated with autoimmunity.

Treatment

- Optimum treatment is uncertain: the cardiac abnormalities define prognosis and repair of these takes priority.
- Irradiated blood should be used until it is known how severe the immunological abnormality is. Children with normal T-cell function are probably at very low risk of developing transfusion-related GvHD.
- Calcium supplementation for hypoparathyroidism.
- As mild immune defects may improve, simple measures such as prophylactic antibiotics may be all that is required. If there is evidence for significant humoral deficiency, then IVIg may be required
- Severe defects, with absent T-cells, should be considered for BMT/HSCT, although in the absence of a thymus it is probable that reconstitution is due to engraftment of mature T-cells.
- Thymic transplants (inserted into muscle peripherally) have been tried and are said to be of value.

Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT)

Wiskott-Aldrich syndrome is an X-linked disorder causing thrombocytopenia with small platelets, eczema, and a progressive immune deficiency. The same gene is also responsible for X-linked thrombocytopenia, a milder variant in which the eczema and immune deficiency is absent.

Cause

- X-linked disease; the gene is located at Xp11.23 encoding WASP (Wiskott-Aldrich-associated protein). The same gene is responsible for X-linked thrombocytopenia.
- WASP is a multifunctional protein, with GTPase binding activity. It is involved in intracellular actin polymerization, intracellular signalling, apoptosis, and phagocytosis.
- Carrier females show non-random X-inactivation, indicating that WASP expression carries a selective advantage in cell development.
- Abnormal O-glycosylation of surface proteins of both lymphocytes and platelets is well described. One such surface antigen is CD43 (sialoglycophorin). However, the gene for CD43 is normal in WAS. N-linked glycosylation is normal.

Presentation

- Presentation is early in childhood in males with severe eczema, which has an atypical distribution compared to atopic eczema. Molluscum contagiosum, warts, and HSV may infect eczematous skin.
- Abnormal bleeding is due to the low platelet count, and bleeding complications occur early.
- Infections develop more gradually, usually affecting the respiratory tract, and are bacterial.
- Autoimmunity (vasculitis and glomerulonephritis) is well described.
- Allergic reactions to food may occur.
- There is often a family history.
- XLT, a mild variant with thrombocytopenia alone, but without eczema and immune deficiency is recognized.
- Two forms of WAS exist: a severe form culminating in early lymphoma, and a milder form compatible with survival to adult life. There is no phenotype-genotype correlation.
- Symptomatic female carriers have been reported (skewed X-inactivation).

Diagnosis

- Clinical features: there is thrombocytopenia (variable in range < 70) with abnormally small platelet volume (<6.0 fl is diagnostic).
- Identification of low/absent WASP with subsequent mutation analysis is required.
- No absolutely diagnostic immunological features.

Immunology

- There is a progressive reduction of T-cells with poor proliferative responses to CD3, CD43, and periodate (specific for O-linked sugars). Proliferation to galactose oxidase and neuraminidase, which act via N-linked sugars, is normal.

• There are reduced IgM and IgA with a normal or high IgG and elevated IgE. There is a progressive loss of antipolysaccharide responses (including isohaemagglutinins) with poor/absent responses to test immunization with polysaccharide antigens. B-cell numbers are normal.
• CD43 (sialophorin) on lymphocytes and gplb on platelets are unstable and tend to fall off cells when they are kept *in vitro*. Abnormalities of the cytoskeleton in T-cells and platelets, with failure of actin bundling, have also been described.
• Variable thymic hypoplasia.

Treatment

• Splenectomy may be beneficial for thrombocytopenia, which is usually resistant to steroids. XLT patients are highly susceptible to overwhelming sepsis if splenectomy is undertaken, even though normally they do not have a problem with infection.
• Platelet transfusions should be avoided unless essential to control bleeding.
• IVIg should be used for patients with poor antipolysaccharide responses, if recurrent bacterial infections are a problem and after splenectomy, as responses to post-splenectomy vaccination schedules will be poor.
• Consider prophylactic antibiotics; mandatory post-splenectomy.
• Treat eczema.
• Avoid aspirin and related drugs (increased risk of bleeding).
• Early (<5 years of age) BMT/HSCT should be considered to prevent the development of lymphoma: it cures all the features of the disease, including eczema.
• The role of BMT/HSCT in adult patients is unclean development of lymphoma is an indication for aggressive chemotherapy followed by transplantation in first remission, with matched unrelated donor for the graft-vs-lymphoma effect.

Outcome

• Death may occur from infection or intracranial haemorrhage.
• Very significant risk of death in late adolescence/early adulthood from high-grade lymphoreticular malignancy - this may be preventable by early BMT.
• Mild variants exist and may have fewer problems with bleeding or infection in adulthood.

Ataxia telangiectasia

Cause

• Cells from AT patients have a disorder of the cell cycle checkpoint pathway, resulting in extreme sensitivity to ionizing radiation. Lymphocytes show frequent chromosomal breaks, inversions, and translocations; the major sites affected are the genes for the T-cell receptors and Ig heavy chains.
• Disease is autosomal recessive; six genetic complementation groups (A, B, C, D, E, V1, V2) have been described and all but V2 map to 11q22-23.
• One of the genes has now been identified and appears to be a DNA-dependent kinase related to phosphatidylinositol kinase-3 (ataxia telangiectasia mutated protein (ATM)). Function appears to be to sense double-stranded DNA breaks.
• ATM phosphorylates BRCA1 (may explain increased risk of breast cancer in carrier females).

Presentation

• Progressive cerebellar ataxia, with typical telangiectasia, especially of the ear lobes and conjunctivae.
• Accompanied by recurrent bacterial sinopulmonary infections.
• Opportunist infections uncommon, but extensive warts are not unusual.

Diagnosis

• Clinical history is usually diagnostic, although the disease may be difficult to identify in the early stage when signs are minimal.
• α-fetoprotein in serum is usually raised.
• Genetic testing is difficult due to the large size of the gene and the lack of clustering of the mutations.

• Spontaneous cytogenetic abnormalities occur, and will be increased by radiation exposure.

Immunology

• Immunoglobulins are variable: there is often a reduction of IgG_2/IgG_4, IgA, and IgE, with poor anti-polysaccharide responses. There is an increased incidence of autoantibodies.

• T-cell numbers and function are usually reduced.

Treatment

• No treatment is effective in what is a relentless disease with progressive neurological deterioration.

• PEG feeding may be required in the later stages when bulbar function deteriorates, to prevent recurrent aspiration.

• IVIg reduces the incidence of infections and improves quality of life but does not affect the outcome.

• Minimize radiation exposure.

Outcome

• Ataxia is progressive and patients become wheelchair-bound early (late teens, early twenties).

• High incidence of malignancy, especially high-grade lymphoma, and the incidence of malignancy is raised in other family members (fivefold increase in breast cancer).

• Death usually ensues in early adult life from infections or neoplasia.

• Heterozygotes for abnormal ATM genes may have an increased risk of chronic lymphocytic leukaemia.

Other chromosomal instability disorders

Ataxia-telangiectasia-like disorder

An AT-like disorder, without telangiectasia has been identified linked to a gene at 11q21, hMre11, also involved with ATM in DNA repair, α-fetoprotein and serum immunoglobulin levels were normal. Patients were radiosensitive.

Nijmegen breakage and Seemanova syndromes

These are syndromes of severe microcephaly, mental retardation (not in the Seemanova syndrome), bird-like face, and recurrent infections. There is increased chromosomal sensitivity to ionizing radiation. Gene has been identified on chromosome 8q21, encoding a protein nibrin (NBS1), involved in DNA repair. Most patients have reduced immunoglobulins and lymphopenia. T-cell responses to mitogens are poor. Cytogenetic analysis shows chromosomal abnormalities, and the most commonly involved chromosomes are 7 and 14, where the Ig and TCR genes are located. Malignancy is increased (lymphoma, neural tumours), and is the major cause of death. There is no specific treatment.

Bloom's syndrome

An autosomal recessive syndrome commonest in Ashkenazi Jews and presenting with marked sun sensitivity, short stature, patchy vitiligo, and very high incidence of cancer. Gene located at 15q26.1, coding for BLM, a nuclear protein, with similarities to helicases. The immunodeficiency is characterized by low IgM, normal or poor specific antibody responses, poor T-cell function, poor B-cell IgM production, and NK-cell defects. Malignancy is the major complication and is difficult to treat because of the inability to repair DNA damage.

Fanconi anaemia

This is an autosomal recessive disease with chromosomal breaks. There are multiple organ defects, bone marrow failure (pancytopenia), radial hypoplasia, abnormal face, and leukaemic transformation. Presentation is usually with short stature, abnormalities of pigmentation, and organ defects. Seven genes *(FANCA-FANCG)* have been identified, on different chromosomes, with eight different complementation groups. Decreased T-cells, NK-cells, increased IgE, and low IgA, and poor responses to polysaccharides have been reported but immunological abnormalities are not a major feature.

ICF (immunodeficiency, centromeric instability, and abnormal fades)
This is due to abnormalities of chromosomes 1, 9, and 16, marked by a dysmorphic face, mental retardation, and malabsorption with failure to thrive. A gene has been identified, *DNMJ3B*, involved in DNA methylation. The immunodeficiency occurs in all patients and is mainly humoral, with severe hypogammaglobulinaemia, but T-cell numbers are usually reduced. However, *in vitro* functional tests of T-cells are usually normal.

Cockayne syndrome
A disorder of nucleotide excision and repair, associated with extreme sun sensitivity. Not normally associated with immunological abnormalities.

Trichothiodystrophy
Similar to Cockayne syndrome.

Xeroderma pigmentosa
Extreme sun sensitivity leading to bullae, keratoses, and squamous cell carcinoma are the features of this syndrome, which is a DNA repair defect. There is an immunodeficiency with low $CD4^+$ T-cells and poor *in vitro* and *in vivo* T-cell function in some patients; low IgG levels may also occur.

DNA ligase I deficiency
A single patient reported with short stature, sun sensitivity, low IgG, absent IgA, poor specific antibody responses, and death from lymphoma. Genetic defect was identified as DNA ligase I deficiency.

Chronic mucocutaneous candidiasis syndromes
Multiple syndromes, presenting with mucocutaneous candidiasis; rarely with invasive candidiasis. Often in association with endocrinopathy.

Cause
- Cause of many cases of CMC is unknown.
- Cytokine abnormalities have been documented.
- Autosomal dominant and recessive forms as well as sporadic cases are all documented.
- AIRE on chromosome 21 (21q22.3) is abnormal in autoimmune polyglandular syndrome type I (APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia).

Presentation
- Early onset of superficial candidiasis affecting nails and mouth and occasionally the oesophagus; persistent; invasive candidiasis is very rare and should raise questions of other diagnoses.
- There may be a family history.
- May be associated with an endocrinopathy causing hypocalcaemia due to parathyroid insufficiency, hypothyroidism, and adrenal insufficiency (consider APECED).
- Other autoimmune phenomena may occur: vitiligo, alopecia, hepatitis, pernicious anaemia.
- Increased susceptibility to bacterial infections (particularly of the respiratory tract), tuberculosis, herpesviruses, and toxoplasmosis. Severe forms may progress to a more generalized combined immunodeficiency, similar to SCID.
- May be associated with thymoma.

Diagnosis
- There is no unequivocal diagnostic test.
- *In vivo* and *in vitro* T-cell responses to *Candida* antigens are poor or absent but anti-candida IgG antibodies are high.
- Acquired immunodeficiency and other risk factors for Candida (diabetes, steroid inhalers, Sjögren's syndrome, proton-pump inhibitor use) should be excluded.
- Presence of superficial candidiasis with an endocrinopathy, either overt or cryptic (autoantibody positive without symptoms), is highly suspicious: AIRE gene should be checked.

Immunology
- Anti-candida antibodies (IgG) are raised, often with multiple precipitin lines on double diffusion tests.

• IgG2/IgG4 are often reduced and there are poor anti-polysaccharide responses with low immunization responses.
• Poor *in vitro* proliferation to *Candida* antigens, with abnormal cytokine production (high IL-6). T-cell responses to mitogens are usually normal. Cutaneous reactivity to *Candida* is absent, although other DTH responses are often normal. T-tymphocyte subsets are usually normal.
• Autoantibodies may be detectable to endocrine organs (parathyroid, adrenal, ovary, thyroid).
• Mannan-binding lectin deficiency has been suggested as a cofactor.

Treatment

• Treatment is difficult: *Candida* will respond well to antifungals (fluconazole or itraconazole) but inevitably relapses when the antifungal is withdrawn. Resistance to these antifungals may occur. Prolonged therapy is undesirable and increases the chances of hepatotoxicy. Newer antifungals are voriconazole and caspafungin - these should be used sparingly.
• Avoid the use of proton-pump inhibitors as these increase the risk of oesophageal candidiasis.
• IVIg should be considered for patients with recurrent bacterial infections. Continuous antibiotics tend to exacerbate the candidiasis.
• γ-IFN may have some beneficial effect..
• BMT/HSCT should be considered for severe forms but the procedure is difficult in heavily infected patients.
• Maintain regular surveillance for significant endocrine disease, in particular adrenal insufficiency, which may be insidious in its onset. Treat endocrine disease normally.

Outcome

• CMC is not as benign as the books would have you believe!
• Cases may die from overwhelming sepsis, in addition to deaths from unrecognized adrenal insufficiency.

X-linked immune dysregulation with polyendocnnopathy syndrome (IPEX) due to F0XP3 deficiency

An X-linked disorder characterized by severe endocrine autoimmunit diarrhoea, eczema, and serious infections.

Cause

• Gene defect identified as FOXP3 gene, also responsible in the mouse for the scurfy phenotype. Gene is close to WASP on the X chromosome, Xp11.23.
• FOXP3 is a forkhead DNA-binding protein.
• Clinical phenotype has also been seen in patients without mutations it FOXP3, indicating that other genes may also be involved.

Presentation

• Presentation early in life with severe watery diarrhoea, failure to thriv severe eczema, and endocrine autoimmunity (early onset diabetes mellitus, thyroid disease).
• Also associated with autoimmune haemolytic anaemia, ITP, neutropenia, splenomegaly.
• Infections may include meningitis and pneumonia.

Diagnosis

• Clinical features.
• Anaemia, thrombocytopenia.
• Eosinophilia.
• Multiple autoantibodies.
• Genetic testing may be possible.

Immunology

• Markedly elevated IgE, with normal IgG, IgA, and IgM; normal specific antibody responses.
• Autoantibody to small bowel antigen AIE-75.
• T- and B-cells usually normal.

Treatment

• BMT/HSCT, preferably before organ damage occurs.

Idiopathic $CD4^+$ T-cell lymphopenia
Initially recognized in adults presenting with AIDS-like opportunist infections but with no evidence of retroviral infection. Unclear whether this is a discrete immunodeficiency or secondary to an unidentified pathogen.
Cause
• Unknown.
• Some cases associated with p56lck and CD45 abnormalities.
Presentation
• Presents with opportunist infections (PCP, mycobacteria, Candida).
• Bacterial sepsis with unusual organisms.
• Increased incidence of lymphomas and autoimmune disease.
• Increased in IV drug users and haemophiliacs.
• May occur in children.
Diagnosis
• Pattern of opportunist infections in the absence of identified retroviral infection.
• Reduced $CD4^+$ T-cell numbers.
Immunology
• Persistently reduced $CD4^+$ T-cell numbers on more than one occasion.
• $CD8^+$ T-cells may also be reduced.
Treatment
• Treat infections.
• IL-2 has been helpful in some cases.

Autoimmune lymphoproliferative syndromes (ALPS; Canale-Smith syndrome)
A series of disorders characterized by failure of apoptosis, leading to uncontrolled lymphoproliferation and autoimmunity.
Cause
Genetic defects have been identified in a number of genes controlling apoptosis.
• Fas (TNFRSF6/CD95/APO-1), ALPS Ia.
• Fas-ligand (TNFSF6/CD95L), ALPS Ib.
• Caspase 8 and Caspase 10, ALPS II.
• No identified abnormality but typical phenotype, ALPS III.
Presentation
• Occurs in males and females, but with skewing to males; usual onset in childhood.
• Occasionally may be asymptomatic.
• Persistent splenomegaly, lymphadenopathy (typically neck), hepatomegaly.
• Autoimmunity: haemolytic anaemia, ITP (made worse by hyperspelehism), hepatitis, uveitis, Guillain-Barré syndrome.
• Increased incidence of lymphoma (Hodgkins > non-Hodgkins) and other malignancies (carcinoma).
Diagnosis
• Unexplained lymphoproliferation with autoimmunity in a child is highly suspicious.
• Lymphocytosis, often with eosinophilia in peripheral blood.
• Abnormal expression of Fas and Fas-ligand or caspases (genetic testing is required but not widely available).
• Abnormal apoptosis assays.
• Histology of lymph nodes shows infiltration of double-negative T-cells, with no evidence of EBV.
Immunology
• Lymphocytosis with increase in peripheral $\alpha\beta^+$, $CD45RA^+$ double-negative T-cells ($CD4^-CD8^-$) > 1% of total lymphocytes.
• Increase in $\gamma\delta^+$ double-negative T cells.

• Poor T-cell proliferative responses and abnormal apoptosis.
• Increased $CD5^+$ B cells; reduced CD27 expression.
• Increased immunoglobulins.
• Multiple autoantibodies.

Treatment

• Splenectomy may be required but increases risks of infection.
• Autoimmune disease may require treatment with corticosteroids and immunosuppressive drugs (all available drugs have been tried).
• Anti-malarial drugs may be helpful (effects on TNF-α).
• BMT/HSCT has been used successfully.

Outcome

• May improve spontaneously over time, with regression of lymphadenopathy and splenomegaly.
• Increased risk of lymphoma.

Cartilage-hair hypoplasia (CHH) syndrome

An autosomal recessive T-cell immunodeficiency associated with short-limbed dwarfism and distinctive fine sparse hair.

Cause

• Autosomal recessive and linked to chromosome 9p21.
• Gene is RNAase RNP (RMRP), involved in the metabolism of RNA primers.
• Occurs particularly in Finland and old-order Amish in America.

Presentation

• Short-limbed skeletal dysplasia, with fine sparse hair and ligamentous laxity.
• Anaemia (macrocytic), neutropenia.
• Infections, especially varicella, a problem in one-third; may rarely present as SCID, with opportunist infections. Recurrent bacterial infections may occur.
• Megacolon (aganglionic).

Diagnosis

• Typical radiographic appearances of joints; sparse hair, and macrocytic anaemia.

Immunology

• Immunology is variable.
• IgA and/or IgG subclass deficiencies are reported.
• Neutropenia, together with a T-cell lymphopenia. B-cell numbers may be normal.
• Poor proliferative responses to mitogens.

Treatment

• IVIg if infections are a problem.
• BMT/SCT if presenting as SCID; will not cure dwarfism.
• Consider varicella vaccine.

Warts, hypogammaglobulinaemia, infection, myelokathexis (WHIM) syndrome

WHIM syndrome is a rare immunodeciency and is the first to be associated with deficiency of a chemokine receptor (CXCR4). CXCR4 is also the co-receptor for HIV.

Cause

• Deficiency of CXCR4 chemokine receptor for stromal-derived factor 1 (SDF-1).
• SDF-1 essential for normal myeloid maturation and differentiation; absence of SDF-1 increases granulocyte apoptosis and causes myelokathexis (white blood cell retention).
• Autosomal recessive; gene located at 2p21.

Presentation

• Presents early in childhood with recurrent bacterial infections, developing into bronchiectasis.
• Warts (papillomavirus infection) develop later and are extensive and confluent; genital warts will predispose to cervical carcinoma.
• Rare patients have cardiac defects.

• Severe granulocytopenia and lymphopenia; bone marrow shows granulocyte hyperplasia.

Diagnosis

• Clinical and laboratory features.

• Protein and molecular diagnosis not yet routinely available.

Immunology

• Hypogammaglobulinaemia; reduced B-cell numbers, especially memory B-cells.

• Neutrophil function is normal.

Treatment

• IVIg for bacterial infections.

• G-CSF may be helpful, even though levels may be raised in some patients, by increasing neutrophil emigration.

γ-Interferon/IL-12 pathway defects

Multiple defects have been identified in association with inherited susceptibility to mycobacterial infection, especially low virulence mycobacteria (BCG, environmental mycobacteria), and salmonellosis. Other infections are rare. Autosomal dominant, autosomal recessive, and X-linked inheritances have been reported.

Causes

Defined genetic disorders identified so far include:

• γ-interferon receptor-1 mutations, leading to autosomal recessive loss of expression of the receptor or loss or γ-IFN binding; these map to the extracellular domains. Partial defects with reduced γ-IFN responses have been reported. Dominant defects have also been reported, involving mutations in the intracellular domain, leading to surface overexpression of a truncated receptor.

• γ-interferon receptor-2 mutations, causing either complete or partial deficiency of the receptor.

• Autosomal dominant mutations in STAT1 (signal transducer and activator of transcription-1) that impair the intracellular signal transduction of γ-IFN.

• IL-12 p40 subunit mutations.

• IL-12R pi deficiency due to mutations in the IL-12RB1 gene.

• An acquired autoimmune syndrome with autoantibodies against γ-IFN has also been described.

Diagnosis

• Lack of surface γ-IFNR can be identified by flow cytometry; but loss of function mutations require genetic identification.

• IL-12R defects so far reported lack surface expression, amenable to detection by flow cytometry.

Treatment

• γ-IFN in high doses may be helpful in autosomal dominant γ-IFNR1 and IL-12R defects, in combination with conventional antimycobacterial treatment; infection tends to recur.

• BMT has been used in γ-IFNR defects, but outcomes have been poor with GvHD, infections, and generalized granulomatous disease.

• IL-12-related defects appear not to lead to recurrent infections.

IRAK-4 deficiency

Deficiency of interleukin-1 receptor associated kinase-4 (a Toll-like receptor) has been reported in association with recurrent pyogenic infections, including *Pneumococcus, Staphylococcus,* and *Shigella,* and poor inflammatory response (absent inflammatory markers). Specific antibody responses are poorly maintained after immunization. Interestingly, infections seem to become less problematic with increasing age.

Major defects of phagocytic cells

- Chronic granulomatous disease:
  - X-linked;
  - autosomal recessive.
- Leucocyte adhesion defects (LAD):
  - LAD-1: defects of CD18, common (β-chain for LFA-1, Mac-1, and CR4 (CD11a, CD11b, CD11c);
  - LAD-2: defects in synthesis of fucose from GDP-mannose; lack of expression of Lewis X ligand.
- Glucose 6-phosphate dehydrogenase (G6PD) deficiency.
- Myeloperoxidase deficiency.
- Secondary granule deficiency.
- Cyclic neutropenia and severe congenital neutropenia.
- Schwachman-Diamond syndrome.
- Chediak-Higashi and Griscelli syndromes.
- Familial lymphohistiocytosis and haemophagocytic syndromes.
- NK-cell deficiency.
- Rare causes: rac2 deficiency.

Chronic granulomatous disease

This is the most significant neutrophil defect, although not the most common. It is also the easiest to diagnose.

Cause

- There is a defect of intracellular bacterial killing in neutrophils and monocytes, due to a failure of superoxide, oxygen radical, and peroxide production.
- X-linked and autosomal recessive forms are described. There is a deficiency of components of cytochrome b558: 91 kDa protein (X-linked; Xp21.1), 22 kDa protein (16q24), or NADPH oxidase p47 (7q11.23) or p67 (1q25).
- As phagocyte hydrogen peroxidase is normal, organisms that are catalase-negative are killed normally, whereas catalase-positive organisms *(Staphylococcus aureus, Aspergillus, Nocardia,* and *Serratia)* cause major problems.
- Kell blood group antigens are encoded adjacent to the X-CGD locus.

