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Primary Immunodeficiency Diseases

Report of a WHO Scientific Group

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PRIMARY IMMUNODEFICIENCY DISEASES

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1 INTRODUCTION

The first barriers to infection are the skin and mucous membranes, and the substances they secrete. When infectious agents penetrate these barriers, other non-specific host factors such as cytokines and complement become involved. These components in conjunction with the specific immune mechanisms of antibodies and lymphocytes constitute the immune system. This complex of interacting factors and cells provides the initial innate non-specific defence and subsequently the acquired specific mechanisms for resistance to infection.

The primary immunodeficiency diseases are the naturally occurring defects of the immune system. As a result of these primary defects, recurrent protozoal, bacterial, fungal and viral infections of varying severity ensue. The immune system can also be adversely affected secondarily by a variety of pathological conditions (including malignancy, metabolic diseases and malnutrition) and drugs; these result in secondary immunodeficiencies.

Both primary and secondary immunodeficiencies result in a similar spectrum of illness — recurrent or persistent infections. Additionally the relationship between immunity and infection is interactive. Infection may cause as well as result from immunodeficiency. Many infectious agents including the human immunodeficiency virus (HIV) have both specific and non-specific effects on the immune system.

The study of patients with primary immunodeficiency diseases has expanded our understanding of immunity. Because of recent progress in immunobiology and genetics, the causes of the Primary Immunodeficiency Diseases can be identified with increasing precision; diagnosis and therapy can be more specific and effective.

2 CELLULAR BASIS OF THE IMMUNE RESPONSE

Like other types of blood cells, the progenitors of T cells, B cells and natural killer (NK) cells are derived from multipotent haematopoietic stem cells (HSC). Cells of the monocyte-macrophage series, Langerhans cells, dendritic cells and other cells that process and present antigen, are important collaborators in the immune responses of T and B cells (see Fig. 1).

Progenitor cells drawn from the circulation into the thymus, interact with thymic stromal cells and their soluble products to undergo cell division and a series of maturation steps. The T lineage cells interact with their microenvironment via cell surface glycoproteins that serve as adhesion molecules and receptors coupled to signal transduction elements. Two pathways of thymocyte differentiation can be identified. Progenitor cells entering the first of these pathways rearrange and express γδ T cell receptor (TCR) genes together with the CD3 complex of proteins ($CD3\gamma\delta\varepsilon\zeta_2$) to become γδ T cells. Precursor cells that fail to achieve a productive VDJγ rearrangement may progress to rearrange VDJβ genes and express the completed β chain together with a surrogate α chain (x) and CD3 proteins. These pre-T cells then rearrange and express α chain genes to become T cells that express the αβ/CD3 receptor complex on their surface. T cells of this sublineage initially express both CD4 and CD8 molecules. Interaction of these molecules with class II or class I molecules on thymic stromal cells are instrumental in determining whether the αβ T cell will survive to become a mature CD4+ T cell or CD8+ T cell. Both positive and negative selection of immature αβ T cells is a consequence of TCR interaction with self antigens presented as peptide fragments within the grooves of class II and/or class I molecules on thymic stromal cells. The γδ T cells do not express CD4 or CD8 molecules during their intrathymic maturation and intrathymic clonal selection may not be required for development of this sublineage. The γδ T cells can be further subdivided into subclasses on the basis of their utilization of either the γ1 or γ2 constant region genes together with separate sets of VDJγ genes. Normal T cell development requires integrity of each of the TCR/CD3 components, CD4, CD8 and their signal transduction partners in addition to key growth factor receptor elements.

Following T-cell migration to peripheral tissues, clones of T cells are selected for growth and further differentiation. On interaction of the T-cell receptor with antigen fragments presented in association with MHC molecules the αβ T cells become activated to produce lymphokines such as IL-2 and express high affinity receptors for this lymphokine. The interaction of IL-2 with its inducible receptor is involved in many effector and regulatory T-cell functions. The role of γδ T cells is presently unknown, but the acquisition of CD8 by γδ T cells in peripheral tissues may enhance interaction with target cells bearing class I (or class I-like) MHC gene products.

The development of B lineage cells is a multifocal process which is concentrated in fetal liver until the bone marrow becomes the major haematopoietic organ. Precursor cells interact with stromal cells to give rise to a rapidly dividing population of pre-B cells that rearrange their VDJ gene segments and produce cytoplasmic