Presentation

- Infections with catalase-positive organisms, especially deep-seated abscesses, osteomyelitis, and chronic granulomata (including orofacial granuloma).
- May mimic inflammatory bowel disease and lead to malabsorption and obstruction of the bowel.
- Liver abscess is a common first presentation, and any child with a liver abscess has CGD until proven otherwise.
- Usually presents initially in childhood but, rarely, first presentation may occur in adults.
- Is a cause of atypical hepatic granulomata in the absence of infection.
- Carriers of XL-CGD are at increased risk of discoid lupus and photosensitivity.

Diagnosis

- Neutrophil oxidative metabolism is abnormal.
- Easiest screening test is the nitroblue tetrazolium reduction (NBT test), but this may miss some cases. The preferred test is a flow cytometric assay using dihydrorhodamine (some patients may have abnormal DHR but normal NBT)
- Bacterial killing will be absent
- X-linked CGD patients should be tested for deficiency of Kell antigens.

Treatment

- Long-term antibiotics (co-trimoxazole and itraconazole) are the mainstay of treatment. Use the liquid formulation of itraconazole (better absorption) and monitor trough levels, adjusting dose accordingly.
- Low-dose prophylactic γ-IFN tends to be used instead.
- Acute infections should be treated promptly with intravenous antibiotics, supplemented with high-dose γ-IFN.
- Drainage of large abscesses may be required.
- Inflammatory bowel disease may be significantly helped by high-dose steroids, particularly where there are obstructive lesions due to granulomata. This increases infection risk.
- BMT/HSCT is the treatment of choice and should be carried out early before infective complications become a threat to life. Results from transplantation in adults are now good.
- Kell-negative XL-CGD patients are a transfusion hazard, and need to be transfused with Kell-negative blood.

Outcome

Outcome has been much improved by use of prophylactic antibiotics and γ-IFN, but it is still a life-shortening illness (death usually by 5th decade).

Leucocyte adhesion molecule deficiency (LAD-1, LAD-2)

Cause

- LAD-1 is due to a deficiency of the β-chain (CD18) for LFA-1 (CD11a), Mac-1 (CD11b), and CR4 (CD11c).
- The gene is located at 21q22.3. There may be variable expression: the severe phenotype has < 1% expression, while in the moderate (incomplete) phenotype there may be as much as 10% of control expression.
- Rare cases may be due to defects in other chains (CD11c) and the Lewis X tigand (LAD-2), caused by an inability to synthesize fucose.

Presentation

- Presentation is variable, depending on the phenotype.
- Delayed umbilical cord separation is a significant feature (> 10 days).
- Skin infections, intestinal and perianal ulcers, and fistulae are typical.
- Periodontitis occurs in older children and may lead to loss of teeth.
- Immunizations may leave scarred nodules.
- Lack of inflammatory change at the sites of infection and an absence of pus formation.

Diagnosis

- Diagnosis is dependent on the demonstration of reduced/absent molecules on lymphocytes and granulocytes by flow cytometry.
- PMA stimulation of granulocytes may be necessary to identify the moderate phenotype in which some upregulation occurs.
- There is usually a peripheral blood neutrophilia, which is often extreme.
- Neutrophil migration is impaired.

Treatment

- Prompt antibiotic therapy is required.
- BMT/SCT is necessary for the severe phenotype: graft rejection is not possible in the absence of LFA-1. This observation led to the use of anti-LFA-1 monoclonal antibodies as anti-rejection therapy. Moderate phenotypes may be more difficult to transplant.
- LAD-2 has been treated with high-dose oral fucose with benefit.

Rac-2 deficiency

An autosomal dominant mutation in the rho-GTPase Rac-2, (22q12.13) has been associated with a presentation similar to that of LAD-1, with delayed umbilical cord separation, absence of pus,

and perirectal ulceration. Chemotaxis was abnormal, and neutrophil granule exocytosis was impaired. BMT was curative.

G6PD deficiency

Cause

- An X-linked (Xq28) condition; the gene is prone to frequent mutations (200 variants have been recorded).
- Absence of functional G6PD (1-5% of normal activity) impairs the NADPH system of oxidative metabolism, with effects similar to those of CGD. However, most variants have enzyme activity of 20-50% normal and have no phagocytic defect.

Presentation and diagnosis

- The presentation is similar to that of CGD, when < 5% enzyme activity is present.
- Haemolytic anaemia is often present, and can be triggered by certain foods (fava beans) and drugs (sulphones such as dapsone; primaquine, salicylates).
- The NBT test is diagnostic and the enzyme activity can be measured.

Myeloperoxidase deficiency

This deficiency is not uncommon (the gene is located at 17q21.3-q23). The prevalence is between 1/2000 and 1/4000 in the USA. Cases are usually asymptomatic, although occasional defects in killing *Candida* have been reported, and infection may occur, particularly if the patient is diabetic.

Schwachman-Diamond syndrome

This is an autosomal recessive syndrome of hereditary pancreatic insufficiency, accompanied by neutropenia, abnormal neutrophil chemotaxis, thrombocytopenia, and anaemia. NK-cell lymphopenia is common. A gene has been identified (*SBDSP*), but the function is as yet unknown. Short stature is usual. Hypogammaglobu-linaemia with recurrent sinopulmonary infections may also occur. Responses to polysaccharide antigens may be absent. Treatment with IVIg may be helpful, and G-CSF has been used, although there is concern over potential risk of myeloid leukaemias. HSCT has also been tried.

Secondary granule deficiency

Neutrophil structure is abnormal with bilobed nuclei. Secondary (lactoferrin) granules are absent and there is a deficiency of other neutrophil enzymes (alkaline phosphatase). This leads to defective neutrophil oxidative metabolism and bacterial killing, resulting in skin and sinopulmonary infections. The diagnosis can be made by careful examination of the blood film, supplemented by cytochemical studies for neutrophil enzymes (NAP score). Some cases have been associated with defects in the *CIEBPe* gene (14q11.2), a CCAAT enhancer protein-binding protein, which acts as a transcription factor in myetopoiesis.

Cyclic neutropenia

This is a rare syndrome characterized by cyclic reductions in neutrophils, but it is perhaps more common than previously thought, with milder variants escaping notice.

Cause

- Autosomal dominant disorder due to mutations in *ELA2* gene, encoding neutrophil elastase. Mutations affect whether elastase localizes to granules or to cell surface membrane. Membrane expression is associated with disease. Similar mutations in *ELA2* also cause severe congential neutropenia.
- Similar disease in dogs (grey collie syndrome) is due to different gene defect and has a 14 day cycle.
- The cycle is usually 21 days ± 3 days, but the molecular cause of cycling is unknown.

Presentation
• Mouth ulceration typically occurs at the neutrophil nadir, more significant invasive infection may occur.
• Mood change just before the nadir is often marked. Symptoms may improve with age.
Diagnosis
• The clue is usually a low neutrophil count during an infective episode.
• Diagnosis is confirmed by serial full blood counts with full differential, 3 times weekly over 4 weeks. Neutrophils may disappear completely. Symptoms usually occur if the count drops below 1×10^9/L.
• There is a compensatory monocytosis at the time of the neutrophil nadir.
Management
• G-CSF prevents a dramatic drop but does not abolish the cycle, which shortens to approximately 14 days.
• There is, however, a risk of myeloid leukaemia with chronic G-CSF therapy and this should be used with circumspection. Data suggest that *ELA2* mutations increase the risk of AML and MDS, and this may be increased further by G-CSF use.
• Prophylactic co-trimoxazole either side of the predicted nadir may be valuable in preventing infection.

Cohen syndrome
This rare autosomal recessive syndrome is due to mutations in the *COH1* gene, whose product is involved in vesicle sorting and intracellular protein transport. Neutropenia is associated with dysmorphic features and mental retardation.

Severe congenital neutropenia (SCN)
Four different genetic variants of SCN have been reported. The majority of cases have defects in the *ELA2* gene, as in cyclic neutropenia, and are at risk of developing myelodysplasia (MDS) and acute myeloid leukaemia (AML). The disorder is autosomal dominant and homozygous defects have not been reported. The neutropenia is, however, static. Rare cases have been associated with autosomal dominant mutations in the transcriptional repressor *Gfi1* gene, causing overexpression of elastase and overflow on to the cell membrane: these cases also have lymphopenia. WASP mutations have been associated with X-linked neutropenia. Mutations have also been reported in the gene encoding the receptor for G-CSF: these cases do not develop MDS or AML. Other possible defects in the ELA2 promoter region may also contribute to SCN cases.

Kostmann's syndrome
This is a congenital severe neutropenia due to a neutrophil maturation defect with arrest at the pro-myelocyte stage. This is genetically distinct from severe congenital neutropenia and has only been reported in one family, with an autosomal recessive pattern of inheritance. It presents with recurrent severe infections. Immunoglobulins are raised; there is a compensatory monocytosis, eosinophilia, and a thrombocytosis. BMT may be used as treatment Co-trimoxazole prophylaxis is necessary.

β-Actin deficiency
Patients with defective β-actin present with recurrent bacterial infections, including abscesses, mental retardation, and joint problems. Neutrophil chemotaxis is abnormal.

Disorders of pigmentation and immune deficiency
There have been major advances in the understanding of the genetics of immune deficiencies that are associated with pigmentary disorders.

Chediak-Higashi syndrome

Cause

- An autosomal recessive disease, due to mutations in the *CHS1* gene (also known as *LYST*- lysosomal trafficking regulator), located at 1q42.1.
- *CHS* is the human equivalent of the beige mutant mouse.
- Exact function of the *LYST* protein is not known.

Presentation

- Benign and aggressive presentations occur.
- Characteristic features are partial oculocutaneous albinism (due to abnormal melanocytes), leading to silver streaks in the hair (prematurely) and pigmentary changes in the iris and also the skin.
- Recurrent infections, especially periodontitis and pyogenic infections.
- CNS abnormalities: peripheral and central, with neuropathy, cranial nerve palsies, parkinsonian features, fits, and mental retardation.
- Hepatosplenomegaly occurs frequently.

Diagnosis

- There are giant primary cytoplasmic granules in leucocytes and platelets.
- Hair shafts show diagnostic clumping of pigment granules.

Immunology

- Granulocyte and monocyte chemotaxis is abnormal with delayed intracellular killing (correctable by ascorbate *in vitro).*
- Defective NK-cell function is common.
- Neutropenia.

Prognosis

Outcome is poor in the accelerated phase with neurological deterioration and a haemophagocytic syndrome like familial lympho-histiocytosis. Expression of CTLA-4 has been associated with development of the accelerated phase. This should be treated with etoposide followed by BMT/HSCT.

Partial albinism (Griscelli syndrome)

This is similar to Chediak-Higashi syndrome but is distinguished from it by the absence of giant granules. Three types have now been identified, and mouse models exist for all types. The genes identified are all involved in the intracellular movement of melanosomes, mechanisms that are also used in granule-containing cells to undertake exocytosis.

- GS type 1 due to mutations in the *MYO5A* gene (15q21.1) encoding myosin Va. This type presents with albinism and severe neurological disease with mental retardation and developmental delay.
- GS type 2 due to mutations in *RAB27A* gene (15q21.1), encoding a GTPase Rab27a involved in the secretory pathway. Patients have hypopigmentation and a severe immune defect, culminating in an accelerated phase of fulminant haemophagocytosis. Treatment is with BMT/SCT.
- GS type 3 due to mutations in the *MLPH* gene, coding for melanophilin, and leading to impaired interaction with Rab27a.

Diagnosis can be confirmed by microscopy of hairs, to examine pigment deposition. Delayed hypersensitivity and NK-cell function are defective; reduced immunoglobulins and neutrophil problems have also been described.

Hermansky-Pudlak syndrome type 2

Type 2 HPS is an autosomal recessive disorder associated with mutations in the *AP3B1* gene. The clinical features are severe congenital (non-cycling) neutropenia, defects of platelet dense bodies, with a bleeding tendency and oculocutaneous albinism. The equivalent gene defect in dogs causes the grey collie syndrome; the mouse mutant is pearl. Other forms of HPS are recognized with distinct gene defects but these do not have neutropenia and infections.

Other pigmentary disorders

Other genetic disorders of pigmentation not associated with immune deficiency include:

- Piebaldism due to mutations in the *KIT* gene.
- Waardenburg syndrome, characterized by piebaldism with sensorineural deafness, caused by multiple gene defects: *PAX3, MITF, SLUG, EDN3, EDNRB, SOX10.*
- Oculocutaneous albinism (four genes: *TYR, P, TYRP, MATP).*
- Hemansky-Pudlak syndrome, types 1 and 3-6 *(HSP1,3-6).*

Familial lymphohistiocytosis, haemophagocytic syndrome in other conditions, and NK-cell deficiency

Familial lymphohistiocytosis (FLH)

FLH is an autosomal recessive disease of childhood, presenting with infiltrations of polyclonal $CD8^+$ T-cells and macrophages into many organs following viral infections. It is associated with the *FLH1* gene (9q21.3) and the *FLH2* gene (10q21), which encodes perforin, the cytotoxic protein of cytotoxic T-cells and NK-cells. *FLH2* defects are particularly common in patients with FLH in Turkey. NK-cell and cytotoxic T-cell function is markedly reduced. Perforin deficiency is the main cause of rapidly fatal FLH. Failure of apoptosis has been suggested as a mechanism for the excessive cellular infiltrates.

Haemophagocytic syndrome in other conditions

Haemophagocytosis has been reported to occur as part of the accelerated phase of type 2 Griscelli syndrome patients, Chediak-Higashi syndrome, and X-linked lymphoproliferative syndrome triggered by EBV exposure. It is also seen in SLE, adult onset Still's, juvenile rheumatoid arthritis, and Castleman's disease. It has been suggested that IL-18 may be responsible in the autoimmune secondary cases.

NK-cell deficiency

Primary NK-cell deficiency has been reported, causing severe or fatal infections with herpesviruses and varicella, usually presenting in the teens or early adult life. Both sexes are affected. The differential diagnosis includes XLPS. Homozygosity for a polymorphism in the FcR-γIIIa receptor (CD16) on NK-cells has been associated with increased susceptibility to herpesviruses; this change also interferes with the ability of certain anti-CD16 monoclonal antibodies to bind.

Immunodeficiency in association with other rare genetic syndromes

Down's syndrome

This is due to trisomy 21. There is a progressive decrease in IgM, dysplastic thymus, low NK activity, and an unusual sensitivity to γ-interferon, whose receptor is located on chromosome 21. There is an increase in TCR γδ cells at the expense of TCR αβ cells. T-cell proliferation to mitogens is reduced, with poor IL-2 production. There is an increase in infections and also in malignancy and autoimmune disease.

Chromosome 18 syndromes

Ring chromosome 18 and deletions of the long and short arms of chromosome 18 are associated with facial hypoplasia, mental retardation, and low or absent IgA.

Immunodeficiency with generalized growth retardation

Schimke immuno-osseus dysplasia

An autosomal recessive syndrome characterized by nephropathy, skeletal dysplasia, and lentigenes. There is a lymphopenia of $CD4^+$ T-cells with poor T-cell mitogen responses. Pancyto-

penia is common. Immunoglobulin levels may be reduced. The gene is *SMARCAL1*, encoding a chromatin remodelling protein.

Immunodeficiency with absent thumbs (TAR syndrome)
There is radial dysplasia, ichthyosis, and anosmia in this syndrome. Recurrent infections and chronic mucocutaneous candidiasis occur. There are poor T-cell mitogen responses and absent IgA, with low IgG and IgM.

Skin disease and immunodeficiency

Dyskeratosis congenita
Dyskeratosis congenita is characterized by cutaneous pigmentation, nail dystrophy, and oral leucoplakia, and is complicated by malignancy and bone marrow failure. Autosomal dominant or recessive and X-linked forms occur. The X-linked form is associated with mutations in the DKCJ gene encoding dyskerin, a nucleolar protein. Variable immune defects are found: hypogammaglobulinaemia and poor delayed-type hypersensitivity. Thymic aplasia may occur. The same gene defect also causes the Höyeraal-Hreidarsson syndrome (microcephaly, growth failure, and pancytopenia, which may mimic SCID).

Netherton syndrome
There is trichorrhexis, ichthyosis, and atopy; some patients have low IgG, abnormal neutrophil function, and poor T-cell mitogens responses. Mutations in the gene *SPINK5*, encoding a serine protease inhibitor, have been identified in some patients.

Acrodermatitis enteropathica This is caused by zinc deficiency, leading to eczema, diarrhoea, malabsorption, and sinopulmonary infections. T-cell numbers and function are reduced and immunoglobulins are low. All the defects are correctable with supplemental zinc.

Anhidrotic ectodermal dysplasia
This family of diseases is associated with defective NFκB signalling. Autosomal recessive or X-linked forms of this syndrome occur. Defects in the *NEMO* gene have been reported in the X-linked form, associated with significant immunodeficiency. Autosomal dominant and recessive forms have mutations in the *EDA 1* gene encoding ectodysplasin (a TNF cyokine family member) or its receptor EDAR, a member of the TNF-receptor superfamily, encoded by *EDA3*. EDAR mutations have been associated with autosomal recessive disease. Hypohydrosis and faulty dentition are the key features. Upper respiratory infections occur. There is variable T- and B-cell function; specific antibody responses may be poor.

Papillon-Lefevre syndrome
Abnormal neutrophil chemotaxis and reduced neutrophil killing have been reported in this syndrome of hyperkeratosis and pyoderma, with periodontitis. The gene defect has been identified as mutations in cathepsin C (also known as dipeptidyl peptidase I), an enzyme involved in protein degradation.

Immunodeficiency with metabolic abnormalities

Transcobalamin II deficiency
Autosomal recessive deficiency of TC II (22q11.2), a vitamin B_{12}-binding protein essential for transport of B_{12}, has been reported in association with diarrhoea, failure to thrive, megaloblastic anaemia, lymphopenia, neutropenia, and thrombocytopenia. Abnormal neutrophil function and hypogammaglobulinaemia are present. All the features are reversible with B_{12} therapy.

Type I hereditary orotic aciduria
This is an autosomal recessive disease of retarded growth, diarrhoea, and megaloblastic anaemia. Fatal meningitis and disseminated varicella may be complications. There is a T-cell lymphopenia and impaired T-cell function.

Biotin-dependent carboxylase deficiency
An autosomal recessive condition characterized by convulsions, ataxia, alopecia, candidiasis, and intermittent lactic acidosis. The severe neonatal form presents with severe acidosis and recurrent sepsis. There is increased urinary P-hydroxypropionic acid, methyl citrate, β-methylcrotonglycine, and 3-hydroxyisovalerate excretion. Decreased T- and B-cells with low IgA are noted. It is treated with biotin.

Mannosidosis
This is a lysosomal storage disease that is associated with abnormal neutrophil (chemotaxis, phagocytosis, and killing) and lymphocyte function (hypogammaglobulinaemia and poor PHA responses).

Hypercatabolism of immunoglobulin

Familial hypercatabolism of IgG
Familial hypercatabolism of IgG has been associated with bone abnormalities, abnormal glucose metabolism, and recurrent infections. IgG is very low with a very short half-life; serum albumin may be normal or low.

Intestinal lymphangiectasia
This is due to a failure of normal lymphatic development in the bowel, with abnormally dilated lymphatics. Similar abnormalities may occur elsewhere, causing localized oedema, effusions, and ascites. There is enteric loss of lymphocytes and malabsorption, particularly of fats. There is a profound lymphopenia, with hypoalbuminaemia and hypogammaglobulinaemia; IgM may be in the normal range and infections may be less severe than the IgG level might predict. Specific responses may be normal but very short-lived. IVIg may be given but weekly therapy may be required to maintain adequate levels. Fat malabsorption may be severe and medium-chain triglyceride supplements may be required.

Splenic disorders

Congenital asplenia and Ivemark syndrome Asplenia may occur alone or in association with partial situs inversus and cardiac defects. Early infections, especially with capsulated organisms, are a typical feature. Sudden unheralded death from overwhelming sepsis may occur and the diagnosis may only be made at post-mortem. Survival into adult life is possible without serious infection. Blood film will show Howell-Jolly bodies; ultrasound will demonstrate absence of the spleen, which can be confirmed by absence of uptake on a labelled white cell scan or on a red cell clearance scan. Initial poor polysaccharide responses may improve after the first 3 years of life. However, there remains a risk of overwhelming sepsis and lifelong prophylactic antibiotics should be administered, together with regular immunizations against pneumococci, *Haemophilus influenzae* type b (Hib), and meningococci.

Tuftsin deficiency
Tuftsin is a tetrapeptide released from the CH_2 domain of IgG by the actions of a membrane leucokinase and a splenic endocarbocypeptidase. It stimulates the bactericidal function of phagocytic cells. Primary tuftsin deficiency has been described in five patients, in association with increased infections. Administration of immunoglobulin replacement may be beneficial. Secondary tuftsin deficiency occurs in splenectomy, asplenia, and hyposplenia (coeliac disease). Tuftsin production may also be impaired by parenteral nutrition, probably by lipid.

Complement deficiencies

Cause

- Genetic deficiencies of all complement components have been described, including the regulatory inhibitors, C1 inhibitor, factor I, and factor H.
- Properdin deficiency is X-(inked; all the others are autosomal recessives, except C1-inhibitor deficiency which is autosomal dominant.
- Factors B, C2, and C4 form part of the extended HLA complex (short arm of chromosome 6, located between HLA-D and HLA-B loci).
- Complement deficiencies are common in South Africa and in the countries of the north African coast and eastern Mediterranean. C2 and C4 deficiencies are relatively common. An increasing number of C4 null alleles increases substantially the risk of developing lupus.

Presentation

- Increased susceptibility to pyogenic infections in C3, factor I, and factor H deficiencies.
- Increased susceptibility to neisserial infections in C5, C6, C7, C8a, C8b, C9, factor D, and properdin deficiencies.
- Recurrent neisserial infection, especially meningitis, should always prompt a screen for complement deficiency; the disease may be milder than in complement replete individuals.
- C9 deficiency is common in Japan and may be asymptomatic, as slow lysis through C5-C8 may take place without C9.
- Increased susceptibility to SLE-like syndrome in C1q, C1r/C1s, C4, C2, C5, C6, C7, C8a, and C8b. C2-deficient lupus is often atypical with marked cutaneous features.
- Factor H, factor I, and MCP deficiencies are associated with the non-diarrhoea-associated haemolytic-uraemic syndrome.

Diagnosis

Diagnosis is by the screening for classic, alternate, and terminal lytic sequence by functional complement assays (classic and alternate pathway CH_{50}-see Part 2), followed by measurement of individual components, as indicated by the screening tests.

Treatment

- No specific treatment is available; use of fresh frozen plasma (FFP) may make the acute problems worse.
- Recurrent neisserial infection may be prevented by prophylactic antibiotics and meningococcal vaccination may help, although there are concerns that disease may be more severe if it occurs in a complement-deficient person after vaccination.
- Prophylactic penicillin (penicillin V 500 mg bd adult dose; erythromycin 250 mg bd if allergic to penicillin) is highly desirable.
- Autoimmune disease is treated in the normal way.

Mannan-binding lectin (MBL) deficiency

MBL (10q11.2) is involved in the initiation of complement activation via the lectin pathway, triggered by binding to mannose residues on bacterial cells' surfaces. Although deficiency of MBL has been associated with infections, many mutations (the gene is highly polymorphic) reducing MBL levels have been identified in asymptomatic families, indicating that MBL deficiency alone is insufficient to cause disease, in the absence of other host-defence problems. It has been estimated that up to 5% of the population lack functional MBL and this has led to suggestions that MBL deficiency may have a beneficial effect in later life. MBL deficiency in cystic fibrosis has been associated with a worse prognosis. The risk of infection in children with low MBL seems to be highest in the first 2 years of life, when specific polysaccharide responses are poor. MBL's value may therefore be early protection, before specific antibody production is optimum, with its role diminishing in later life, replaced by antibody.

Hereditary angioedema and C4-binding protein deficiency

Hereditary angioedema

Cause

- Two main types of HAE are recognized: type I (85%; gene deletion, no protein produced) and type II (15%; point mutation in active site of enzyme). The gene is located on chromosome 11.
- Condition is autosomal dominant as one normal gene is insufficient to protect against symptoms.
- An X-linked type III HAE has been proposed. The abnormality is unknown.
- There is failure of inactivation of the complement and kinin systems, but the angioedema is most likely to be due to the latter (bradykinin) rather than the former.

Presentation

- There is an angioedema (deep tissue swelling) of any part of the body, including airway and gut; the latter presents with recurrent abdominal pain and repeated laparotomies may be undertaken before the diagnosis is made.
- There is no urticaria or itch, although patients often describe an uncomfortable prodromal tingling.
- Attacks begin in later childhood/teenage years and may be precipitated by trauma (beware dental work and operations) and infections.
- Frequency of attacks may be increased by oral contraceptives and by pregnancy. There is an increased risk of immune complex disease.

Diagnosis

- Typically C4 and C2 are undetectable during an acute attack and low/absent in between.
- In type I, there will be a low C1-inhibitor level immunochemically and this will become undetectable in an acute attack.
- In type II, there will be a normal or high level of inhibitor measured immunochemically, but function will be low or absent.
- Angioedema may be acquired secondary to SLE or lymphoma and these may be distinguished from HAE by the reduction in C1q, although this is not always reliable.

Treatment

- Treat major attacks with purified (steam-treated) inhibitor (1000-1500 IU, i.e. 2-3 ampoules) by slow intravenous injection.
- FFP may be an emergency alternative but there are the usual risks of transmitted infection and it is also possible for FFP to exacerbate attacks by providing more substrate.
- Tracheostomy may be required if there is significant laryngeal oedema.
- Prophylaxis may be obtained with modified androgens (danazol, 200-600 mg/day; or stanozolol, 2.5-10 mg/day) or anti-fibrinolytics (tranexamic acid 2-4 g/day). Regular liver function tests and liver ultrasounds are required for monitoring therapy with all these agents.
- Prophylactic purified inhibitor should be used before high-risk surgical procedures, although tranexamic acid may be adequate for minor procedures.
- Dental work should always be carried out in hospital in view of the risk of developing oral oedema and airway obstruction.
- Abdominal attacks respond poorly to purified inhibitor—treatment should be conservative: analgesia (NSAIDs), IV fluids, and avoidance of unnecessary laparotomies (unless there is good evidence for other pathology).

C4-binding protein (C4bp) deficiency

C4bp inhibits the classical pathway of complement activation by blocking the formation of the C3 convertase C4bC2a. Deficiency of C4bp may cause angioedema and, because of its interaction also with protein S, may be a cause of purpura fulminans.

C4-binding protein (C4bp) deficiency

Classification of secondary immunodeficiency

- Viral infections
  - HIV, CMV, EBV, rubella, echoviruses, coxsackieviruses, measles, influenza
- Acute bacterial infections
  - Septicaemia
- Chronic bacterial and parasitic infections
  - Tuberculosis, leishmaniasis
- Malignancy
- Plasma cell tumours and related problems
  - Myeloma, plasmacytoma, Waldenstrom's macroglobulinaemia
  - Amyloidosis
- Lymphoma/leukaemia
  - Hodgkin's disease, non-Hodgkin's lymphoma, chronic lymphocytic leukaemia, other chronic and acute leukaemias
- Extremes of age
  - Prematurity, old age
- Transfusion therapy
  - Whole blood; clotting factors
- Drugs and biologicals
  - As an undesirable side-effect; immunosuppressive drugs
- Physical therapies
  - Plasmapheresis and variants, radiation (see Chapter 16)
- Nutrition
  - Starvation, anorexia; iron deficiency
- Chronic renal disease
  - Uraemia, dialysis, nephrotic syndrome
- Gastrointestinal disease
  - Protein-losing enteropathies; secondary to cardiac disease
- Metabolic disease
  - Diabetes mellitus, glycogen storage disease, mannosidosis
- Toxins
  - Cigarettes, alcohol, other chemicals
- Splenectomy
  - In conjunction with other diseases (lymphoma; coeliac disease; sickle-cell disease); traumatic (congenital asplenia)
- Burns
- Myotonic dystrophy

Human immunodeficiency virus 1 and 2

HIV-1 and HIV-2 are retroviruses, responsible for the acquired immunodeficiency syndrome AIDS.

Immunological features

- Virus enters the cells via a cognate interaction of the gp120 env with $CD4^+$ and a chemokine receptor, either CxCR4 or CCR5.
- It also infects other $CD4^+$ cells (macrophages, dendritic cells) and other cells expressing CD4-like surface proteins (neuronal cells).
- Macrophage tropic viruses use CCR5, and infect T-cells poorly; T-cell tropic viruses use CXCR4 for entry and form syncytia.
- Resistance to viral infection is associated with polymorphism in the chemokine receptors.
- A viral isolate entering T-cells via $CD8^+$ has been described.

• Uptake of virus into phagocytic cells may be augmented by antibody, and complement. HIV activates complement.
• High levels of viral replication may take place in lymph nodes.
• Initial viraemia after infection is controlled by $CD8^+$ cytotoxic T-cells (increased cell numbers). The asymptomatic phase is characterized by strong cytotoxic responses, but viral replication still detectable intermittently, HIV is not a true latent virus.
• The antibody response to major viral proteins appears after a lag phase of up to 3 months and persists through the asymptomatic phase but declines in late-stage disease.
• Marked B-cell dysfunction with polyclonal increase in immunoglobulins and the appearance of multiple autoantibodies.
• In the seroconversion illness there is a dramatic fall in $CD4^+$ T-cells and rise of $CD8^+$ T-cells. The levels of $CD4^+$ T cells may drop to a level at which opportunist infections may occur at this early stage (poor prognostic indicator). Levels then usually recover to within the low normal range. There is then a slow decline of absolute $CD4^+$ T-cell count over time (years) following infection.
• Passage to the symptomatic phase is characterized by a rapid drop in $CD4^+$ T-cells, loss of cytotoxic activity, and switch of virus type from slow-growing, non-syncytial-forming strains to rapidly growing, syncytial-forming strains (quasi-species evolving through lack of replica-tive fidelity and under immunological selection pressure). This is accompanied by the occurrence of opportunist infections.
• Activation of T-cells enhances viral replication and hence $CD4^+$ T-cell destruction. Therefore, opportunist infections enhance the self-destruction of the immune system. Long-term non-progressors and patients responding to highly active antiretroviral therapy (HAART) show good proliferative responses to *gag* proteins. Progression has been associated with a switch from Th1 to Th2 responses.
• HIV preferentially infects $CD45RO^+$ cells but the depletion of T-cells affects principally $CD45RA^+CD62L^+$ naive T-cells.
• T-cell depletion is caused by increased apoptosis, impaired production (HIV effects on thymus), and destruction of both infected and uninfected cells.
• HIV replication is suppressed by natural CCR5 chemokine ligands, RANTES, MIP-1α, and MIP-1β, which are secreted by $CD8^+$ T-cells. SDF-1α is the natural ligand for CXCR4. High levels of chemokine production have been associated with resistance to infection.

Diagnosis and monitoring

• Diagnosis depends on the detection of antiviral antibody and/or viral antigen, not on immunological markers. Screening tests for anti-HIV antibody are followed up by PCR-based tests. Informed consent must be obtained.
• The most accurate monitoring of disease is now available through measurements of viral load by quantitative PCR (viral load). Lymphocyte surface markers (CD4 count) must not be used as a way of HIV testing without consent.
• $CD4^+$ T-cell numbers will be reduced and $CD8^+$ T-cells increased in most acute viral infections and in seriously ill patients in the intensive care unit setting.
• In the acute seroconversion illness, there is a sharp fall in absolute $CD4^+$ T-cell numbers and increase in $CD8^+$ T-cell numbers with T-cell activation markers increased (IL-2 receptor (CD25) and HLA class II (DR)); this normally returns rapidly to normal as evidence of viral replication disappears. Persistent $CD4^+$ T-cell lymphopenia after seroconversion illness is a poor prognostic sign indicating rapid progression to terminal illness.
• Sequential monitoring of the $CD4^+$ T-cell numbers provides guidance on the rate of progression of disease and identifies levels at which therapeutic interventions may be indicated (*Pneumocystis* prophylaxis at 0.2×10^9/l $CD4^+$ T cells).
• Once the $CD4^+$ T-cell count falls below 0.05×10^9/l, further monitoring is of little clinical value (except psychologically to patients, who view cessation of monitoring as doctors giving up).

• Successful treatment with HAART will lead to a rise of $CD4^+$ T cells to within the normal range and suppression of viral load.
• Immune function will recover in patients with a good response to HAART.
• Serum immunoglobulins are usually polyclonally elevated (IgG levels > 50 g/l may be recorded); serial measurements have no clinical utility. Most of the antibody is either 'junk' or relates to an anamnestic response.
• Autoantibodies may be detected (including anti-nuclear and dsDNA antibodies, anti-neutrophil cytoplasmic antibody (ANCA), and anti-cardiolipin). The presence of autoantibodies may cause serious diagnostic confusion, especially if the clinical presentation is atypical.
• Rare patients, usually children, may suffer from panhypo-gammaglobulinaemia or specific antibody deficiency, presenting with recurrent bacterial infections: these patients may derive significant benefit from IVIg. It has been more difficult to demonstrate specific antibody defects in adults, although a subpopulation of adult patients do have recurrent sinopulmonary infections with *Haemophilus* and *Pneumococcus:* IVIg seems to be less helpful.
• Serum β_2-microglobulin levels may be elevated, as a marker of increased lymphocyte turnover; however, the range of elevation in HIV patients is small compared to that seen in lymphoproliferative disease, and its value (except where CD4 T-cell counts are unavailable) is small. Serum and urinary neopterin, a marker of macrophage activation, may also be elevated. There is little to choose between these two surrogate markers.

Immunotherapy

• Mainstay of therapy at present is the use of antiretroviral agents. Mono or dual agent therapy is not recommended. Complex multi-agent regimes are now used. The reader is advised to consult the current HIV literature for information on the current state of therapeutic options. Most regimes require strict timing of administration and high levels of compliance. Multiresistant HIV strains have been reported.
• IVIg may be helpful in certain HIV infants, although not in adults.
• Other immunotherapies (interferons, IL-2) have been uniformly disappointing and are not used routinely, α-IFN enjoyed a vogue in the treatment of Kaposi's sarcoma (due to HHV-8), but the latter responds better to cytotoxic therapy and radiation.
• Use of passive immunotherapy has been disappointing.
• No reliable vaccine is yet available, although trials are continuing on a number of candidate vaccines.

Epstein-Barr virus

EBV is associated with infectious mononucleosis (glandular fever), Hodg-kin's disease, Burkitt's lymphoma, and nasopharyngeal carcinoma. Rare EBV-positive T-cell lymphomas have also been described (T/NK-lethal midline granuloma).

Immunological features

• EBV is a transforming B-lymphotropic virus of the herpes family, binding to the cells via CD21 (C3d) receptor and HLA class II antigens. This receptor is also expressed on follicular dendritic cells and pharyngeal and cervical epithelium. All these tissues are targets. Pharyngeal epithelium is usually affected first, with infection spreading to B cells in the adjacent lymphoid tissue of Waldeyer's ring.
• Following infection there is a B lymphoproliferation, triggered by cross-linking of the CD21, CD19, CD81 complex by virus, which is controlled rapidly by cytotoxic T-cells, which form the 'atypical mononuclear cells' seen on smears. Both HLA-restricted and unrestricted cells are produced, with the latter directly recognizing a virally induced antigen on the cells (LYDMA, lymphocyte-determined membrane antigen). The viral BZLF1 protein is a major target antigen.
• Viral persistence occurs, with reactivation of infection in the immunocompromised (immunosuppressed patients, transplant recipients, HIV-infected patients), giving oral hairy leucoplakia, lymphocytic interstitial pneumonitis, and lymphoma. Nasopharyngeal carcinoma also occurs, although other cofactors are likely to be involved.

• In patients with a genetic predisposition (Duncan's syndrome, NK-cell deficiency), severe or fatal infection can occur on first exposure to EBV.
• Although infectious mononucleosis (glandular fever) is usually a self-limiting illness, some patients fail to clear the virus and develop an appropriate sequence of IgG antibodies: such patients have persistently positive IgM antibodies to EBV and have chronic symptoms (fatigue, malaise, sore throats).
• In the acute phase of EBV infection there is suppression of mitogen and allogeneic responses. NK function is also abnormal even though cell numbers are increased. It has been shown that EBV-transformed cells secrete a homologue of IL-10. Monocyte chemotaxis is also abnormal.
• EBV infection may cause severe B-cell lymphoproliferative disease in immunosuppressed patients, and in patients after BMT. It also causes B-cell lymphomas, especially in solid organ transplant recipients on long-term immunosuppression.

Immunological diagnosis

• Usual screening test (Monospot) for acute EBV infection relies on the production of heterophile antibodies that agglutinate sheep cells. This test may miss cases. IgM antibodies are detected and are then succeeded rapidly by IgG antibodies to early antigen (EA) and viral capsid antigen (VCA); antibodies to EBV nuclear antigen (EBNA) appear weeks to months after infection.
• Initial lymphopenia is followed by lymphocytosis of $CD8^+$ T cells, which give rise to the atypical lymphocytes seen on blood films. However, monitoring of lymphocyte subpopulations is of little value, except in unusual variants of EBV infection.
• There is usually an acute polyclonal rise in immunoglobulins, which may be associated with the production of autoantibodies.

Immunotherapy

• None is required normally. However, in patients with a persistent EBV syndrome, high-dose aciclovir (800 mg 5 times daily for 14 days) may lead to remission of symptoms and disappearance of the IgM anti-EBV antibodies.
• Vaccines are in development, including peptide vaccines.
• Adoptive immunotherapy with EBV-specific CTL is undergoing trials, especially in immunosuppressed or immunodeficient patients.

Cytomegalovirus (CMV)

CMV behaves similarly to EBV, with an early $CD8^+$ T-cell lymphocytosis giving atypical lymphocytes on a blood film. Proliferative responses are reduced during acute infections. CMV infection of monocytes with production of an IL-1 inhibitor may be important. However, congenital CMV infection leads to a prolonged suppression of T-cell function and may also suppress antibody production. In BMT recipients, there may be prolonged suppression of myeloid differentiation. Reactivation of the disease may occur in the context of immunosuppression (HIV, drug therapy). High-titre anti-CMV antibodies in the form of IVIg may help to prevent infection, although once infection is established treatment with antivirals (ganciclovir, foscarnet, cidofovir) is necessary. Vatganciclovir is an oral prodrug.

Rubella

Congenital rubella, but not acute infection, causes poor lymphocyte responses (reduced PHA proliferation) and may lead to long-term depressed humoral immune function. Hypogammaglobulinaemia and a hyper-IgM syndrome, with transiently reduced CD40 ligand expression, have been reported. Rubella appears to directly infect both T- and B-cells.

Measles

Measles virus is capable of infecting both lymphoid and myeloid cells. Acute measles depresses cutaneous type IV reactivity (tuberculin reactivity); this is transient. Similar effects occur with measles vaccines. There is also suppression of NK activity and immunoglobulin production. It is

possible that acute measles may lead to reactivation of TB due to immunosuppression. Acute measles may cause a transient lymphopenia; PHA- and PPD-driven proliferation is reduced. Neutrophil chemotaxis may be impaired transiently (significance). Early inactivated measles vaccines led to a response predominantly against viral haemagglutinin but not to the fusion protein, sometimes leading to an atypical wild-type infection as a result of the inappropriate immune response.

Influenza virus
Acute influenza may give a marked but transient lymphopenia, accompanied by poor T-cell proliferative responses.

Hepatitis B virus
Non-specific immunosuppressive effects are seen, which may be due to liver damage or to virus. Congenital infection with HBV leads to tolerance of the virus and chronic carriage. 5% of normal subjects do not make a humoral response to HBV vaccines after the normal 3-injection course. Some may respond if a different brand is used. Alternatively, a double dose may be given (40 μg), either alone or in combination with γ-IFN 2 000 000 IU. Subjects should be warned of severe flu-like symptoms, Interleukin-2 (1 000 000 IU) has also been used successfully.

Post-viral fatigue syndromes
- Chronic fatigue syndromes, accompanied by muscle/joint pains and neuropsychiatric symptoms, may occur after a range of viral infections, including enteroviruses.
- Immunological abnormalities include: variable lymphopenia, IgG subclass abnormalities; atypical anti-nuclear antibodies.
- May be transient or persistent.

Acute bacterial infections
Acute bacterial sepsis may lead to profound changes in immune function on a temporary basis.
Immunological features
- Neutrophil migration and chemotaxis are increased, while phagocytosis is normal or decreased.
- Lymphopenia affecting $CD4^+$ and $CD8^+$ cells may be marked. Significant and temporary hypogammaglobulinaemia may be present (release of immunosuppressive components from bacteria).
- Massive acute-phase response with elevation of C-reactive protein (CRP) and other acute-phase proteins (complement, fibrinogen, protease inhibitors, $a_2$macroglobulin (IL-6 carrier)) and a reduction in albumin (negative acute-phase protein).
- Complement components will be consumed rapidly, but synthesis will be increased (all are acute-phase proteins), so measurements may be difficult to interpret. Functional assays of complement are usually highly abnormal.
- Toxic shock may follow certain types of bacterial infections (staphylococci, streptococci), due to release of 'superantigenic' toxins, which activate many clones of T cells directly, bypassing the need for MHC on antigen-presenting cells by binding directly to the T-cell receptor. Effects are likely to be due to cytokine excess.

Immunological investigation
- The most important investigations are microbiological, to identify the pathogen, by culture and rapid antigen or PCR tests.
- Monitoring of the acute-phase response (CRP) gives a good indication of response to therapy. Acute measurement of immunoglobulins and complement is usually misleading and may lead to erroneous diagnoses of antibody or complement deficiency. It is best to leave these investigations until convalescence. Functional assays of complement may take 2-3 weeks to normalize after acute sepsis.
- Acute measurement of cytokines in toxic shock is currently impractical and the diagnosis is a clinical one.

Chronic bacterial sepsis

Immunological features

• Hypergammaglobulinaemia is usual, often with small and sometimes multiple monoclonal bands developing, representing the immune response against the pathogen. Chronic antigenaemia will cause immune complex reactions and secondary hypocomplementaemia (subacute bacterial endocarditis (SBE)).

• The acute phase becomes a chronic phase: anaemia of chronic disease, iron deficiency due to sequestration (defence against pathogen); see below. There is the risk of amyloid development.

• T-cell function may be significantly impaired. The best example is mycobacterial infection, with anergy to PPD and third-party antigens. 10% of TB cases do not respond to tuberculin. Mycobacterial products (arabino-D-galactan) interfere with *in vitro* proliferative responses to PHA, PWM, and PPD; the effect is possibly via macrophages and may involve prostaglandins (inhibitable by indomethacin). There is often a lymphopenia. Persistently raised CRP may also be suppressive.

• Miliary TB may cause neutropenia, generalized bone marrow suppression, and leukaemoid reactions.

• Untreated leprosy is a potent suppressor of cell-mediated immunity: T-cell responses to mitogens and antigens are reduced. This defect disappears with appropriate antibiotic therapy and appears to be mediated by a glycolipid. The underlying bias of the immune system towards either Th1 (cellular) orTh2 (antibody) responses determines whether the response to leprosy is tuberculoid (Th1) or lepromatous (Th2). Other immunological features include the development of vasculitis (erythema nodosum) and glomerulonephritis (assumed to immune complex with IgG and complement).

Immunological monitoring

• Acute-phase markers provide the best guide to progress and response to therapy (but beware of elevations from drug reactions). The erythrocyte sedimentation rate (ESR) is less useful because of its long half-life.

• Low complement (C3) and elevated C3d indicates immune-complex reaction (renal involvement likely); monitoring of functional haemolytic complement is not valuable.

• Immunoglobulins are usually high (polyclonal stimulation, monoclonal bands). Electrophoresis also shows elevated α_2-macroglobulin, reduced albumin; beware apparent monoclonal 'bands' from very high CRP (use specific antisera on immunofixation to demonstrate this).

• Hypogammaglobulinaemia is rare: consider underlying immunodeficiency.

Measurement of *in vitro* T-cell function and lymphocyte markers is not valuable unless there is a suspicion that the infections are due to an underlying immunodeficiency.

Immunotherapy

γ -Interferon offers some possibilities for modifying the Th1:Th2 balance in chronic mycobacterial infections and in leishmaniasis.

Fungal infections

Except for cutaneous infections, invasive fungal infections are usually the markers of, rather than the cause of, immunodeficiency, indicating defective neutrophil/macrophage and T-cell immunity.

Parasitic infections

Immunological features

• Malaria has no overt effect on cell-mediated immunity but reduces the humoral immune responses to bacterial antigens (tetanus toxoid, meningococcal polysaccharide, and *Salmonella O* antigen), presumably through effects on the spleen. There appears to be little interaction between

HIV infection and malaria where the two diseases overlap. Tropical splenomegaly due to vivax malaria is associated with a $CD8^+$ T-cell lymphopenia and raised IgM.
• Trypanosomes suppress cellular responses, but there is often a polyclonal increase of non-specific immunoglobulin, especially IgM.
• Visceral leishmaniasis is characterized by a polyclonal nypergammag-lobulinaemia, often massive, but with absent cell-mediated immunity until after treatment. Splenomegaly may be massive and there is often lymphopenia. The cachexia and lymphopenia are mediated by release of tumour necrosis factor-α (TNF-α) by infected macrophages.
• Many parasites, including malaria and trypanosomes, escape immunological surveillance by antigenic variation. This occurs under selection pressure from the immune system. Other avoidance mechanisms include shedding of surface antigen complexed with antibody.
• Autoimmunity may occur as a consequence of the chronic infection: schistosomiasis is associated with anti-nuclear antibodies including anti-calreticulin antibodies. Onchocerciasis is also associated with anti-calreticulin antibodies (which cross-react with an onchocercal antigen).
• Parasitic infections are associated with excess eosinophil and IgE responses.
Immunological monitoring There is little value in monitoring anything other than the acute-phase response.

Malignancy
Immunological features
• Malignancy, especially lymphoid, is very common in primary immunodeficiencies (Wiskott-Aldrich syndrome, CVID, DNA repair defects) and in secondary immunodeficiencies (HIV, EBV). Some viruses are directly oncogenic (hepatitis B, EBV).
• Malignancy is also increased in patients with autoimmune disease, possibly secondary to immunosuppressive drug therapy, and in transplant patients who are immunosuppressed (skin tumours, carcinoma of the anogenital tract).
• Abnormalities of T- and NK-cell function have been described in patients with solid tumours, leading to the suggestion that impaired immune surveillance is related to the development of tumours, although they might equally be a consequence, not a cause. T-cell defects include reduction of IL-2 and TNF-α production, and activation markers such as CD71 (transferrin receptor). Cancer cells may alter immune function through the release of TGF-β, which reduces T-cell proliferative responses and macrophage metabolism, and through inhibitors of complement.
• Some tumours have a propensity to generate autoimmune responses due to inappropriate expression of antigens; these may lead to paraneoplastic phenomena, such as the Lambert-Eaton myasthenic syndrome (small cell lung carcinoma) due to an autoantibody against voltage-gated calcium channels, and neuronal and retinal autoantibodies in breast, ovarian, and colonic tumours.
• Major immunosuppression may result from radio- and chemotherapy. This may be prolonged and lead to secondary infective complications.
Immunological monitoring
• There is little value in immunological monitoring of aspects such as NK-cell numbers or function.
• Patients with significant and persistent infective problems post-treatment may warrant investigation of cellular and humoral immune function, depending on the type of infections. Lymphocyte surface markers, immunoglobulins, IgG subclasses, and specific antibodies to bacteria and viruses may be appropriate.
• Paraneoplastic phenomena may suggest a search for unusual autoantibodies (voltage-gated calcium channels, cerebellar Purkinje cells, retinal antigens).
Immunotherapy
• Immunotherapy of solid tumours has a chequered career. IL-2 therapy has been proposed for certain tumours (renal and melanoma) but there are no good controlled trial data to support this and it is very toxic. *In vitro* stimulation of non-specific killers (LAK cell therapy) by IL-2, using

either peripheral blood cells or tumour-infiltrating cells, has also been claimed in small open trials to be beneficial but is even more toxic.
• Other immunotherapies tried have included the use of non-specific immunostimulants such as BCG, *Corynebacterium parvum*, and *Bordetella pertussis*, often given intralesionally. Occasionally spectacular results have been achieved. α-IFN has been used with success in certain lymphoid disorders (hairy-cell leukaemia, plateau-phase myeloma).
• Monoclonal antibodies are now being introduced targeted against tumour-specific antigens, such as CD20 (rituximab) in lymphoma, anti-CD52 in CLL, and anti-Her-2 (trastuzumab) in breast cancer. Monoclonal antibodies have also been used to target radiopharmaceuticals to tumours where the antibody itself may kill tumour cells poorly (anti-CD20 monoclonals labelled with yttrium).
• A major benefit of immunotherapy has been in the use of colony-stimulating factors to protect the bone marrow, allowing higher doses of conventional cytotoxic agents to be used. However, there are concerns that this approach may increase the risk of secondary myeloid leukaemias.

Myeloma

Immunological features

• Myeloma is a tumour of plasma cells, leading to clonal proliferation. A single isolated lesion in bone is referred to as a plasmacytoma. Waldenstrom's macroglobulinaemia is a clonal proliferation of IgM-producing lymphocytes.
• Staging of disease depends on bone marrow features, paraprotein level, calcium, and haemoglobin.
• There may be a genetic background (HLA-Cw2, -Cw5) and IgA paraproteins may be associated with a translocation t(8;14). Other translocations may occur, Ig gene rearrangements are detectable.
• Myeloma cells often express both lymphocyte and plasma cell antigens simultaneously. Abnormal B cells may be detectable in the peripheral blood, expressing high levels of CD44 and CD54. Cells also express CD56 (NCAM), an adhesion molecule, and soluble levels of NCAM are elevated in myeloma.
• IL-6 plays a key role as either autocrine or paracrine factor stimulating proliferation. CRP may be raised in consequence. Osteoclast-activating factors are also produced, leading to bone destruction (IL-1, IL-6, TNF-β).
• Monoclonal immunoglobulin production parallels the frequency of B cells: 52% IgG; 22% IgA; 25% free light chain only; and 1% IgD. IgE myeloma is exceptionally rare and is found with plasma cell leukaemia. Biclonal myeloma and non-secreting tumours may be found.
• Synthesis of heavy and light chains is often discordant and whole paraprotein may be accompanied by excess free light chains. Free light chains are readily filtered but nephrotoxic. IgD myeloma often presents in renal failure.
• Hyperviscosity is common with high levels of IgM and IgA paraproteins but is rare with IgG and free light-chain paraproteins. IgA frequently polymerizes *in vivo* (dimers and tetramers). Paraproteins may have autoantibody activity and may be cryoglobulins (types I and II). Complexes of paraproteins (especially IgM) with coagulation factors may cause bleeding.
• Although myelomatous change probably arises in spleen or lymph node, these are unusual sites for disease, which is usually found in bone and bone marrow. Excess clonal plasma cells will be found in the bone marrow.
• Normal humoral immune function is impaired and there is suppression of non-paraprotein immunoglobulin (arrest of B-lymphocyte maturation). Specific antibody responses are poor.
• T-cell function is also impaired, leading to viral infections.
• Low levels of monoclonal paraproteins are found in other lymphopro-liferative conditions, chronic infections, connective tissue diseases, and old age.

• Where a paraprotein is present without other features of myeloma (no increase in plasma cells in bone marrow), the term 'monoclonal gammopathy of uncertain significance' is applied. A proportion of these patients develop myeloma with time and all should be monitored at intervals.
• Heavy-chain disease is rare (μ, γ, and α). α-Heavy-chain disease is the most common. All are associated with lymphoma-like disease.
• POEMS syndrome (polyneuropathy, organomegaly, endocrine abnormalities, monoclonal gammopathy, and skin rashes) appears to be a plasma-cell variant of Castleman's disease, a hyperplasia of lymph nodes, which may occur with autoimmune diseases. It is associated with high circulating levels of IL-1, IL-6, VEGF, and TNF.

Immunological diagnosis and monitoring

• Diagnosis of a paraproteinaemia depends on accurate electrophoresis of serum and urine, followed by immunofixation. Immunochemical measurements of immunoglobulin levels (by radial immunodiffusion (RID) or nephelometry) may be misleading due to polymerization or, in the case of IgM, monomeric paraprotein.
• Paraprotein levels are best determined by scanning densitometry, provided that the total protein in serum can be measured accurately. There are difficulties if the M-band overlaps the (β-region.
• Urinary light-chain excretion may be helpful as a prognostic monitor of tumour cell burden, but there are difficulties in the calculation of this and renal function affects the output. Measurement of serum free light chains (binding site assay) appears to be a more sensitive marker of clonality and tumour burden.
• Serum β_2-microglobulin is a valuable marker of tumour-cell activity. CRP may be a surrogate for IL-6 production and soluble NCAM may be of prognostic value.
• Degree of humoral immunodeficiency should be assessed by measurement of exposure and immunization antibodies, followed by test immunization with protein and polysaccharide antigens.

Immunotherapy

The disease is probably not curable at present. Standard chemotherapy includes melphalan and prednisolone; other agents used include vincristine, doxorubicin (or related drugs), and carmustine (BiCNU). α-interferon has a major effect in prolonging the plateau phase. BMT (allogeneic and autologous purged marrow) may also prolong remission but it is doubtful if it is curative. Colony-stimulating factors should be used with caution as they may enhance tumour cell growth.
• Waldenstrom's macroglobulinaemia may be treated with fludarabine or cladribine; rituximab is also helpful.
• Radiotherapy may be required for localized plasmacytomas.
• IVIg may be beneficial in dealing with secondary infective problems but should be used with great caution in patients with renal impairment and those with rheumatoid activity of their paraproteins (both may lead to renal failure). Prophylactic antibiotics may be an alternative.
• Plasmapheresis may be required to deal with hyperviscosity and/or cryoglobulinaemia.

Lymphoma: Hodgkin's disease

Immunological features

• Hodgkin's disease (HD) is a lymphoma seen predominantly in the young. It is characterized by the presence of typical Reed-Sternberg cells (CD15, CD30 positive).
• Three major types (nodular sclerosing, mixed cellularity, and lymphocyte depleted) are recognized. Lymphocyte predominant may well be a separate disease, as it occurs later and often relapses to non-Hodgkin's lymphoma. Staging depends on the number of sites affected and by the presence or absence of constitutional symptoms.
• EBV genome is often found in HD, and Reed-Sternberg (RS) cells are usually positive. RS cells are thought to be the true neoplastic cell, possibly derived from interdigitating reticulum cells.

• T- and B-cell numbers are reduced. Immunoglobulins are often raised, especially IgE. 10% of patients will have hypogammaglobulinemia (severe disease). There may be poor specific antibody responses; primary antibody responses are impaired, whereas secondary responses may be normal.
• T-cell proliferation is reduced (reversible by indomethacin, suggesting a possible macrophage defect). Cutaneous anergy is common. Responses to Pneumovax may be present even if there is a lack of DTH responses.
• In some cases the defects have been shown to precede the development of the disease and also to persist long-term after successful treatment (although the role of the cytotoxic regimes in this is poorly understood). It is difficult then to distinguish from a primary immunodeficiency complicated by lymphoma.
• Bacterial infections are common (*Pneumococcus* and *Haemophilus influenzae*), related to poor humoral function and possibly also to poor neutrophil function.
• Before CT scanning became widespread, splenectomy for staging was common. This is now only undertaken for symptomatic hypersplenism. Splenectomy has a very significant effect on immune function in lymphoma, and patients may become unresponsive to bacterial vaccines.

Immunological diagnosis and monitoring
• Diagnosis is made on histological examination of excised lymph node, supplemented by the use of immunocytochemistry to identify populations of cells. This may be useful in the identification of scanty RS cells. However, RS cells may also be found in association with glandular fever, reactive hyperplasia, and some non-Hodgkin's lymphomas. There is usually a reactive expansion of $CD4^+$ T-cells.
• Molecular techniques should be used to look for evidence of EBV genome.
• HD is associated with an acute-phase response, with elevated ESR, CRP, and caeruloplasmin. This may be a poor prognostic indicator.
• All patients with lymphoma should be monitored for evidence of humoral immune deficiency: serum immunoglobulins, IgG subclasses, and specific antibodies. Test immunization is appropriate. Particular attention should be paid to apparently cured patients, who may still have a persisting immunodeficiency.

Immunotherapy
• Treatment is with radiotherapy and/or chemotherapy. The latter is used for patients with constitutional ('B') symptoms. There are many regimes for combination chemotherapy.
• Most regimes are myelosuppressive and impose a temporary secondary defect through neutropenia.
• Relapse may be treated with autologous bone marrow transplant (harvested in remission) or with a stem-cell transplant.
• Secondary neoplasms may occur (myelodysplasia, acute myeloid leukaemia) and the risk is related to the intensity of treatment.
• IVIg may be required for those with a persisting symptomatic humoral defect after treatment.

Non-Hodgkin's lymphoma
Immunological features
• This category includes all those lymphoid and histiocytic lymphoid malignancies that are not Hodgkin's disease. There are many classifications, but the two used most often in the UK are Kiel and the Working Formulation. A WHO classification was introduced in 1999. Morphology and cellular origin play a major role in classification.
• Tumours are also divided on the basis of their clinical grade (= aggressivity). Low-grade B-cell tumours overlap with chronic lymphocytic leukaemia. Waldenstrom's macroglobulinaemia is often referred to as an immunocytic lymphoma. Both T- and B-cell lymphomas are recognized, as well as tumours derived from histiocytic elements.

• Retrovirus, HTLV-1, has been associated with T-cell lymphomas in areas where it is endemic (Japan and the Caribbean).
• EBV has been associated with certain B-cell lymphomas, particularly associated with immunosuppression and endemic Burkitt's lymphoma, which is found in malarial areas. This tumour, but also others, is associated with chromosomal abnormalities, normally translocations t(14;8).
• Many other translocations have been identified. It is thought that these translocations allow dysregulated activity of cellular oncogenes, such as *bcl-2* and *abl*, by placing them in proximity to active promoters. In Burkitt's lymphoma it is the oncogene *c-myc*.
• Often sites of translocations involve the heavy- and light-chain genes for immunoglobulin and the genes for T-cell receptors.
• Secondary lymphomas are usually non-Hodgkin's lymphoma (NHL). These are found with:
  - primary immunodeficiencies (WAS, CVID, AT, Chediak-Higashi, DNA repair defects);
  - connective tissue diseases (rheumatoid arthritis, Sjogren's syndrome, SLE);
  - phenytoin therapy;
  - post-transplant (ciclosporin therapy).
• In the case of primary immunodeficiency, it is likely that the chronic infections lead to an abortive immune response that predisposes to lymphoma. Perhaps earlier diagnosis and better treatment will prevent this.
• Studies of humoral and cellular function have shown abnormalities that have not always correlated with the type of lymphoma. Abnormalities are more likely in high-grade tumours. Both hypo- and hypergammag-lobulinaemia may occur and may persist after treatment. Monoclonal bands, often IgMK, may be found in association with B-cell tumours. Autoantibody activity may be noted.
• Acquired angioedema is often associated with an underlying B-cell lymphoma with a paraprotein.
  - Paraprotein binds to and inhibits C1-esterase inhibitor.
• As in Hodgkin's disease, splenectomy may have been undertaken in the past, imposing an additional immunological defect. These patients require careful supervision.

Immunological diagnosis and monitoring

• Diagnosis requires histological examination of lymphoid tissue, accompanied by immunohistochemistry, using panels of monoclonal antibodies to identify the predominant cell type.
• Clonality will be established by molecular techniques looking at Ig and Tcr gene rearrangements.
• Humoral immune function should be monitored, as for Hodgkin's disease.
• Serial β_2-microglobulin measurements may be helpful as a marker of lymphocyte turnover.
• Electrophoresis will demonstrate the presence of paraproteins. If autoimmune phenomena are present, then association with the paraprotein can be shown by light-chain restriction on immunofluorescence.
• Sometimes abnormalities of immunoglobulins precede overt disease. In contrast to primary immunodeficiency, IgM disappears first, followed by IgG and IgA.
• Finding an isolated but marked reduction of IgM in an older person should lead to a review for evidence of lymphoma (selective IgM deficiency is vanishingly rare!).

Immunotherapy

• Treatment depends on the type of treatment and its grade. Localized disease may be amenable to radiotherapy, while disseminated disease will require chemotherapy. Aggressive chemotherapy of high-grade tumours may result in some cures.
• Autologous bone marrow transplantation may be helpful in relapse. IVIg may be required if there are infective problems.
• The monoclonal antibody rituximab, with or without attached radioisotope, is valuable for treating CD20-positive lymphomas.

Chronic lymphocytic leukaemia (CLL)

Immunological features

• CLL is a clonal proliferation of small lymphocytes. It is the most common form of lymphoid leukaemia. 95% are B-cell in origin; 5% are T-cell in origin. Other variants include prolymphocytic leukaemia (B-PLL), hairy cell leukaemia (HCL), and splenic lymphoma with circulating villous lymphocytes (SLVL). Cell counts may become very high (> 100 x 10^9/l). It is predominantly a disease of the elderly (95% of patients are over 50).

• Different variants may be distinguished by flow cytometry:

- B-CLL is usually $CD5^+$, $CD23^+$, $FMC7^-$, $CD22^+$, with weak surface Ig. Clonal restriction can usually be demonstrated with anti-light-chain antisera;
- B-PLL has the phenotype $CD5^+$, $CD23^-$, $FMC7^+$, $CD22^+$, slg^+;
- HCL is $CD5^-$, $CD23^-$, $FMC7^+$, $CD22^+$, slg^+;
- SLVL is $CD5^+$, $CD23^+$, $FMC7^+$, $CD22^+$, slg^+;
- Circulating lymphoma cells may be distinguished because they often express CD10 (CALLA);
- T-PLL is rare: cells are usually $CD4^+$, $CD8^-$, but dual-positive or $CD4^-$, $CD8^+$ variants may occur;
- Large granular lymphocytic leukaemia has the phenotype $CD4^-$, $CD8^+$, $CD11b^+$, $CD16/56^+$, $CD57^+$. The cells may be highly active in an NK assay.

• Bone marrow examination shows an excess of lymphocytes.

• Chromosomal abnormalities are common: trisomy 12 and deletions of the long arm of chromosome 13 in B-cell disease, and chromosome 14 abnormalities (inversion or tandem translocation) or trisomy 8q in T-cell disease. Recent studies have demonstrated that some CLL patients have an abnormal *ATM* gene (ataxia telangiectasia mutated).

• Recurrent bacterial infections are a major problem.

• Humoral function is impaired and response to Pneumovax® is a better predictor of infection than total IgG.

• Studies of normal B-cell function is difficult *in vitro* due to the predominance of the aberrant clone.

• Electrophoresis may show small bands (usually IgM). T-cell numbers may be increased ($CD4^+$ T-cells), but function may be poor with low/absent PHA responses.

• Viral infections may be a problem (shingles with dissemination, HSV).

• Autoimmune phenomena are common: ITP and haemolytic anaemia. Splenectomy may be required and this exacerbates the immune deficit.

• Vaccine responses are frequently entirely absent in this situation and patients must have prophylactic antibiotics.

• HCL may be associated with vasculitis.

Immunological diagnosis and monitoring

• Diagnosis of straightforward CLL is usually possible from the white count and examination of the film. Confirmation requires flow cytometry and examination of the bone marrow.

• Studies of humoral immune function are necessary and should include test immunization with Pneumovax®. As these diseases are chronic, monitoring should be carried out at regular intervals to identify deterioration.

Immunotherapy

• Treatment is with cytotoxic agents. Chlorambucil is the usual agent but fludarabine, deoxycorfomycin, and 2-chlorodeoxyadenosine are highly effective. The last named produces a state similar to ADA deficiency. This leads to a profound immunosuppression, with T-cell lymphopenia and a significant risk of opportunist infections. Patients treated with these agents should have regular T-cell counts by flow cytometry and receive prophylactic co-trimoxazole and irradiated blood products (risk of engraftment).

• α-Interferon is very effective in HCL Rituximab® is valuable in combination with fludarabine and cyclophosphamide. The humanized monoclonal antibody Campath-1H® (alemtuzimab) has

been used in resistant cases with success, but causes profound immunosuppression. Younger patients may be candidates for BMT.
• Recurrent infections may require prophylactic antibiotics or IVIg. Monthly treatment is usually adequate (dose 200-400 mg/kg).

Chronic myeloid leukaemia (CML) and myelodysplastic syndromes
Immunological features
• Chromosomal abnormalities occur in almost all cases of CML and myelodysplastic syndromes. The Philadelphia chromosome (t(9;22)) is the most common, but others have been described, including the 5q-syndrome, monosomy 7, trisomy 8,19, or 20, and deletions on other chromosomes (12 and 20). The deletions of chromosome 5 are of interest because they map to the region containing the genes for IL-3, -4, -5, G-CSF, and GM-CSF.
• There is a high incidence of progression to acute myeloid leukaemia.
• Abnormal neutrophil function is well described: neutropenia is common in myelodysplasia. Even if the neutrophil count is normal, function is often not, with abnormalities of adhesion, chemotaxis, phagocytosis, and bacterial killing being well documented. This occurs particularly with monosomy 7 in childhood.
• Infections are common.

Acute leukaemias
Overview
• Acute leukaemia is a common malignancy of childhood, and accounts for about 30-40% of paediatric malignancy. 80% of cases are due to acute lymphoblastic leukaemia (ALL).
• Certain primary immunodeficiencies are risk factors for ALL (Bloom's syndrome, ataxia telangiectasia, Schwachmann's syndrome, xeroderma pigmentosa). Most ALLs are B cell in origin.
• T-ALL is associated strongly with HTLV-1 infection in areas where this virus is endemic. A number of chromosomal translocations have been described, including the Philadelphia translocation (t(9;22)), which is common in adult ALL. T-ALL is often associated with translocations involving the T-cell receptor genes.
• ALL is classified according to the FAB classification, on the basis of cytological appearance, into L1, L2, and L3 types. Immunophenotyping allows the distinction of B-, T-, and null (rare) ALLs.
• Acute myeloid leukaemia has also been classified by the FAB group into M0-M7, depending on the predominant cell type identified by morphology and cytochemistry. Cases of AML may be secondary to Wiskott-Aldrich syndrome, Chediak-Higashi syndrome, and Fanconi anaemia, as well as to the use of cytotoxic drugs such as cyclophosphamide.
• Occasionally, biphenotypic leukaemias may be detected, defined as the presence of at least two markers from each lineage (e.g. lymphoid and myeloid). They account for 5-10% of acute leukaemia and tend to have a poor prognosis. Often they present as AML, but have evidence of clonal rearrangements of immunoglobulin and Tcr genes.
Immunological features
• In ALL the immune system is usually normal, although primary IgM responses to some antigens (viruses), may be poor. Secondary responses are usually normal. Non-neoplastic cells are normally present in normal numbers. The leukaemic clones rarely have functional activity, although there have been reports of cytokine production. Rare cases may be hypogammaglobulinaemic at presentation.
• Chemotherapy is profoundly immunosuppressive, affecting both T- and B-cell function and rendering patients neutropenic. Careful attention to prevention of infection (isolation, irradiation of food, gut decontamination) is essential.
Immunological diagnosis
• Diagnosis of leukaemia is usually made on the basis of suspicious blood films, supplemented by immunophenotyping of both peripheral blood and bone marrow, to identify the characteristics

of the leukaemic clone. This is supplemented by genetic analysis to identify any translocations: probes to the sites of recombination for these translocations give a very sensitive tool for detecting minimal residual disease in bone marrow after treatment.

- Leukaemia phenotyping is best undertaken by haematologists who will have access to supportive evidence from blood films, bone marrow smears and trephines, as well as cytochemical enzymatic studies. They will also undertake the therapy.
- Monitoring of humoral and cellular function post-treatment, and especially after BMT, is essential.

Immunotherapy

- The management of ALL involves intensive chemotherapy and radiotherapy to sanctuary sites such as the nervous system (often with intrathecal methotrexate). For relapse or high-risk patients bone marrow or stem cell transplantation is used, either matched unrelated donors or purged autologous if an HLA-identical donor is not available. There is a high risk of long-term development of non-Hodgkin's lymphoma and acute myeloid leukaemia.
- AML is treated similarly with intensive chemotherapy, with the option for BMT/HSCT when remission is obtained. Acute promyelocytic leukaemia associated with the t(15;17) translocation may be treated with all-trans retinoic acid, which allows differentiation of the blocked cells to mature neutrophils, although BMT is still required.
- Certain cytokines may have a role as adjunctive agents, allowing intensification of chemotherapy.

Bone marrow and stem cell transplantation

- Bone marrow (BMT) or stem cell (HSCT) transplantation is part of the treatment for a variety of inherited diseases (SCID and SCID variants, CGD, HIGM, Wiskott-Aldrich syndrome, osteopetrosis, Gaucher's disease) in addition to its role in the acute leukaemias and CML with blast transformation.
- BMT leads to an immediate severe immunodeficiency, due to the conditioning required to allow 'take'. All blood products must be irradiated to prevent viable lymphocytes engrafting and must be CMV$^-$.
- There follows a period of gradually improving immune function while the immune system reconstitutes. This recapitulates immunological ontogeny.
- T-cell function reconstitutes early, but full B-cell function may take up to 2 years. IgG_2 levels may remain depressed and there are frequently poor responses to polysaccharide antigens.
- Degree of reconstitution is affected by the degree of mismatch and by graft-versus-host disease.
- Return of T-cell function *in vitro* (positive PHA) is usually taken to define the time when release from isolation is safe, but this is usually the last parameter to normalize. Anti-CD3 stimulation responses usually return early.
- Appearance of recent thymic emigrants may be detected by measurement of TRECs (T-cell receptor excision circles) and by the use of CD45RA and CD27 to define naive and effector $CD4^+$ T-cell re-appearance.
- While B-cell function is poor during the acute phase and for the first year thereafter, IVIg prophylaxis is essential.
- Return of B-cell function can be monitored by IgA/IgM levels and development of isohaemagglutinins. Reappearance of class-switch memory B cells is also valuable, especially in patients transplanted for hyper-IgM syndromes.
- Once off IVIg, a full programme of immunizations should be undertaken, starting with killed vaccines (killed polio, DPT, Hib, and Pneumovax®). The response to these can be assessed (pre- and post-levels are required, and remember that antibody from IVIg may persist for up to 6 months or longer).
- Once there is a good response to killed/subunit vaccines, then live vaccines can be administered (MMR).

• Immunological function in chronic GvHD is markedly abnormal, with a persisting risk of invasive infections of all types. The gastrointestinal involvement superimposes a severe nutritional defect, which further reduces immune function.

Extremes of age: prematurity

• At birth, infants are dependent for the first 6 months of life on maternally transferred immunoglobulin (IgG only).

• Immune function gradually develops, although there is usually a physiological trough in IgG levels at around 6 months: if this is prolonged, then transient hypogammaglobulinaemia of infancy results.

• Additional protection to the neonatal gut is provided by breastfeeding, particularly in the first few days when the IgA-rich colostrum is produced.

• Maternal antibody transfer is an active process in the placenta that begins at around 14 weeks' gestation and accelerates markedly after 22 weeks. The process can take place against a concentration gradient and is selective for some IgG subclasses: IgG_2 is transferred relatively less well.

• Antibody-deficient mothers will also be at risk of producing hypogam-maglobulinaemic infants, who will require IVIg for the first 6 months of life. Good replacement therapy during pregnancy will obviate the need for this.

• Premature delivery interrupts the placental transfer and leaves the infant deficient in immunoglobulins and with a relatively less mature humoral and cellular immune system. Breastfeeding is rarely possible, but oral administration of colostrum is desirable to prevent necrotizing enterocolitis. Infections are often problematic, although other factors, such as ITU nursing, venous and arterial lines, and lung immaturity, all contribute. Group B streptococcal infections are particularly troublesome.

Immunological features and diagnosis

• All immunoglobulins will be low, as will be IgG subclasses. However, the 'normal' ranges are calculated from full-term delivery. Provided that there are no major complications, the immune system rapidly catches up after delivery and there are rarely long-term sequelae.

• Responses to immunization schedules may be poor.

Immunotherapy

• The role of IVIg replacement as routine for premature infants has been investigated extensively, with conflicting results, and a consensus as to the value is difficult to obtain.

- Differences in products and batches may relate to highly variable levels of anti-group B streptococcal antibodies.
- Better products, enriched for specific antibodies to the problem pathogens, may be required.
- Oral, IgA-rich products have also been used to reduce the risk of enterocolitis.

• Immunization of the premature causes problems in timing, as there may be very poor responses if routine immunizations are given at intervals calculated from date of delivery uncorrected for gestational age.

Extremes of age: the elderly

Immunological changes in the elderly are multifactorial, relating to the decline in normal immunoregulatory processes, the increased incidence of disease, and the increased use of drugs. There is no relationship to chronological age.

Immunological features

• There is no significant change in lymphocyte numbers or subsets in the healthy elderly, although lymphoid organs show a reduction of germinal centres.

• Mucosal immunity seems to be reasonably intact, although the non-specific inflammatory response is reduced.

• Aged lymphocytes have metabolic abnormalities such as reduced 5'-nucleotidase activity (also associated with CVID), and there are changes in the expression of surface antigens.

• Immunoglobulin levels change with age: IgG and IgA tend to rise while IgM and IgE fall. Primary humoral responses are reduced and secondary responses give lower peak titres and a more rapid fall with time. Antibody affinity may also be poorer. Some studies have shown that vaccine responses in the elderly may be as good as in younger people.
• CVID may present for the first time post-retirement, but this diagnosis should only be entertained when other secondary causes have been eliminated.
• With increasing age there is an increasing incidence of small monoclonal bands on electrophoresis (MGUS), such that 20% of 95-year-olds will have bands. These are present at low levels and are rarely of great significance.
• There is a parallel increase in autoantibodies of all types. These are usually oresent at low titres and are not associated with disease. Normal ranges for antibody titres should be adjusted to take account of these changes.
• Cell-mediated immunity, as tested by mitogen responses and by DTH testing, are also reduced in the elderly. Thymic function is probably better than previously thought and new thymic emigrants can be detected in the elderly.
• Biologically, the healthy very elderly (> 85 years old) represent a special group. There may be combinations of MHC genes that can be associated with survival (in Japan, a high frequency of DR1 and low frequency of DR9), but this might be due to selection out of those individuals with less favourable MHC types associated with autoimmune disease.
• Coexisting disease imposes additional strains on the immune system, for example, chronic lung disease from smoking, cardiac failure with pulmonary oedema and malnutrition. These often tip the balance away from the immune system in favour of invading pathogens.
• Diseases such as influenza have a disproportionate effect on the elderly through the risks of secondary bacterial infection and exacerbating pre-existing underlying diseases. Infections common in early childhood, such as meningitis, are also more common in the elderly

Immunological diagnosis

• The investigation of the elderly for immunodeficiency should be symptom-driven.

Immunotherapy

• Preventative vaccination of at-risk groups is thought to be helpful, for instance with influenza vaccine and Pneumovax®. Risk groups are those with underlying significant disease, particularly chronic lung disease. However, protection may be poor because of the underlying decay of immune function.
• Consideration should also be given to ensuring that other vaccines such as tetanus are kept up to date (this tends to be forgotten in the elderly) as tetanus antibodies may fall below protective levels. Keen gardeners are at most risk.
• Immunoglobulin therapy may be required for those with significant symptomatic hypogammaglobulinaemia. Occasional elderly patients may prefer the simplicity of weekly IMIg at their GP's surgery rather than regular hospital trips.

Transfusion therapy

• In addition to immediate reactions to blood products due to transfused white cells, pre-formed antibodies (to HLA or IgA), etc., there is evidence for an immunosuppressive effect. This is most noticeable in the effect on renal allograft survival.
• IVIg has complex immunoregulatory properties when used in high doses.
• Crude factor VIII concentrates were immunosuppressive, although this may relate as much to chronic hepatitis due to hepatitis C. High-purity FVIII is much less immunosuppressive.
• Other infections transmissible by blood, such as HIV and CMV, can have major immunosuppressive effects.
• The use of unirradiated blood in the immunocompromised (with poor/absent cell-mediated immunity; CMI) may lead to engraftment of viable lymphocytes and the development of graft-versus-host disease.
• Lymphocytes may be viable for up to 2 weeks in bank blood.

Chronic renal disease (nephrotic syndrome and uraemia)

Nephrotic syndrome

- Renal protein loss should always be considered when investigating hypogammaglobulinaemia.
- Investigation of humoral function is essential if there is significant proteinuria.
- In the nephrotic syndrome there is an increased susceptibility to Pneumococcus and other streptococci.
- Typical pattern is loss of immunoglobulins in order of ascending molecular weight, depending on the selectivity of the proteinuria, with preferential loss of IgG, then IgA, and preservation of IgM until gross nephrosis ensues.
- The IgG synthetic rate is normal or increased and the IgM catabolic rate is normal.
- Responses to Pneumovax® are poor but responses to influenza are normal.
- Poor neutrophil chemotaxis and opsonization are also described.
- Loss of complement proteins such as C3 and factor B may also contribute to poor bacterial handling through decreased opsonization.

Uraemia

- Chronic uraemia is immunosuppressive with poor humoral and cellular immune responses. The molecules responsible for this are uncertain.
- Lymphopenia is common, affecting $CD4^+$ and $CD8^+$ T-cells; DTH and mitogen responses are reduced.
- Immunoglobulins and specific antibody responses to pneumococcal and hepatitis B vaccines may be low. Double doses of HBV vaccines (40 μg) are advised.
- Lymph nodes show a loss of secondary follicles.
- Neutrophil function shows defective chemotaxis and phagocytosis, with impaired oxidative metabolism, leading to poor bacterial killing.
- Certain types of dialysis membrane (cellophane, now no longer used) activated the alternate pathway of complement, with release of anaphylotoxins and neutrophil activation leading to severe circulatory and respiratory problems.
- Dialysis patients often have a $CD4^+$ T-cell lymphopenia; increased expression of CD11b/CD18 is seen on neutrophils. Increased T-cell apoptosis may occur.

Renal transplantation

Renal transplant recipients will be on long-term immunosuppressive therapy. They have an increased risk of papillomavirus-induced skin tumours, EBV-induced lymphomas and B-lymphoproliferative disease, and poor humoral and cellular immune function. Monitoring is required, especially if lymphocytotoxic agents such as azathioprine are used.

Protein-losing enteropathy and liver disease (cirrhosis)

Protein-losing enteropathy

Causes Secondary hypogammaglobulinaemia may be due to protein-losing enteropathy, for which there are many causes:

- Menetrier's disease (giant rugal hypertrophy)
- Coeliac disease and other types of sprue
- Inflammatory bowel disease (Crohn's disease)
- Infections-hookworm, TB
- Fistulae post-gastrectomy syndrome
- Neoplasms
- Allergic gut disease (eosinophilic gastropathy)
- Secondary to constrictive pericarditis and gross right heart failure
- Whipple's disease
- Chylous effusions
- Intestinal lymphangiectasia (dilated lymphatics).

Immunological features

• Immunoglobulins are low, with a short half-life, but the synthetic rate I * may be increased.
• Specific antibody responses may be normal, although they may decline rapidly.
• Lymphopenia is associated with dilated or blocked lymphatics (intestinal lymphangiectasia, constrictive pericarditis, right heart failure). This may lead to poor mitogen responses and DTH reactions.

Diagnosis

• Proof that the bowel is the source of immunoglobulin and cellular loss is difficult as most laboratories are singularly unkeen on trying to measure faecal immunoglobulin excretion! Whole bowel perfusion studies may make this more tolerable.
• Radiolabeled albumin excretion in the faeces will quantitate the loss.
• Full studies of humoral and cellular function are required, together with investigation for the underlying cause (radiology, endoscopy, and biopsy).

Liver disease (cirrhosis)

• Increased infections are seen (especially with alcohol which directly impairs macrophage function) with bacteria and mycobacteria.
• Complement components are reduced (decreased synthesis).
• Neutrophil phagocytosis and chemotaxis occur.
• T-cell function is poor.

Metabolic disorders

A number of metabolic diseases are associated with concomitant immunological impairment.

• Glycogenosis type Ib: neutropenia and neutrophil migration defect. Recurrent infections a problem: septicaemia, wound infections, osteomyelitis, and sinusitis.
• Mannosidosis: recurrent severe infections; impaired neutrophil chemo-taxis. Poor T-cell responses to PHA and concanavalin A (ConA).
• Galactosaemia: increased risk of Gram-negative septicaemia due to abnormalities of neutrophil motility and phagocytosis.
• Myotonic dystrophy: hypercatabolism of IgG but not albumin, IgA, or IgM may occur, although infections are not usually a major problem.
• Sickle-cell disease: increased susceptibility to meningitis and septicaemia. There is an acquired splenic dysfunction, due to infarction. Tissue hypoxia also contributes to bacterial infection. Serum immunoglobulins and vaccine responses are usually normal, even to polysaccharide antigens. It is recommended that all patients should be treated as other asplenic or hyposplenic patients and should receive Pneumovax and Hib vaccines, and be considered for prophylactic antibiotics.
• Coeliac disease: this may be accompanied by splenic atrophy and these patients should be investigated and treated as other asplenic patients.
• Prolidase deficiency: rare autosomal disorder with rashes, skin ulceration, dysmorphic features, splenomegaly, and recurrent infections. It also appears to be associated with a risk of developing SLE. It is diagnosed by the presence of iminopeptiduria.

Diabetes mellitus

Immunological mechanisms

• There is an underlying genetic susceptibility to type I diabetes (the HLA type is shared with CVID) and consequent immune dysregula-tion. As a result of the association with the A1, B8, DR3, C4Q0 haplo-type, there is an increased incidence of C4 deficiency in diabetes.
• In established disease, the raised glucose itself interferes with both innate and specific immune functions. Most of the research has been done on chemically induced or genetic diabetes in mice; much less work has been done on human diabetes.

• Humoral function is impaired: IgG levels may be reduced, while IgA may be increased. Specific antibody responses may show poor primary immune responses and the non-enzymatic glycation of immunoglobulin may interfere with function. Both T-dependent and T-independent antigens are affected.
• Lymphoid organs are essentially normal but peripheral blood lymphocytes may show variable abnormalities. CMI may be depressed with poor DTH responses and abnormal mitogen responses and poor cytokine production (IL-2).
• Macrophage and neutrophil function is also reduced.
• Type I diabetes is strongly associated with coeliac disease: hyposplenia may occur.
• Infections with *Candida* and other fungi, TB, and pneumococci are more common in diabetes. Staphylococcal colonization of the skin is higher in diabetics than in normal individuals.
• Abnormalities of immune function are more marked in type I diabetes but correlate poorly with blood glucose levels. It is possible that immune dysfunction relates to glycation of surface antigens on immunologically important cells.

Diagnosis and treatment

• Recurrent infections in diabetics should be investigated in the normal way and not merely accepted, particularly if diabetic control is not bad. This should include humoral and neutrophil function.
• Regular pneumococcal vaccination is recommended.

Iron deficiency and nutritional status

Iron deficiency

• Induced sideropenia due to sequestration is part of the body's response to chronic infection, as iron is essential to bacteria. However, it is also essential to host defences.
• Iron deficiency due to loss or inadequate intake impairs neutrophil bactericidal activity, as it is essential for the activity of myeloperoxidase. There is often a T lymphopenia. Immunoglobulins are usually normal but specific antibody production is reduced. All the changes are reversible with iron.

Nutritional status

• The immunodeficiency of malnutrition is difficult to disentangle because it is usually accompanied by multiple other health problems, which make identification of cause and effect impossible.
• Marasmus is total nutritional deficiency, while kwashiorkor is protein deficiency in a high-calorie diet.
• Both are usually accompanied by vitamin deficiency.
• Increased susceptibility to infection seems to be the rule.
• Non-specific barriers are impaired (especially in vitamin A deficiency).
• There may be variable abnormalities of neutrophil bactericidal activity, but these may well be secondary to infection.
• Immunoglobulins are often normal or high, even if the albumin is low. IgE levels may be elevated, even in the absence of significant parasitic infections, suggesting dysregulation of the Th1:Th2 axis.
• Mitogen responsiveness is reduced in kwashiorkor. Lymph nodes show germinal centre depletion and there is thymic atrophy, although the latter is also a feature of infection.

Asplenia

• Acquired asplenia (surgery, trauma) or hyposplenia (sickle-cell disease, coeliac disease) is associated with an increased susceptibility to overwhelming infection with capsulated organisms, *Capnocytophaga canimorsus* (dog bites), and problems in handling malaria *Babesia* and *Bartonella* (all intra-erythrocytic organisms).
• Risk appears to be lifelong, and is not limited to the first 2-3 years after splenectomy, as was previously thought.

• Degree of compromise also depends on the reason for splenectomy. For example, individuals splenectomized for lymphomas often have a more severe defect than those splenectomized for trauma.
• Ideally, all patients undergoing elective splenectomy should be immunized with Pneumovax and Hib vaccine and probably with the meningococcal A and C or meningococcal conjugate vaccine preopera-tively. If this is not possible, then immunization prior to discharge may be adequate, although responses immediately post-surgery will be reduced.
• All asplenic patients should be on prophylactic antibiotics (penicillin V, 500 mg twice a day, for choice) and should have their antibodies to Pneumovax® and Hib measured. Those with suboptimal levels should be (re)-immunized. Those with poor responses to Pneumovax should receive the conjugated pneumococcal vaccine Prevenar®. Annual influenza immunization is essential to reduce risks of secondary bacterial sepsis
• Asplenic patients may also not maintain adequate levels following vaccination for more than 3-5 years and regular checks should be carried out to ensure that protection is adequate. Note that the licence for Pneumovax does not indicate that it can be given this frequently. However, provided steps are taken to avoid immunizing patients with high antibody levels, the risk of adverse events appears to be low.
• Dog bites are dangerous and patients must seek immediate assistance: antibiotic prophylaxis is essential.
• Specific advice is required for foreign travel to malarial areas.

Drugs and toxins
Drugs
The major drugs is immunosuppression, other drugs have also been reported to cause immunodeficiency. In many cases the evidence is poor, because pre-existent immunodeficiency has not been excluded. However, anticonvulsants, especially phenytoin and carbamazepine, both have strong associations with humoral immune deficiency, which may or may not resolve on withdrawal of the drugs. Newer anticonvulsants may also be associated with humoral immunodeficiency.

Toxins
• Smoking suppresses mucosal immune responses, improving some allergic diseases such allergic alveolitis.
• Illegal drugs have considerable immunosuppressive potential, in part due to contaminants. Cannabis is particularly dangerous to severely immunocompromised patients as it may contain fungal spores.
• Alcohol in excess suppresses macrophage function, and as result increases the risk of tuberculosis.

Burns
• Burns cause a highly significant acquired T- and B-cell immunodeficiency.
• Disruption of the integrity of the normal cutaneous barriers and associated non-specific defences is a serious problem. Complement levels will be reduced by loss.
• In severe burns neutrophil function is impaired and there is a lymphopenia, with depletion of lymphoid organs.
• Mitogen, and allogeneic responses are reduced.
• These changes may be stress-related, due to excessive endogenous steroid production (Curling's ulcer is also associated) or to the release at the burned site of bacterial products.
• Immunoglobulin levels fall, often dramatically, due to reduced synthesis and increased loss through exudation. However, there is no benefit from IVIg replacement therapy.
• The best treatment is good intensive care and rapid grafting to re-establish normal barrier function.

Thoracic duct drainage

- This used to be used as an immunosuppressive technique for the treatment of rheumatoid arthritis.
- It usually occurs now as an unintended consequence of radical oesophageal surgery; chylothorax results, usually draining through the surgical drains.
- Loss of the circulating lymphocyte pool occurs within 48-72 hours and leads to severe prolonged lymphopenia, followed by severe panhypo-gammaglobulinaemia.
- Opportunist infections (PCP, *Candida)* occur.
- Chylothorax should be drained to the abdominal cavity if possible to allow conservation of lymphocytes. IVIg will be required, together with prophylactic co-trimoxazole, antivirals, and antifungals.
- Reconstitution of the immune system is dependent on thymic function and may take up to 2 years. Once normal lymphocyte numbers are achieved, a programme of re-immunization is required, commencing with killed or subunit vaccines.

Physical and environmental factors

Radiotherapy and ionizing radiation

- Specific immune responses are affected (T- and B-cells); neutrophil and macrophage function is usually spared unless there is radiation damage to bone marrow.
- T-cell proliferative responses to mitogens and antigens remain depressed after irradiation for years.
- Lymphopenia, particularly of $CD4^+$ T-cells, is common; humoral immune function is reduced.

Ultraviolet light

Photoimmunosupprression occurs even with low levels of UV light exposure; apoptosis may be increased. NFκB in T-cells is activated by UV light.

Chronic hypoxia (attitude)

- Increased infections are noted at high altitude.
- Hypoxic therapy has been used as an adjunct to sports conditioning: this is associated with measurable alterations in circulating lymphocyte profiles, although the patterns are not consistent.

Trauma and surgery

- Systemic inflammatory response syndrome (SIRS) is recognized in trauma and major surgery. It is caused by cytokine release (TNF-αβ, IL-1β, IL-6) and may lead to multi-organ failure.
- A Th1 to Th2 switch and reduced T-cell proliferation have been noted but this is in part mediated by prostaglandin release from macrophages.
- Antibody production is inhibited by β-endorphin (reversible by naloxone).
- Prolonged administration of anaesthetic agents in ITU may contribute to worse immune function.
- Preventing tissue hypoxia is crucial (supplemental oxygen).

Proteus syndrome

Proteus syndrome is a hamartomatous condition with partial gigantism of hands and feet, with pigmentary lesions, bony abnormalities, multiple benign tumours (lipoma, haemangioma), and developmental delay. Hypo-gammaglobulinaemia has been reported.

Immunotherapy

Introduction

The aim of this chapter is to giver an overview of the very complex but exciting area of immunotherapy. Despite great advances in the basic science, the results of clinical immunotherapy have not been as good as had been hoped. Nonetheless, the advances in basic immunology continue to provide new avenues to explore.

Major mechanisms of immunomodulation

- Immunization:
  - active;
  - passive.
- Replacement therapy:
  - immunoglobulin (IM, SC, IV);
  - C1-esterase inhibitor;
  - α_1-antitrypsin;
  - plasma.
- Immune stimulants:
  - drugs;
  - cytokines.
- Immune suppressants:
  - drugs;
  - monoclonal antibodies;
  - cytokines and antagonists;
  - IVIg;
  - antibody removal (plasmapheresis).
- Desensitization:
  - domestic allergens;
  - pollen allergens;
  - bees and wasps;
  - other allergens.
- Anti-inflammatory agents:
  - NSAIDs;
  - anti-cytokines (anti-TNF); IL-1 ra;
  - anti-complement mAbs;
  - anti-endotoxin mAb;
  - anti-T-cell/anti-B-cell mAbs.
- Adoptive immunotherapy:
  - bone marrow transplantation;
  - stem-cell transplantation;
  - thymic transplants.

Passive immunization

Protection is provided by transfer of specific, high-titre antibody from donor to recipient. The effect is transient (maximum protection 6 months). Protection is immediate (unlike active immunization).

Problems

- Risk of transmission of viruses.
- Serum sickness (including demyelination); acute reactions.
- Development of antibodies against infused antibodies reduces effectiveness.
- Identification of suitable donors (Lassa fever, rabies).

Types
- Pooled specific human immunoglobulin.
- Animal sera (antitoxins, antivenins).
- Monoclonal antibodies (anti-endotoxin).

Uses
- Hepatitis A prophylaxis (but new vaccine provides active immunization and longer prophylaxis).
- Hepatitis B (for needlestick injuries), tetanus, rabies, Lassa fever.
- Botulism, VZV (especially during pregnancy and in the immunocompromised), diphtheria, snake bites (post-exposure).
- Rhesus incompatibility (post-delivery anti-D).

Active immunization

The purposes of active immunization are to:
- stimulate the production of protective antibody (opsonization, complement fixation, enhanced phagocytosis, blocking uptake (virus neutralization));
- stimulate antigen-specific T-cells: whether these are Th1 or Th2 cells depends on the type of pathogen and the optimal protective response;
- produce long-lasting immunological memory (T- and B-cells):
  - mediated by the retention of antigen on follicular dendritic cells in lymph nodes, leading to a long-term depot;
  - hence antibody levels often persist years after the primary course of immunizations have been completed, rather than decaying to zero;
- produce 'herd immunity': the generation of a sufficiently large pool of immune individuals reduces the opportunity for wild-type disease to spread, increasing the effectiveness of the immunization programme;
  - over 75% immunization rates are required to achieve this.

Active immunization can use:
- purified component, e.g. toxin (inactivated = toxoid);
- subcomponent;
- live attenuated pathogen (e.g. BCG, polio).

Active immunizationcan be combined with passive immunization (although wis may reduce the development of long-term immunological memory).
- This approach is used for tetanus and rabies as a strategy for treatment, post-exposure.

Toxoid/subcomponent vaccines
- Immune response frequently requires augmentation with adjuvants.
- May be side-effects from adjuvants.
- No risk that disease will be produced.
- Inactivation may damage key epitopes and reduce protection.
- Safe to use in the immunocompromised but responses (and protection) unpredictable.

Attenuated vaccines
- Usually more immunogenic and do not require adjuvants.
- Risk of reversion to wild type (e.g. polio).
- Side-effects from culture contaminants (demyelination from duck embryo rabies).
- May produce mild form of disease (measles, mumps).
- Contraindicated in immunosuppressed (paralytic polio in antibody deficiency).
- Unexpected viral contaminants (SV40 and polio; hepatitis B and yellow fever).

General problems of active immunization

Active immunization has a number of problems, including the following:

• Allergy to any component (e.g. residual egg protein, often in viral vaccines from the growth media).
• Reduced or absent responses in immunocompromised (including splenectomy).
• Delay in achieving protection (primary and secondary immune responses require multiple injection schedules).
• Preferred route of administration (site of IM; SC, ID):
  - route of administration may determine the type of immune response;
  - route to be relevant for the route of infection of the pathogen (e.g. need for mucosal immunity to enteric organisms).
• Storage: most live vaccines require refrigerated storage to maintain potency; this may be a problem, especially in tropical countries.
• Age at which a vaccine is administered may alter the response, e.g. responses to polysaccharide antigens are poor in:
  - children under the age of 24 months;
  - the elderly.
• Maternal antibody, passive immunization, concomitant medical illness, and associated drug therapy may reduce the response.
• Ideally, responses should be checked serologically in patients where there may be a poor response.
• Serological unresponsiveness does not preclude good T-cell immunity (hepatitis B).
• Anti-self immune response to immunization (e.g. autoimmunity after meningococcus group B polysaccharide administration).
• Multiple immunogenic strains of target organism (e.g. *Meningococcus, Pneumococcus).*

Additional stimulation of the immune system

Poorly immunogenic antigens may be used if combined with agents that non-specifically increase immune responses ('adjuvants').
• Adjuvants:
  - 'depot' of antigen (alum-precipitated; oil);
  - non-specific stimulation (Freund's; MDP); Freund's adjuvant is too potent to be used in humans. It has however been safely used in microlitre quantities;
  - polymerization (liposomes; ISCOMS—Quil A);
  - expression in vectors (e.g. use of vaccinia; chimeric viruses; BCG, *Salmonella).*
• Use of immunogenic carrier proteins conjugated to primary antigen:
  - tetanus toxoid;
  - diphtheria toxoid.

Modern approaches to vaccine development

Development of more potent but safer vaccines is always the goal.
• Molecular techniques have been used to modify pathogens through site-specific mutation, reducing pathogenicity, or by inserting the gene into a carrier (vaccinia, *Salmonella).*
• Development of the host response to the carrier organisms means that it can only be used once.
• Molecular techniques allow the safe synthesis of bulk quantities of antigen (e.g. hepatitis B surface antigen).
• Recombinant technique needs to be selected carefully to ensure appropriate post-translational glycosylation of the antigen.
• Recombinant organisms may also be used to target antigens to particular cells, for example:
  - *Salmonella* are rapidly taken up by macrophages;
  - inserted gene products will also be directed straight to antigen-presenting cells.
• Conjugation of poorly immunogenic antigens (such as polysaccharides) to immunogenic proteins (tetanus, diphtheria toxoids).
• Humoral immune response to the polysaccharide switches from IgG_2 to IgG_1 and IgG_3.
• Specific peptides are being used experimentally to try to stimulate specific T-cell responses.

• Epitope mapping of antigens to determine the T- and B-cell epitopes is required.
• Direct injection into muscle of nucleic acid (RNA, DNA) coding for specific genes, coupled to gold microsphere carriers or in plasmids, generates an immune response.
• Nucleic acid is not degraded but is taken up into myocytes and specific protein production can be detected for several months thereafter, leading to an excellent depot preparation.
• Concern over risk of bystander attack by the immune system on myocytes containing the injected DNA.

Generation of effective response

To generate an effective immune response, both host and pathogen factors need to be taken into account Factors encouraging the development of an effective vaccine involve both infectious agent factors and host factors.

• Infectious agent factors:
  - one or a small number of serotypes; little or no antigenic drift;
  - pathogen is only moderately or poorly infectious;
  - antigens for B- and T-cell epitopes are readily available;
  - the immune response can be induced readily at the site of natural infection;
  - wild-type infection is known to produce protective immunity;
  - availability of animal models to test vaccine strategies.
• Host factors:
  - humoral and cellular immunity is readily induced;
  - HLA background of population is favourable to a high response;
  - proposed antigens induce appropriate Th1 or Th2 response.

Factors in the infectious agent that mitigate against an appropriate immunization response and therefore prevent the development of good vaccines include the following.

• Marked antigenic variation/drift; many serotypes causing disease: this limits the ability to generate an effective vaccine (e.g. pneumococcal disease).
• Potential for change in host range of the pathogen (e.g. change in cell tropism of viruses such as HIV).
• Infection may be transmitted by infected cells that are not recognized by the immune system even after immunization.
• Integration of viral DNA into the host genome (latency).
• Natural infection does not induce protective immunity.
• Pathogen uses 'escape' mechanisms:
  - resistant external coats, e.g. mycobacteria;
  - poorly immunogenic capsular polysaccharides;
  - antigenic variation in response to host immune recognition, e.g. influenza virus, malaria;
  - camouflage with host proteins, e.g. CMV and β_2-microglobulin;
  - production of proteins similar to host proteins, e.g. enterobacteria; may give rise to autoimmunity;
  - extracellular enzyme production to interfere with host defence (staphylococcal protein A);
  - production of molecules that disrupt immune responses, e.g. superantigens.
• Pathogen-induced immunosuppression (HIV).
• Failure to form appropriate response (e.g. complement-fixing antibodies).
• No suitable animal model.

Host factors that mitigate against an appropriate immunization response and therefore prevent the development of good vaccines include the following.

• Immune response is inappropriate, e.g. antibody when cellular response is required (e.g. leishmaniasis).

• Immune response enhances infection, e.g. antibody formation may enhance infection through increased uptake into macrophages (yellow fever, HIV).
• Cells of immune system are target of infection.
• 'Wrong' HLA background predisposes to a low response or autoimmunity.

The ultimate goal of any immunization programme is the eradication of the disease. This requires that:

- the infection is limited only to humans;
- there is no animal or environmental reservoir;
- absence of any subclinical or carrier state in humans;
- a high level of herd immunity can be established to prevent person-to-person spread:
  - this requires considerable infrastructural support to ensure that all at-risk populations are targeted for immunization;
  - this has only been achieved for smallpox;
  - however, herd immunity for smallpox has waned as the immunization programme have stopped; bioterrorism with smallpox is a significant threat.

Replacement therapy

This is used for treatment of primary and some secondary immune deficiencies.

Replacement therapies for some primary and secondary immune deficiencies

Type of deficiency	Replacement therapy
Antibody deficiency	Immunoglobulin (IV, SC)
α_1-antitrypsin deficiency	α_1-antitrypsin
Complement deficiency	C1-esterase inhibitor Fresh-frozen plasma (virally inactivated)
Cellular immune deficiency	Bone marrow transplantation (stem cell transplant) Cord blood transplant Thymic transplant
Combined immune deficiency (SCID)	Bone marrow transplantation (stem cell transplant) Cord blood transplant Thymic transplant Gene therapy (ADA) Red cells (ADA) PEGyiated-ADA Immunoglobulin (IV, SC) Cytokines (IL-2, γ-IFN)
Phagocytic defects	Bone marrow transplantation (stem cell transplant) Cord blood transplant Granulocyte transfusions Cytokines (G-CSF, GM-CSF, IL-3)

Intravenous immunoglobulin (IVIg) for replacement therapy 1

Manufacture and specification

• IVIg is a blood product, prepared by cold ethanol precipitation of pooled plasma.
• Donors are screened for transmissible infections (HIV, HCV, HBV).
• Plasma is not used currently (risk of prion disease); no test currently available to identify prion disease in donors.
• Donated plasma is usually quarantined until donor next donates (avoids undetected infection at time of first donation).

• Donor pool usually >1000 donors, to ensure broad spectrum of antibody specificities.
Subsequent purification steps vary between different manufacturers but all are based on the original Cohn fractionation process.
• The IgA content is variable.
Significant levels of IgA may be important when treating IgA-deficient patients, who may recognize the infused IgA as foreign and respond to it, leading to anaphylactoid responses on subsequent exposure.
Uncertain how much of a problem this, and there is no standardized method for detecting clinically significant anti-IgA antibodies.
All current products have low/undetectable IgA, except for lyophilized Sandoglobulin®.
• Product must have low levels of pre-kallikrein activator, Ig fragments, and aggregates as these three can cause adverse events on infusion.
• Variations of IgG subclasses do not seem to make significant differences to the effectiveness as replacement therapy.
• Comparing the presence of functional antibodies in individual products I is difficult as there are no internationally standardized assays, but IVIg must have intact opsonic and complement-fixing function.
• All licensed products must have at least two validated antiviral steps:
  - cold ethanol precipitation;
  - pH4/pepsin;
  - solvent/detergent treatment;
  - pasteurization;
  - nanofiltration.
• No product should be viewed as virally 'safe'.
Full counselling about risks and benefits must be given to patient, with written information, and this must be recorded in the medical notes.
Written consent must be obtained prior to therapy and retained in the medical notes.
• Liquid preparations are now preferred for ease of administration.

Uses

• IVIg (or SCIg) is mandatory for:
  - replacement in the major antibody deficiencies (XLA, CVID);
  - in combined immunodeficiencies (pre- and immediately post-BMT).
• IVIg is also recommended in patients with secondary hypogammaglobulinaemia, such as CLL and myeloma, post-chemotherapy.
• The role of IVIg in IgG subclass and specific antibody deficiency is less secure, and regular prophylactic antibiotics might be tried first, with IVIg reserved for continuing infection despite therapy (assess risk-benefit).
• Where there is doubt, a 1-year trial is reasonable, with monitoring of clinical effectiveness through the use of symptom diaries.
• To ensure a realistic trial, adequate dosing and frequency of infusions must be undertaken to ensure that benefit will be obvious.

Dose regime

• Treatment should provide 0.2-0.6 g/kg/month given every 2-3 weeks for primary antibody deficiency, or as an adjunct in combined immunodeficiency.
• Older patients with CLL may manage on monthly infusions.
• Most patients on monthly schedules become non-specifically unwell or develop breakthrough infections after 2-3 weeks.
Under these circumstances the interval should be shortened.
• Rare hypercatabolic patients, or those with urinary or gastrointestinal loss, may require weekly infusions of large doses to maintain levels.

• Adjust dose according to the trough IgG level, aiming to achieve a trough IgG level within the normal range (6-16 g/l).
• Aim for higher trough in patients with established bronchiectasis or chronic sinusitis (target trough 9 g/l), as this will reduce lung damage.
• Breakthrough infections are an indication to re-assess interval and target trough level.

IVIg for replacement therapy 2: adverse reactions and risks of infection

Adverse reactions

• Most adverse reactions are determined by the speed of infusion and the presence of underlying infection.
• Untreated patients receiving their first infusions are most at risk.
• Reactions are typical immune complex reactions:
  - headache;
  - myalgia;
  - arthralgia;
  - fever;
  - bronchospasm;
  - hypotension;
  - collapse;
  - chest pain.
• Pre-treatment of the patient with antibiotics for 1 week prior to the first infusion reduces antigenic load and reaction risk.
• Hydrocortisone (100-200 mg IV) and an oral antihistamine (cetirizine, fexofenadine) given before the infusion are also of benefit.
• The first infusion should be given at no more than two-thirds of the manufacturer's recommended rate.
• Start slowly and increase rate in steps every 15 minutes.
• Similar precautions may be required before the second infusion.
• Reactions may occur in established recipients if:
  - there is intercurrent infection;
  - there is a batch or product change.
• Other adverse events include
- urticaria;
- eczematous reactions;
- delayed headache and fatigue (responds to antihistamines!);
- medical problems from transferred antibodies (e.g. ANCA-uveitis).
• Products should only be changed for clinical not financial reasons.
  - Severe anaphylactoid reactions have been reported after switching products.
  - IVIg products are not interchangeable.

Risks of infection

• Infection remains a major concern.
- Hepatitis B is no longer an issue.
- There have been a significant number of outbreaks of hepatitis C.
- Other hepatitis viruses (HGV) may cause problems.
- No risk of HIV transmission, as the process rapidly destroys the virus.
- Safety in respect of prion disease is not known but risk will be cumulative with continuing exposure.
- Antiviral steps reduce but do not eliminate risk.
• Batch exposure needs to be kept to a minimum.

IVIg for replacement therapy 3: monitoring and home therapy

Monitoring

- Check HCV PCR and baseline LFTs pre-treatment.
- Store pre-treatment serum long-term.
- Monitor trough IgG levels on all patients regularly (alternate infusions).
- Monitor liver function (alternate infusions, minimum every 3-4 months)—transmissible hepatitis.
- Repeat HCV PCR if any unexplained change in LFTs.
- Monitor CRP-evidence of infection control.

Record batch numbers of all IVIg administered.

- Use symptom diaries in appropriate patients to monitor infective symptoms, antibiotic use, and time off work/school.
- In the event of a significant adverse reaction:
  - immediate blood sampling for evidence of elevated mast-cell tryptase, complement activation (C3, C4, C3d);
  - send sample for anti-IgA antibodies (if IgA deficient);
  - screen for infection (CRP, cultures);
  - rare antibody-deficient patients seem to react persistently to IVIgs: changing to a different product may sometimes assist. Occasionally continued prophylactic antihistamines, paracetamol, or even steroids may be required before each infusion to ensure compliance with therapy.

Home therapy

- For patients with primary immunodeficiencies, home treatment is a well-established alternative to hospital therapy.
- Criteria for entry to home therapy programmes are laid down in approved guidelines.
- Patients should not be sent home on IVIg without formal training and certification by an approved centre.
- The centres will also arrange for long-term support, with trained home therapy nurses and support from community pharmacy suppliers.
- Home therapy is not available in all countries for legal and/or financial reasons.

Criteria for home therapy

Criteria for home therapy	Comments
4-6 months hospital therapy	Must be reaction-free
Must have good venous access (IVIg)	Consider SCIg if venous access poor
Patient must have a trainable long-term partner	Infusions must *never* be given alone
Patient must have access to telephone at site where infusions wilt be given	To call for assistance if problems
GP must be supportive	Rare for GP to be called
Patient must accept regular follow-up at Training Centre	Annual supervised infusions are advisable
Patient must agree to keep infusion logs with batch records	Essential for dealing with batch recalls, look-back exercises
Patient and partner must complete training programme, with written assessment	Training manual must be provided

Intramuscular and subcutaneous immunoglobulins for replacement therapy

Intramuscular immunoglobulin (IMIg)

There is no role for IMIg in replacement therapy. Administered doses are too low to be effective in preventing infection. However, occasional older patients prefer the convenience of a weekly

injection at their GP's surgery to hospital-based infusions. IMIg has been associated with an adverse reaction rate of 20%.

Subcutaneous immunoglobulin (SCIg)

For those with poor venous access, high-dose SCIg replacement is at least equivalent to IVIg in terms of maintaining adequate trough IgG levels and preventing infection.

- 16% solution of immunoglobulin is used.
- Specific licensed SCIg preparations are now available from several manufacturers.
- It is administered in a weekly dose of 100 mg/kg via a syringe driver, in multiple sites.
- Two infusion pumps (Graseby battery-powered drivers for 10 ml syringes) may be used simultaneously.
- Rate is usually set to 99 ml/h (maximum).
- 10 ml per site is the usual maximum tolerated dose per site.
- Tolerability is reasonable, with local irritation being the only significant side-effect.
- Regular trips to hospital will be required for trough IgG and LFT monitoring.
- Trough levels tend to run approximately 1 g/l higher than the same dose given as IVIg on a 2-3 weekly cycle.
- Syringe drivers must be checked at least annually by a qualified medical electronics technician.

C1-esterase inhibitor for replacement therapy

Deficiency of C1-esterase inhibitor causes episodic angioedema, which may be fatal if involving the upper airway.

- Purified C1 inhibitor is available on a named-patient basis as Berinet-P.
- These are blood-derived products and carry the same risks as IVIg in respect of tranmissible infections.
- Products undergo viral inactivation steps (steam-treatment).
- A recombinant product is undergoing clinical trials.
- Patients should have samples checked for LFTs and HCV status prior to each course of treatment.
- Appropriate consent should be obtained if possible.
- Batch numbers must be recorded.
- Indications for treatment include:
    - attacks above the shoulders;
    - surgical prophylaxis (including major dental work).
- It is less effective against bowel oedema, but if pain is severe one dose should be given.
- Attacks involving the bowel should be treated with fluids, analgesics, and NSAIDs.
- Surgery should be avoided unless there is good evidence for pathology unrelated to HAE.
- Dose is 500-1000 U (1-2 ampoules) administered as a slow bolus IV.
- Levels of C1-esterase inhibitor level in the serum should rise to above 50% for several days.
- Same dose is used for prophylaxis.
- When used as treatment, it will prevent attacks progressing, but does *not* lead to a dramatic resolution of symptoms; accordingly laryngeal oedema may require other measures, such as tracheostomy, as urgent procedures.
- Plasma can be used if the purified concentrate is unavailable, but is less effective and may even increase the oedema by providing fresh substrate for the complement and kinin cascades.
- Pooled virally inactivated FFP is now available, and may carry a reduced risk of infection, although this is debated.
- Experimental treatments with kinin inhibitors are undergoing trials.

α_1-Antitrypsin (α_1-AT) for replacement therapy

α_1-AT deficiency leads to progressive emphysema and to liver disease. Purified a1-AT is now available on a named-patient basis. Trials have not been that encouraging, but this is because late-stage patients with established disease have been involved. No good prophylactic trials have been undertaken in asymptomatic patients, so it is not yet known how effective the drug is in preventing complications. Supplementation may also be useful during acute infections when high local levels of trypsin are released by activated neutrophils. Infusions need to be given at least weekly to maintain enzyme activity.

Immune stimulation

Specific immunostimulation

Specific immune stimulation = immunization.

Non-specific immunostimulation

- A number of agents are now available that act as non-specific immune stimulators and have significant clinical benefit.
- Specialist literature should be consulted for current dose regimes.

BCG

- Direct stimulant of immune system; activates macrophages.
- Only found to be of value in bladder cancer.
- As it is a live agent, it should not be administered to those with concomitant immunosuppression (lymphoid malignancy, drug-induced).
- Derivative of cell wall muramyl dipeptide (MDP) licensed in Japan to enhance bone marrow recovery after chemotherapy.
- Other bacterial products are under investigation (extracts of *Corynebacterium)* as immunostimulants.

Cimetidine

- Known to have immunoregulatory properties, not related to anti-H2 activity, as actions do not appear to be shared with other anti-H2 drugs such as ranitidine.
- Thought to reduce T suppressor activity.
- Has been used with success in the hyper-IgE syndrome to reduce IgE and in CVID to increase IgG production.

Glatiramer (Copaxone)

- An immunostimulatory drug, comprising synthetic peptides, administered by injection.
- Used in the treatment of relapsing and remitting multiple sclerosis.
- Side effects include chest pain, allergic reactions, and lymphadenopathy.

G-CSF/GM-CSF

- Act on bone marrow precursors to increase production of mature neutrophils.
- Used as adjuncts to chemotherapy to prevent or reduce neutropenic sepsis.
- Used to mobilize stem cells for apheresis.
- Used to treat congenital neutrophil disorders: Kostman's syndrome, cyclic neutropenia, idiopathic neutropenia.
- PEGylated form of G-CSF available to increase duration of action.
- May increase the risk of myeloid malignancy.
- Do not use in Kostman's syndrome where there are cytogenetic abnormalities, as risk of malignant transformation is increased.
- Bone pain may be a side-effect.

Interleukin -2 (Aldesleukin)
- Licensed for use in metastatic renal cell carcinoma.
- Can produce tumour shrinkage, but no increase in survival.
- Intravenous administration is associated with a severe capillary leak syndrome.
- Rarely used.

α-interferon
- Main use now is in treatment of hepatitis C, in combination with ribavirin.
- PEGylated interferon is available, to increase duration of action.
- Also valuable in carcinoid tumours, hairy cell leukaemia and lymphomas, myeloma, melanoma, and hepatitis B.
- Side-effects can be severe and include severe flu-like symptoms and 3 severe depressive state (suicide may be provoked): patients with a history of depression should not receive α-IFN.
- Dose is determined by the condition treated.

β-interferon
- Used in the treatment of relapsing and remitting multiple sclerosis.
- Side-effects similar to those of a-interferon but may also cause humoral immune abnormalities, and monitoring of serum immunoglobulins pre- and post-treatment is recommended.

γ-Interferon (Immukin®)
- γ-IFN-1b is used as adjunctive therapy in patients with chronic granulomatous disease for prevention and treatment of infections.
- It can also be used to increase response rate to HBV vaccination in poor responders.
- Side-effects include severe flu-like symptoms.
- Dose is usually 50-100 $\mu g/m^2$ three times a week.

Levamisole
- Originally introduced as an anti-parasitic drug.
- Found to increase circulating T cells, activate macrophages, and enhance DTH reactions.
- Used in RhA and as adjuvant in colonic cancer (FDA approved).
- May cause agranulocytosis (in HLA $B27^+$ patients).

Immunosuppressive/ immunomodulatory drugs: corticosteroids

These drugs are based on endogenous products of the adrenal cortex and form the mainstay of immunosuppressive therapy; synthetic compounds are more potent Natural steroids are highly plasma bound (cor-ticosteroid-binding globulin; albumin). Actions of corticosteroids are manifold.

Actions
- Transiently increased neutrophils (decreased margination and release of mature neutrophils from marrow).
- Decreased phagocytosis and release of enzymes (lysosomal stabilization).
- Marked monocytopenia and reduced monokine (IL-1) production.
- Alteration of cellular gene expression (high-affinity cytoplasmic receptor translocated to nucleus) via NFκB inhibition through increased cytoplasmic concentrations of IκBa.
- Release of lipomodulin (inhibits phospholipase A2 with reduction of arachidonic acid metabolites).
- Lymphopenia due to sequestration in lymphoid tissue and interference with recirculation ($CD4^+$ T-cells > $CD8^+$ T-cells > B-cells) and lymphocytotoxic effects (high doses).
- Decreased T-cell proliferative responses (inhibit entry into G1 phase).
- Reduced serum IgG and IgA.
- No effect on NK or antibody-dependent cell-mediated cytotoxicity (ADCC) activity.

Side-effects

• Carbohydrate metabolism: poor cellular glucose uptake; increased hepatic gluconeogenesis; and glycogen deposition.

• Increased lipolysis and free fatty acid (FFA); increased selective fat deposition.

• Inhibit protein synthesis and enhance protein catabolism. , Increase glomerular filtration rate (GFR) and sodium retention; decrease calcium absorption, inhibit osteoblasts.

• Clinical side-effects are multitudinous:

- diabetes;
- hyperlipidaemia;
- obesity;
- poor wound healing;
- growth arrest (children);
- myopathy;
- hypertension;
- purpura and skin thinning;
- cataracts;
- glaucoma;
- peptic ulcer;
- allergy (synthetic steroids);
- avascular necrosis;
- psychiatric complications;
- thrush.

• Patients on medium- to long-term treatment require regular checks o1 blood pressure, blood sugar, and bone mineral density.

• Prophylactic therapy against bone loss is highly desirable, especially in females and all older patients. Optimal therapy is yet to be determined but includes hormone replacement therapy (HRT; if not contraindicated by underlying disease), vitamin D and calcium, or oral bisphosphonate.

• Doses of over 20 mg/day for long periods may also increase the risk o opportunist infection with *Pneumocystis carinii;* consideration should therefore be given to the use of low-dose co-trimoxazole.

Uses

• Autoimmune diseases, e.g. SLE, vasculitis, rheumatoid arthritis.

• Polymyalgia rheumatica, giant-cell arthritis.

• Allergic diseases (asthma, hay fever).

• Inflammatory diseases (Crohn's disease).

• Malignant disease (lymphoma).

• Allograft rejection.

• Other immunological diseases (ITP, glomerulonephritis).

Dosage regimes

• Prednisolone is the stock drug. Other steroids have little advantage but the BNF has equivalency tables.

• Enteric-coated tablets are kinder to patients, but may lead to erratic absorption.

• If plain steroids are used, gastroprotection with either an H2-antagonist or proton-pump inhibitor is most effective in preventing ulceration.

• Low-dose steroids for RhA (prednisolone 7.5 mg/day) have been used: most patients are now on methotrexate or anti-TNF agents.

• For SLE higher doses (up to 30 mg/day) are required (larger doses for cerebral lupus).

• Life-threatening immunological disease requires much higher doses, 1-2 mg/kg, orally or IV.

• Various pulsed regimes using IV methylprednisolone have been advocated, especially in acute vasculitis. The evidence is split over their effectiveness: 10 mg/kg is advocated, pulsed at varying intervals, often in combination with IV cytotoxics.

• Topical steroids with limited absorption are invaluable in controlling local allergic symptoms (asthma, hay fever).
• Where patients who have been on long-term steroids are being tailed off, they will remain adrenally suppressed for up to 6 months after cessation of therapy and should therefore have steroid cover for illnesses, operations, etc. Patients should carry warning cards.

Immunosuppressive/ immunomodulatory cytotoxic drugs: azathioprine
Azathioprine is converted *in vivo* to 6-mercaptopurine and acts to inhibit the synthesis of inosinic acid (precursor of purines) thus inhibiting DNA synthesis and reducing cell replication.
Actions
• Preferentially inhibits T-cell activation rather than B-cell activation.
• Reduces the circulation of large 'activated' lymphocytes, but has little effect on small resting lymphocytes.
• Long-term use causes significant lymphopenia (T- and B-cells).
• Hypogammaglobulinaemia, due to inhibition of B-cell proliferation (less or no effect on T-cell proliferation).
• Suppression of monokine production.
Side-effects
• Causes profound bone marrow suppression: this is related to deficiency alleles of the enzyme thiopurine methyltransferase (TPMT) involved in its metabolism.
• Screening for enzyme levels is widely available.
• Toxic hepatitis (related to TPMT deficiency).
• Opportunist infections (including HSV, papillomaviruses).
• Gastric upset (probably unrelated to TPMT deficiency).
• Teratogenicity.
• Malignancy (lymphoma).
• PUO syndrome (hypersensitivity).
Uses
• Autoimmune diseases (SLE, rheumatoid arthritis, vasculitis, liver disease, myasthenia, inflammatory bowel disease).
• As a 'steroid-sparing' agent.
Dosage
• In view of the potential for severe/fatal side-effects, azathioprine must be introduced carefully.
• Initial dose should not exceed 1 mg/kg and the patient should receive weekly full blood counts with a differential white count for the first month of therapy.
• If there is any drop in platelets or white cells, it is unsafe to continue with therapy.
• Deficiency alleles for TPMT are present in 1 in 300 of the population: pre-treatment screening is helpful.
• If it is tolerated, then the dose can be increased to 2.5 mg/kg (or in special circumstances to 4 mg/kg).
• The need for continuing therapy needs to be reviewed regularly.
• Hypogammaglobulinaemia may be severe and require replacement therapy, although recovery may eventually occur over several years.
• Liver function tests must also be monitored.
• Allopurinol is contraindicated with azathioprine as it interferes with elimination and raises blood levels.

Immunosuppressive/ immunomodulatory cytotoxic drugs: cyclophosphamide and chlorambucil (alkylating agents)
These drugs bind to and cross-link DNA and possibly also RNA, thus 'interfering with DNA replication and transcription. Effects are dependent on the phase of the cell cycle during exposure and the competence of the DNA repair mechanisms.

Actions
• Dose-dependent lymphopenia (T ($CD8^+ > CD4^+$) and B cells).
• Reduced B-cell proliferation and antibody synthesis (reduced IgG and IgM).
• Lesser effect on T-cells ($CD8^+ > CD4^+$, which may actually enhance T-cell responsiveness under certain circumstances).
Side-effects
• Bone marrow depression.
• Alopecia.
• Haemorrhagic cystitis (caused by acrolein)—ensure good hydration and use mesna for doses above 200 mg/kg. May be less risk with pulsed IV therapy.
• Sterility (males and females-offer males sperm banking and warn all patients of reproductive age and record in notes).
• Secondary lymphoid neoplasms ((11-fold increase), bladder tumours (10% long-term), skin tumours (fivefold increase), and acute myeloblasts leukaemia).
• Opportunist infections (use co-trimoxazole and antifungal prophylaxis).
• Nausea and vomiting (high dose).
Major uses
• Vasculitis, especially Wegener's granulomatosis, PAN, MPA, CSS.
• SLE and RhA.
• Glomerulonephritis (including Goodpasture's).

Dosage
• There is considerable debate over the optimal regimes for use of cyclophosphamide.
- Continuous low-dose oral cyclophosphamide (2-4 mg/kg/day).
- Intravenous pulse therapy (10 mg/kg/pulse or 0.75-1 g/m^2 body surface area; intervals determined by protocol and blood counts).
• Long-term side-effects may be higher with continuous oral therapy, but this may reflect the higher total dose, and lower doses may be effective.
• In life-threatening conditions, high-dose IV pulses, with IV steroids, will have a faster effect (remember mesna cover and IV fluids).
• Chlorambucil is used orally in doses of 0.03-0.06 mg/kg/day and is less toxic to the bladder than cyclophosphamide, but probably equally toxic to the marrow in therapeutic doses.
• Dosages of both drugs will require adjustment in renal impairment. Regular blood counts are required, according to current local protocols.

Immunosuppressive/ immunomodulatory cytotoxic drugs: methotrexate (MTX)
Methotrexate is a competitive inhibitor of the enzyme dihydrofolate reductase and impairs synthesis of tetrahydrofolate from folic acid (required as cofactor for thymidine synthesis). It therefore interferes with DNA synthesis. It is also thought to interfere with other intracellular enzymes. As an immunosuppressive agent it is used in low weekly doses | (much lower than the doses used as an antineoplastic agent). Low-dose MTX probable inhibits the enzyme 5-amino-imidazole-4-carboxamide ribonucleotide (AICAR) transformylase, leading to the accumulation of ICAR and increased adenosine.
Actions
• Variable effects on lymphocyte numbers in peripheral blood.
• Possible inhibition of monokine production.
• Inhibition of lipo-oxygenase pathway.
• Reduction in antibody synthesis.
• Converted to methotrexate polyglutamates, which persist long-term intracellularly and are potent inhibitors of AICAR tranformylase and dihydrofolate reductase.

Side-effects
- Mucositis, nausea, and vomiting.
- Bone marrow suppression (megaloblastic-may be reversed by folinic acid rescue): may be worse if other anti-folate drugs are co-administered, e.g. sulphasalazine, co-trimoxazole.
- Hepatic fibrosis (dose-related).
- Pneumonitis (5% of patients), a hypersensitivity reaction.
- Sterility.

Uses
- RhA; psoriatic arthropathy.
- Polymyositis/dermatomyositis.
- GvHD in BMT.
- Adjunctive therapy with infliximab.

Dosage
- The drug is given *weekly* either orally or IM.
- Initial dose is 7.5 mg (adult), increased stepwise depending upon side-effects and clinical benefit to a maximum of 20-30 mg/week.
- Dose may need reducing in renal impairment.
- Care needs to be taken with co-administration of other drugs.
- Folinic acid may reduce risk of bone marrow toxicity and mucositis (given 24-36 hours later). Folic acid will not be effective as MTX inhibits its conversion to the active form.

The most frequent cause of side-effects, including death, is the inadvertent daily administration of the drug-always check carefully. Do not rely on pharmacy systems to spot errors.
- Baseline tests should include FBC, LFTs, chest radiograph, and lung function.
- Monitor weekly (initially) pre-dose full blood counts and regular liver function tests.
- If abnormal LFTs are noted, the drug dose should be reduced or the drug stopped.
- It is thought that the risks of monitoring for liver disease by biopsy outweigh any benefit
- Development of pneumonitis is an absolute indication for withdrawing the drug; high-dose steroids may be required.

Immunosuppressive/ immunomodulatory cytotoxic drugs: 2-chlorodeoxyadenosine (2CDA, cladribine) and fludarabine
- Mode of action is by inhibition of adenosine deaminase, i.e. effectively producing ADA-deficient severe combined immunodeficiency.
- Primary use is in the treatment of haematological malignancy (B-cell tumours), but they have the potential, with careful use, to be potent immunosuppressive agents.
- Both produce a profound and long-lasting B-cell and $CD4^+$ T-cell lymphopenia.
- Prophylactic antibiotics (co-trimoxazole) and antifungals are advisable.
- Long-term monitoring of lymphocyte surface markers and serum immunoglobulins is advisable after treatment.

Immunosuppressive/ immunomodulatory drugs: ciclosporin (CyA)/tacrolimus (FK506)/sirolimus (rapamycin)

These three agents are macrolide antibiotics derived from fungi. They act specifically on T-helper cells, but leave other cell types unaffected. Target cells are inhibited but not killed (therefore effects are fully reversible on cessation of treatment).

Modes of action
- Ciclosporin (CyA) interacts with cyclophilin, a 17 kDa protein (peptidyl-prolyi *cis-trans* isomerase) and tacrolimus interacts with FK-BP12, a 12 kDa protein, similar to cyclophilin.
- Complex prevents calcineurin, a calcium- and calmodulin-dependent protein, from dephosphorylating the nuclear factor of activated T cells (NF-AT), reducing transcription of the IL-2 gene.
- Immunosuppressive activity is not directly related to this activity.

• CyA and tacrolimus inhibit IL-2 and IL-2Rα gene expression and prevent T-cell activation (cells arrested at G0/G1).
• Sirolimus has no effect on IL-2 production; it binds FK-BP, but the complex does not inhibit calcineurin; it appears to block calcium-independent signalling (via CD28) and inhibits mTOR.
• May interfere with macrophage function (lymphokine release and receptor synthesis).
• CyA interferes with B-cell proliferation and antibody production.

Side-effects

• Hypertension.
• Hirsutism.
• Nephrotoxicity.
• Hepatotoxicity.
• Lymphomas (NHL-85% EBV^+).
• Opportunist infections (CMV, papillomaviruses, HHV-8).
• Neurotoxicity.
• Multiple drug interactions (CyA and tacrolimus induce cytochrome P450(IIEI)).
• Diabetes (inhibition of insulin release).
• Headache.

Uses

• Combination therapy for allografts.
• RhA, SLE.
• Autoimmune diseases (uveitis, Behcet's, inflammatory bowel disease).

Dosage

• CyA and tacrolimus are available orally and intravenously.
• Dosage for CyA depends on the circumstances, but 10-15 mg/kg/day may be required for allograft rejection, while 5 mg/kg may suffice for autoimmune disease.
• Tacrolimus dosage is 0.1-0.3 mg/kg/day for allograft rejection; experience of this drug is less in autoimmune disease, but doses up to 0.1 mg/kg/day in divided doses appear to be effective.
• Monitoring of drug levels is desirable for both drugs: this can prevent toxicity and monitor compliance.
• Sirolimus dosage is 6 mg as a stat. dose followed by 2 mg daily, with drug monitoring and appropriate dosage changes, in allografts. Dosages for use in autoimmune disease are not defined.
• Because it operates on different activation pathways it may make a useful combination treatment.
• Creatinine and electrolytes, liver function, and blood pressure should be monitored regularly.

Immunosuppressive/ immunomodulatory drugs: mycophenolate mofetil (MMF) and brequinar

Mycophenolate mofetil (MMF)

This drug is a prodrug of mycophenolic acid. In its actions it is similar to azathioprine.

Actions

• Blocks inosine monophosphate dehydrogenase and blocks synthesis o guanine, but has no effect on salvage pathway.
• It acts predominantly on lymphocytes, to prevent proliferation, but ha: little effect on non-lymphoid cells.

Side-effects

• Lymphopenia
• Opportunist infections (CMV, HSV)
• Lymphoma
• Hepatotoxicity.

Uses
- Prophylaxis of allograft rejection
- Autoimmune disease: SLE nephritis; uveitis.

Dosage
- Dosage is 1 g twice a day, increased to maximum of 1.5 g twice daily.
- FBC and LFTs should be monitored regularly.

Brequinar

Brequinar is an experimental drug that inhibits pyrimidine synthesis. It has the greatest effects on B-cells and antibody production, especially IgE. It is effective in animal models of allograft rejection.

Immunosuppressive/ immunomodulatory drugs: leflunomide
- After oral administration is converted to an active metabolite that inhibits dihydro-orotate dehydrogenase (involved in pyrimidine synthesis and required by T cells).
- T cell proliferation is blocked.
- Used in RhA: as effective as methotrexate.
- Onset of action is slow (4-6 weeks).
- Side effects include severe hepatotoxicity and Stevens-Johnson syndrome.

Drug can be 'washed-out' with cholestyramine or activated charcoal (binds it in the gut).
- It is teratogenic: effective contraception is required.
- Dose is 100 mg daily for 3 days; then 10-20 mg daily.
- Monitoring with regular FBC and LFTS mandatory.

Immunosuppressive/ immunomodulatory drugs: D-penicillamine and gold

D-Penicillamine This enigmatic drug comes and goes in terms of its utility as an immunomodulator.
- Precise immunological actions are not known.
- It decreases antibody production.
- It inhibits T-cell proliferation, possibly through enhanced hydrogen peroxide production or through sulphydrylation of the surface receptors of the lymphocytes.
- Neutrophil chemotaxis and oxidative function are impaired.
- Macrophage antigen-presenting function and monokine production are reduced.
- Also believed to inhibit collagen synthesis; hence its use in scleroderma.
- Very slow in its onset of action.
- Main side-effects include a range of autoimmune diseases, including myasthenia gravis, renal disease (nephrotic), SLE, polymyositis, and Goodpasture's syndrome.
- Mainly used in RhA and scleroderma, but has fallen out of favour due to side-effects.
- Dosage is 250 mg daily increased to 1 g daily.
- Regular monitoring (every 1-2 weeks initially) of renal function, urinalysis, and FBC required.
- Penicillin-allergic patients may be at increased risk of reacting to penicillamine.

Gold
- Gold may be given either as IM injections or orally: evidence suggests that parenteral therapy is more effective.
- Concentrated selectively in macrophages, and reduces monokine production (IL-1); hence also reducing T- and B-cell responses.
- Impairs endothelial expression of adhesion molecules and hence reduces cellular traffic to sites of inflammation.
- Profile of side-effects is similar to that of penicillamine, and the onset of action is also slow.
- It is used only in RhA: now mainly replaced by anti-TNF agents and methotrexate.

• Dosage is dependent on the route and expert advice on current regimes should be sought from a clinician experienced in its use.

Immunosuppressive/ immunomodulatory drugs: hydroxychloroquine/mepacrine

• Antimalarials have a particular role to play in trie management of joint and skin complaints in connective tissue diseases.
• Effective in relieving the fatigue experienced in association with connective tissue diseases such as SLE and Sjogren's syndrome.
• Mode of action is uncertain, but there is evidence that they interfere with the production of cytokines and reduction of production of granulocyte lysosomal enzymes.
• Anti-platelet action demonstrated: may be valuable in anti-phospholipid syndrome.
• Onset of action is slow.
• Well-tolerated with few side-effects.
• Haemolysis may occur in G6PD-deficient patients.
• Retinal toxicity is possibly a problem with hydroxychloroquine, and definitely a problem with chloroquine (not used in rheumatic disease now).
• Evidence that there is a significant risk of ocular toxicity from hydroxychloroquine is minimal.
• Loss of colour discrimination may be an early sign.
• Hydroxychloroquine may cause nausea and vomiting which can be severe and usually occurs during the initiation phase.
• Mepacrine stains the skin yellow.
• Preferred antimalarial is hydroxychloroquine.
• Starting dose for hydroxychloroquine is 400 mg/day (adult), reducing to maintenance of 200 mg/day. Alternatively the maximum daily dose is calculated as 6.5 mg/kg lean body mass.
• Mepacrine is an unlicensed drug in the context of autoimmune disease, and the dose is 50-100 mg per day.

Immunosuppressive/ immunomodulatory drugs: thalidomide/oxypentifylline

• Thalidomide is an interesting drug that reduces the severity of conversion reactions due to treatment of lepromatous leprosy.
• It is a potent inhibitor of TNF-α production by monocytes, due to interference with gene transcription.
• It decreases the expression of adhesion molecules.
• Circulating $CD4^+$ T cells are reduced.
• It inhibits -y-interferon production by T cells, due to preferential stimulation of Th2 cells.
• Side-effects include its well-documented teratogenicity, neuropathy,
and drowsiness (which may limit dose).
• It is used in the management of ulceration in Behcet's syndrome and in reducing the severity of GvHD.
• Dosage is 100 mg/day, reducing to 50 mg on alternate days.
• Nerve conduction studies should be carried out as a baseline pre-treatment, and at intervals on treatment.
• Women of child-bearing age must be formally counselled over the risks and agree to take appropriate contraceptive measures. This must be recorded in the notes.
• Oxypentifylline is a drug originally introduced for peripheral vascular disease. It has similar but weaker actions to thalidomide in inhibiting TNF-α production.

Immunosuppressive/ immunomodulatory drugs: sulphasalazine and colchicine

Sulphasalazine

• Sulphasalazine was originally introduced as an antibiotic.

• It comprises sulphapyridine coupled to 5-aminosalicylic acid via a diazo bond that can be split by enteric bacteria.
• The precise mode of action as an immunomodulatory agent is uncertain.
• It has minor immunosuppressive activities, mainly localized to the gut, but little effect on peripheral blood lymphocyte numbers or function.
• It is used predominantly in inflammatory bowel disease, rheumatoid arthritis, and seronegative arthritides, in doses of 500-2000 mg/day in divided doses.
• It has a significant array of side-effects including male infertility, macrocytic anaemia, rash, Stevens-Johnson syndrome, and secondary hypogammaglobulinaemia.
• Regular monitoring of FBC is required.

Colchicine
• Colchicine is a useful anti-inflammatory.
• Precise immunological role is uncertain, but it inhibits microtubule assembly and therefore inhibits mitosis.
• Concentrated in neutrophils and inhibits chemotactic activity, thus reducing the accumulation of non-specific inflammatory cells.
• Valuable in Behcet's syndrome and familial Mediterranean fever.
• Value is limited by side-effects at therapeutic doses (diarrhoea and gastrointestinal discomfort).
• Usual dose is up to 0.5 mg four times daily, if tolerated.

Immunosuppressive/ immunomodulatory drugs: dapsone
• Used to treat dermatitis herpetiformis.
• Inhibits fi-integrin-mediated neutrophil adherence to endothelium by binding to intracellular G-protein.
• Main side-effect is haemolysis, which is marked in G6PD-deficient patients.
• Always check G6PD status prior to use and monitor FBC for signs of haemolysis (reduced haptoglobins are a sensitive indicator of haemolysis).
• Dose is usually 1-2 mg/kg/day.

Immunosuppressive/ immunomodulatory biologicals: high-dose IVIg
High-dose IVIg is widely used as an immunomodulatory agent in autoimmune diseases. The precise mechanisms of action clinically are uncertain, although many mechanisms have been postulated.
Actions
• Fc-receptor blockade on phagocytic cells.
• Inhibition of cytokine production.
• Inactivation of pathogenic autoantibodies (anti-idiotypes).
• Inhibition of autoantibody production by B-cells.
• Decreased T-cell proliferation.
• Actions of soluble cytokines, cytokine receptors, and antagonists present in IVIg.
• Inhibitory actions of stabilizing sugars.
Side-effects
• Aseptic meningitis (elevated lymphocytes and protein in CSF) secondary to sugars.
• Renal failure (secondary to osmotic load from sugars).
• Massive intravascular haemolysis (secondary to IgG isoagglutinins).
• Hyperviscosity (CVAs, MI).
• Immune complex reactions in patients with high-titre rheumatoid factors, cryoglobulins (types II and III), or other immune complex disease, e.g. SLE.
• Anaphylactoid reactions (IgA deficiency; infected).
Main uses
• Replacement therapy for primary and secondary immunodeficiencies.

• Autoimmune cytopenias (ITP, AIHA).
• Vasculitis: Kawasaki syndrome; Wegener's granulomatosis.
• Neurological diseases (acute Guillain-Barre, CIDP, pure motor neuropathy, myasthenia gravis, LEMS, MS).
• Factor VIII and factor IX inhibitors.
• Anti-phospholipid antibodies in pregnancy.
• Autoimmune skin disease (pemphigus, pemphigoid, epidermolysis bullosa acquisita).
• Eczema.
• Polymyositis, dermatomyositis.

Dosage

• Dosage regimes range from 0.4 g/kg/day for 5 days, through 1 g/day for 2 days to 2 g/kg/day as a single dose.
• Minimal evidence is available to distinguish between the schedules.
• Fewer doses may be required for ITP.
• My own practice is to start all high-dose regimes on the 5-day schedule to assess tolerability and then increase to 1 g/kg/day for 2 days subsequently.
• In adults and children, risks of renal impairment and aseptic meningitis are highest with the ultrarapid infusion schedule of 2 g/kg/day, and
I avoid this if possible.
• Rapid infusions should be avoided in all elderly patients because of the risks of hyperviscosity.
• Pre-treatment IgA deficiency and high-titre rheumatoid factors should be excluded, and renal function assessed.
• Pre-treatment FBC, LFTs, and hepatitis serology (HBV, HCV) should also be measured.
• IgA-deficient patients require special care, and should be on products low in IgA (Octagam®, Flebogamma®, Gammagard-SD®, Vigam-5®, Sandoglobulin-NF®).
• If there is renal impairment to start with, daily creatinines should be measured and, if there is a rise of 10% or more, then therapy should be discontinued.
• FBC should be repeated during the course to ensure that haemolysis does not take place (haptoglobin is a sensitive indicator of intravascular haemolysis).
• Infusion rates should follow manufacturers' guidelines.
• There must be no switching of products.

Immunosuppressive/ immunomodulatory biologicals: polyclonal antibodies, high-dose anti-D immunoglobulin, and blood transfusion effect

Polyclonal antibodies

• Xenogeneic antisera raised by the immunization of animals with purified human T cells or thymocytes (rabbit anti-thymocyte globulin (ATG) and rabbit anti-lymphocyte globulin (ALG)) are potent immunosuppressive agents.
• They cause a profound lymphopenia.
• They are difficult to standardize and have significant batch-to-batch variation.
• They also contain cross-reactive antibodies that react with other cells types, including platelets.
• Utility is limited by the development of a host-anti-rabbit response, which both neutralizes the xeno-antiserum and also gives rise to a serum sickness reaction.

Actions

• Complement-mediated lymphocyte destruction.
• Cause marked but variable lymphopenia.
• Reduced T-cell function.

Uses

• Acute graft rejection or GvHD.
• Diamond-Blackfan syndrome.

Dosage

• Dosage depends on batch but is usually within the range of 5-30 mg/kg/day.
• Effect can be monitored by absolute lymphocyte counts or flow cytometric analysis of peripheral blood lymphocytes.

High-dose anti-D immunoglobulin
• High dose anti-D immunoglobulin can be used to control autoimmune haemolytic anaemia in Rhesus D^+ patients.
• Mechanism of action is unclear.

Blood transfusion effect
• In renal allografts it has been well-documented that both donor and random blood transfusions reduce the risks of graft rejection.
• Immunological mechanism is uncertain.

Immunosuppressive/ immunomodulatory biologicals: monoclonal antibodies 1
Since the first edition of this book there has been an explosion of monoclonal antibodies, now mainly chimeric or humanized molecules, and these are replacing murine monoclonal antibodies, which have a high rate of induction of anti-mouse antibodies, which abolish the effectiveness.

Infliximab (Remicade®)
• Chimeric monoclonal antibody to TNF-α.
• Licensed for use in Crohn's disease, rheumatoid arthritis, ankylosing spondylitis.
• Major hazard of use is significant increase in tuberculosis.
• Screening for TB prior to administration is advised.
• Use of Quantiferon Gold may be valuable in establishing previous tuberculous infection.
• Development of PUO, cough, weight loss should trigger search for TB.
• Induces development of anti-nuclear antibodies.
• Must be used in combination with MTX to prevent the development of anti-chimera antibodies.
• Dose 3 mg/kg as IV infusion, repeated at 2, 6, and then 8 week intervals.
• Cessation of therapy in Crohn's disease may lead to loss of effect when re-introduced.

Adalimumab (Humira®)
• Another monoclonal antibody to TNFa.
• Side-effects, dosing, and indications are similar to those for infliximab.
• Co-therapy with MTX is recommended.
• Dose is 40 mg on alternate weeks subcutaneously.

Alemtuzumab (MabCampath®)
• Humanized anti-CD52 monoclonal (Campath®).
• Lytic antibody: targets predominantly B cells.
• Used in B-cell lymphomas and B-lymphoproliferative disease (EBV^+) in the immunosuppressed.
• Causes tumour lysis and cytokine release syndrome (capillary leak), as for rituximab.

Rituximab (MabThera®)
• A humanized anti-CD20 monoclonal antibody, expressed on normal cells, censed for use in non-Hodgkin's B-cell lymphomas.
• Licensed for use in non-Hodgkin's B-cell lymphomas.
• Can cause massive tumour lysis syndrome, with cytokme release (antibod/-complement mediated lysis) and capillary leak syndrome 1-2 hours after infusion.
• Fever and chills, nausea and vomiting, and hypersensitivity reactions are common.
• Analgesic and antihistamme should be administered before treatment.
• Full resuscitation facilities should be available.

• Synergistic with chemotherapy.
• Likely to be valuable IP the treatment of autoimmune diseases where autoantibody plays a significant role (autoimmune haemolytic anaemia, ITP).
• Also shoeing promise as adjunctive therapy in SLE.
• Side-effects include severe and prolonged hypogammaglobulinaemia due to destruction of normal B-cells.

Omalizumab (Xolair®)
• A humanized monoclonal antibody against IgE Fc region that prevents binding to the high-affinity IgE receptor.
• Trials in asthma have been disappointing but it will probably be more valuable in allergic rhinoconjunctivitis, where IgE is more definitively involved.

Basiliximab (Simulect®)
• A humanized monoclonal antibody that binds to the α-chain of the IL-2 receptor and prevents T-cell proliferation.
• Used in the prophylaxis of acute organ rejection in renal allografts, in combinations with cyclosporin and co rti co steroids.
• Main side-effect is severe hypersensitivity.
• Dose is 20 mg 2 hours before transplant and 20 mg 4 days after surgery.

Immunosuppressive/ immunomodulatory biologicats: monoclonal antibodies 2
Daclizumab (Zenapax®)
• A humanized monoclonal antibody that binds to the -y-chain of the IL-2 receptor and prevents T-cell proliferation.
• Used in the prophylaxis of acute organ rejection in renal allografts, in combinations with cyclosporin and corticosteraids.
• Main side-effect is severe hypersensitivity.
• Dose is 1 mg/kg by IV infusion in the 14 hours pre-transplant, then continued daily for 14 days.

Gemtuzumab (Mylotarg®)
• A monoclonal antibody against the CD33 antigen expressed on myeloid leukaemic blasts and normal myeloid cells.
• Used to treat AML in first relapse.

Anti-CD154
• Anti-CD154 (gp39, CD40 ligand} blocks the binding of CD154 on activated but not resting T-cells with CD40 on B-cells and APCs.
• It will induce a slate of immunodeficiency similar to HIGM.
• Experimental models have shown benefit in allograft rejection and autoimmunity.

Trastuzumab
• A humanized antibody against the epidermal growth factor receptor Her-2.
• Blocks and downregulates the receptor.
• Valuable in the treatment of metastatic breast cancer where the tumour overexpresses Her-2.
• Her-2 is also expressed on other tumours.

Abciximab (ReoPro®)
• Monoclonal antibody binding to platelet glycoprotem IIb/IIIa receptors.
• Used once only as an adjunct to heparin and aspirin in high-risk patients undergoing percutaneous transluminal coronary artery interventions to prevent thrombotic complications.

OKT3 (muromonab-CD3®)
- Murine antibody against T-cell CD3 ε-chain.
- Used to treat allograft rejection.
- Associated with high incidence of the development of HAMA (human anti-mouse antibodies) that reduce its effect and cause a serum sickness reaction.
- Now used less, as humanized alternatives are available.

Digoxin-specific antibody (Digibind®)
- Fab' fragments of antibody against digoxin.
- Used to treat digoxin poisoning by tending to the drug.

Other immunosuppressive/immunomodulatory biologicals
Other biologicals include cytokine antagonists and fusion proteins.

Etanercept (Enbrel®)
I Etanereept is a soluble fusion protein of the ligand binding portion of the type 2 TNF receptor (p75) coupled to the Fc portion of human IgG_1.
- It binds both TNF-α and TNF-β.
- It is synergistic with MIX.
- It is licensed for use in RhA, psoriatic arthritis, and juvenile arthritis.
- It may be of value m Behçet's disease and uveitis.
- Demyelination has been reported; risk of immunosuppression is significant.
- Dose is 25-50 mg twice a week by subcutaneous injection for 12 weeks, reducing to 25 mg twice a week with a maximum duration of therapy of 24 weeks.
- If there is no response by 12 weeks, then it should be discontinued.

Anakinra (Kineret®)
- A recombmant version of the naturally occurring IL-1 receptor antagonist.
- Blocks the action of IL-1.
- Has been used in RhA, but evidence of benefit is not compelling.
- Causes neutropenia and headache.
- Dose is 100 mg daily subcutaneously.

CTLA4Ig
- This is a fusion protein of CTLA4, a T-cell surface molecule, and human IgG_1.
- CTLA4 binds to B7 on APC and blocks the binding of B7 to T-cell CD28, preventing the co-stimulatory stimulus required for T-cell activation and inducing anergy.
- Currently undergoing trials in psoriasis and lupus (not promising!).

Other experimental biological agents
Many other biological agents are in clinical trials. Not all will prove clinically valuable. Some are shown in the table: many others are being tested.

Some immunosuppresswe/immunomodulatory biological agents in clinical trials

Agent	Target
Pegsunercept	Pegytated soluble TNF receptor type I (RhA)
Atlrzumab	Humanized anti-IL-6 monoclonal antibody (RhA)
Natalfeumab	Humanized antibody against cefl adhesion molecules 4-1 and 4-7; trials in MS. RJA may lead to progressive multifocal leucoencephalopathy (PML)
Alefocept	CD2 antagonist
HuMAX-IL-15	Anti-IL-15 monoclonal antibody
Pexelumab and eculizumab	Complement C5 Inhibitors, in trial for paroxysmal nocturnal haemoglobinuria

Total lymphoid irradiation (TLI)

- TLI is experimental as an immunosuppressive therapy, having been used previously for the treatment of lymphoid malignancies.
- Produces profound impairment of T-cell numbers and function, although there is a small population of radio resistant small lymphocytes.
- A more modem variant is to use UV sensitizing agents (psoralens) and then irradiate leucocytes in an extracorporeal circulation (photopheresis).
- TLI may be of benefit in intractable rheumatoid arthritis, multiple sclerosis, and severe SLE.

Side-effects

- Severe leucopenia.
- Thrombocytopenia.
- Opportunist infections.
- Lymphoma (NHL).

Thoracic duct drainage

- Has been used in the past for treatment of severe RhA.
- Causes a severe and long-lasting immunosuppression
- Similar effects are seen from accidental thoracic duct damage in oesophageal arid cardiac surgery, where a chylous effusion is allowed to dram unchecked.
- A profound and persistent lymphopenia of both T-and B-cells is caused/
- Recovery of immune function may occur over a long period.
- Prophylaxis against *Pneumocysris carinii* pneumonia and fungal infections will be required if the drainage is accidental.

Plasmapheresis

- Plasmapheresis is the removal of plasma constituents using automated cell separators. The plasma components are removed by either centrifugation or membrane filtration.
- Erythrocytes and other cellular components are re-infused and the removed plasma replaced with either FFP or FFP + IVIg to maintain circulating volume.
- About 50% of the plasma is removed each time.
- A therapeutic course is usually 3-5 daily treatments.
- The amount of antibody removed depends on volume of distribution. 90% of IgM but only 20% of IgG is removed each time as only 40% of the IgG is within the vascular space.
- Plasmapheresis also has the advantage of the removal of immune complexes and small mediators (toxins, anaphylotoxins, cytokines), in addition to the antibodies.
- Plasmapheresis is only suitable for urgent therapy, as antibody levels return rapidly and frequently overshoot to higher levels after plasm apheresis is discontinued. It is important to commence conventional immunosuppression at the same time.

Side-effects
- Leakage/air embolism.
- Anticoagulation (citrate toxicity)/thrombocytopenia.
- Reactions to replacement fluids.

Uses
- Hyperviscosity (Waldenström's macroglobulinaemia, IgA myeloma).
- Goodpasture's syndrome/Wegener's granulomatosis.
- Cryoglobulinaemia.
- Myasthenia gravis.
- Guillain-Barre syndrome (but IVIg is as good).
- It has been tried in other autoimmune diseases (RhA, FVIII antibodies, MS, lupus nephritis) with variable anecdotal success.
- A limiting factor in its use is access to appropriate equipment.

Immunoadsorption
- Selective removal of autoantibodies has been attempted using an extracorporeal circuit including a column of inert beads coated with protein A or protein G for specific adsorption of IgG.
- This treatment is experimental and it is not widely used in clinical practice.

Allergy interventions: drugs

Treatment for allergic disease is divided into three major target areas mast cells, released mediators, and the specific immune response. Treatment can be topical or systemic: topical is preferred if this is effective. The underlying ctironic inflammatory component especially of asthma, needs always to be addressed rather than just using symptomatic agents. Corticosteroids and antihistamines are more effective as prophylactic agents, taken before allergen exposure.

Mast-cell active drugs
- Corticosteroids (interfere with synthesis of leukotrienes).
- Mast-cell stabilization: cromoglycate'nedocromil/ketotifen (prevent allergen-triggered calcium flux and hence prevent degranillation).

Released mediators
- β-Agonists (smooth muscle relaxation, some anti-inflammatory effect (salmeterol)).
- Antihistamines: use long-acting high-potency non-sedating drugs without cardiotoxicity (loratidme, desloratidine, levocetirizine, cetinzine. fexofenadme].
- Corticosteroids.
- Anti-PAF drugs (clinical results disappointing).
- Leukotriene (LTD_4) antagonists, monteleukast, zafirleukasl (useful adjunctive treatment in asthma).
- 5-lipoxygenase inhibitor, zileuton (asthma).
- Kinin antagonists: icatibant (anti-bradykinin B2 receptor antagonist), WIN64338, FR173657 (orally active B2 antagonist).

Specific IgE
- Desensitiiation
- Peptide therapy (experimental)
- Anti-FcRe therapy (experimental)
- Omalizumab, anti-lgE.

Allergy interventions: desensitization (immunotherapy)
Mechanism of benefit
- Mechanism of desensitization is uncertain: specific IgE may rise in early stages of treatment then

fall.
• The role of 'blocking antibodies' (IgG_4) is unknown.
• One hypothesis suggests that sequential exposure gradually switches the $CD4^+$ T-cell response from Th2 to Th1, reducing IgE production and the levels of the pro-allergic cytokmes IL-4 and IL-5.

Indications

Desensitization should be considered for patients with:

- anaphylaxis to insect venoms;
- rhinoconjunctivitis not controlled with maximal medical therapy, used correctly, and including repeated courses of oral steroids.

Asthma may be amenable to treatment but carries high risks.

Exclusions

Current guidelines suggest that desensitization is **inapropriate** for those with:

- multiple allergies;
- severe asthma (FEV_1 < 75% predicted): seasonal wheeze only induced by pollen is not a contraindication to desensitization out of the pollen season;
- heart disease;
- hypertension requiring β-blockade (difficult to resuscitate in emergencies);
- use of angiotensm converting enzyme (ACE) inhibitors] in creased risk of angioedema, local and systemic);
- during pregnancy;
- excess alcohol consumption (increased risk of side-effects).

• Age > 50 years is associated with poor responses (exception is venom immunotherapy).

Schedules

• Traditional desensitization is done with weekly injections of increasing doses of aqueous allergen until maintenance doses are reached at 14-18 weeks.
• Once on maintenance doses, intervals between injections are spaced out to 4-6 weeks for 3 years.

Short course (4 or 6 injections over the winter) of adsorbed allergens (Pollmex® and Pollinex Quattro®) are as effective for pollen allergies: course is repeated annually for 3 years.

• Allergens available include:

- venoms: bee, wasp, bumblebee (for market gardeners who use bumblebees for pollination);
- pollens: grass, birch pollen;
- animals: cat (potent allergen associated with high incidence of side-effects), horse, dog (but this is not as effective as the others);
- house dust mite.

• Highly purified allergens should always be used: whole insect extracts or multiple allergens combinations are not recommended.
• All except Pollinex® are unlicensed at present and are therefore administered on a named-patient basis. Formal written consent is required.
• Precise protocol and total duration of therapy varies depending or ths allergen.
• All require long-term commitment from patients.
• Schedules are onerous and the leeway for changes to accommodate holidays are minimal.
• Patients must not undertake vigorous exercise after injections (increased risk of side-effects).
• Injections cannot be given if patient is unwell.
• Pre-treatment with antihistamines reduces risk of local reactions.
• Peak flow should be monitored pre- and 30 minutes post-injection.
• All patients must stay for at least 1 hour post-injection (no exceptions).
• Rush and ultra-rush schedules have been devised, but are rarely re quired in practice and significantly increase the risks of reactions.
• Desensrtotion must be carried out in hospital and staff must be conversant with emergency

management of anaphylaxis and cardiac arrest procedures.

Side-effects

• Main-side effects are pain and swelling at site of injections (pre-treat with antihistamine and supply oral steroid).

• Systemic reactions may occur (cough is often the first sign): treat as for any acute allergic reaction.

• Risk of reactions is increased:

- during the up-dosing period (aqueous allergens);
- in patients treated with cat allergen;
- if patient has mtercurrent infection (defer injection);
- if patient is extremely anxious (use sedation if necessary);
- if patient is exposed to allergen naturally during the up-dosing period;
- if patient is drinking excess alcohol;
- if patient is started on ACE inhibitor

Sublingual therapy

• Sublingual desensitiialion is widely practised in Europe.

• Allergen drops or tablets are placed under the tongue on a daily basis.

• Local tingling and minor swelling may occur.

• Experience from Europe suggests that this is safe and effective and can be administered at home.

Adoptive immunotherapy

Interleukin-2 and LAK therapy

• LAK therapy (lymphokine-activated killer cells) has been proposed as a treatment for malignant disease.

• Peripheral blood lymphocytes are harvested and then stimulated *in vitro* with high-dose IL-2 and re-infused into the patient with additional IL-2.

• Side-effects are severe and tumoricidal activity limited.

• A better approach may be to expand tumour-invading lymphocytes (TIL).

• The therapy is moderately toxic in therapeutic doses (fluid retention; capillary leak syndrome).

• There is some evidence of benefit in melanoma and renal cell carcinoma (salvage therapy).

Questions and answers

1. Wich cytokines regulate neutrofil production?

GM-CSF, G-CSF

2. What is the proportion of each immunoglobulin class in the circulation?

IgG	6.8-15.4 g/l
IgA	0.84-2.97 g/l
IgM (males)	0.54-1.90 g/l
IgM (females)	0.81-2.30 g/l
IgG subclasses	
IgG_1	4.2-10.8 g/l
IgG_2	1.5-6.0 g/l
IgG_3	0.5-1.9 g/l
IgG_4	0.2-1.4 g/l
IgA subclasses	
IgA_1	0.7-2.4 g/l
IgA_2	0.2-0.9g/l
Total IgE	<100 kU/l or <100 IU/ml
IgD	0.01-0.04 g/l

3. What are the clinical features of immunodeficiency?

Recurrent infections.

There is no universally accepted definition of what constitutes 'recurrent infection' and therefore it is difficult to be categorical about who should be investigated for immunodeficiency. The following should be used as guidance.

- Two major or one major and recurrent minor in 1 year.
- Unusual organisms (Aspergillus, Pneumocystis).
- Unusual sites (liver abscess, osteomyelitis).
- Chronic infections (sinusitis).
- Structural damage (bronchiectasis).

4. What is treatment of X-linked agammaglobulinaemia?

- Iintravenous IgG should be started at the earliest opportunity; dose of 200-600mg/kg/month given at intervals of 2-3 weeks. Longer intervals do not give satisfactory replacement.
- Subcutaneous immunoglobulin given weekly (same total dose) is an alternative.
- Trough IgG levels should be monitored regularly, with the aim of maintaining a level well within the normal range (6-9 g/l). Early institution of IVIg and adequate trough levels preclude the development of bronchiectasis.
- Prompt antibiotic therapy (course of 10-14 days) for upper and lower respiratory tract infections together with physiotherapy and postural drainage if lung damage has already occurred. Ciprofloxacin is a valuable antibiotic (though not licensed for small children). Use cystic fibrosis approach of zero tolerance to cough.
- As children get older and can comply, perform regular lung function testing, including transfer factor.
- High resolution CT scanning (HR-CT) is useful for identifying subclinical bronchiectasis, but imposes a significant radiation burden and should not be overused.
- Do not give oral poliovaccine as patients often fail to clear it, which increases the risk of reversion to wild type, with consequent paralytic disease.
- Genetic counselling of the patient and family once genetic basis confirmed. Identify and counsel carriers.
- Long-term immunological follow-up (plus additional specialist input as required).

5. What is immunology selective IgA deficiency?

• The IgA will be undetectable (< 0.05 g/l), but total IgG and IgM will be normal. IgG subclasses may be reduced (G_2 and G_4). Secreted IgA will be absent (secretory piece deficiency is vanishingly rare), but testing for this is of little clinical value.
• T-cell function is normal (PHA and antigens).
• Autoantibodies may be present (anti-IgA antibodies). There will be an increased IgE in the presence of atopic disease.
• In the absence of IgA, IgM and IgG appear on mucosal surfaces.

6. How do diagnosis of chronic mucocutaneous candidiasis syndromes?

There is no unequivocal diagnostic test.
• *In vivo* and *in vitro* T-cell responses to *Candida* antigens are poor or absent but anti-candida IgG antibodies are high.
• Acquired immunodeficiency and other risk factors for Candida (diabetes, steroid inhalers, Sjögren's syndrome, proton-pump inhibitor use) should be excluded.
• Presence of superficial candidiasis with an endocrinopathy, either overt or cryptic (autoantibody positive without symptoms), is highly suspicious: AIRE gene should be checked.

7. What is treatment of chronic mucocutaneous candidiasis syndromes?

• Treatment is difficult: *Candida* will respond well to antifungals (fluconazole or itraconazole) but inevitably relapses when the antifungal is withdrawn. Resistance to these antifungals may occur. Prolonged therapy is undesirable and increases the chances of hepatotoxicy. Newer antifungals are voriconazole and caspafungin - these should be used sparingly.
• Avoid the use of proton-pump inhibitors as these increase the risk of oesophageal candidiasis.
• IVIg should be considered for patients with recurrent bacterial infections. Continuous antibiotics tend to exacerbate the candidiasis.
• γ-IFN may have some beneficial effect..
• BMT/HSCT should be considered for severe forms but the procedure is difficult in heavily infected patients.
• Maintain regular surveillance for significant endocrine disease, in particular adrenal insufficiency, which may be insidious in its onset. Treat endocrine disease normally.

8. What is treatment of chronic granulomatous disease?

• Long-term antibiotics (co-trimoxazole and itraconazole) are the mainstay of treatment. Use the liquid formulation of itraconazole (better absorption) and monitor trough levels, adjusting dose accordingly.
• Low-dose prophylactic γ-IFN tends to be used instead.
• Acute infections should be treated promptly with intravenous antibiotics, supplemented with high-dose γ-IFN.
• Drainage of large abscesses may be required.
• Inflammatory bowel disease may be significantly helped by high-dose steroids, particularly where there are obstructive lesions due to granulomata. This increases infection risk.
• BMT/HSCT is the treatment of choice and should be carried out early before infective complications become a threat to life. Results from transplantation in adults are now good.
• Kell-negative XL-CGD patients are a transfusion hazard, and need to be transfused with Kell-negative blood.

9. What are immunological features human immunodeficiency virus 1 and 2

• Virus enters the cells via a cognate interaction of the gp120 env with $CD4^+$ and a chemokine receptor, either CxCR4 or CCR5.

• It also infects other $CD4^+$ cells (macrophages, dendritic cells) and other cells expressing CD4-like surface proteins (neuronal cells).
• Macrophage tropic viruses use CCR5, and infect T-cells poorly; T-cell tropic viruses use CXCR4 for entry and form syncytia.
• Resistance to viral infection is associated with polymorphism in the chemokine receptors.
• A viral isolate entering T-cells via $CD8^+$ has been described.
• Uptake of virus into phagocytic cells may be augmented by antibody, and complement. HIV activates complement.
• High levels of viral replication may take place in lymph nodes.
• Initial viraemia after infection is controlled by $CD8^+$ cytotoxic T-cells (increased cell numbers). The asymptomatic phase is characterized by strong cytotoxic responses, but viral replication still detectable intermittently, HIV is not a true latent virus.
• The antibody response to major viral proteins appears after a lag phase of up to 3 months and persists through the asymptomatic phase but declines in late-stage disease.
• Marked B-cell dysfunction with polyclonal increase in immunoglobulins and the appearance of multiple autoantibodies.
• In the seroconversion illness there is a dramatic fall in $CD4^+$ T-cells and rise of $CD8^+$ T-cells. The levels of $CD4^+$ T cells may drop to a level at which opportunist infections may occur at this early stage (poor prognostic indicator). Levels then usually recover to within the low normal range. There is then a slow decline of absolute $CD4^+$ T-cell count over time (years) following infection.
• Passage to the symptomatic phase is characterized by a rapid drop in $CD4^+$ T-cells, loss of cytotoxic activity, and switch of virus type from slow-growing, non-syncytial-forming strains to rapidly growing, syncytial-forming strains (quasi-species evolving through lack of replicative fidelity and under immunological selection pressure). This is accompanied by the occurrence of opportunist infections.
• Activation of T-cells enhances viral replication and hence $CD4^+$ T-cell destruction. Therefore, opportunist infections enhance the self-destruction of the immune system. Long-term non-progressors and patients responding to highly active antiretroviral therapy (HAART) show good proliferative responses to *gag* proteins. Progression has been associated with a switch from Th1 to Th2 responses.
• HIV preferentially infects $CD45RO^+$ cells but the depletion of T-cells affects principally $CD45RA^+CD62L^+$ naive T-cells.
• T-cell depletion is caused by increased apoptosis, impaired production (HIV effects on thymus), and destruction of both infected and uninfected cells.
• HIV replication is suppressed by natural CCR5 chemokine ligands, RANTES, MIP-1α, and MIP-1β, which are secreted by $CD8^+$ T-cells. SDF-1α is the natural ligand for CXCR4. High levels of chemokine production have been associated with resistance to infection.

10. What is Basiliximab (Simulect®)?
• A humanized monoclonal antibody that binds to the α-chain of the IL-2 receptor and prevents T-cell proliferation.
• Used in the prophylaxis of acute organ rejection in renal allografts, in combinations with cyclosporin and co rti co steroids.
• Main side-effect is severe hypersensitivity.
• Dose is 20 mg 2 hours before transplant and 20 mg 4 days after surgery.

Websites
Clinical Immunology Society *www.clinimmsoc.org*
European Society for Immunodeficiencies *www.esid.org*

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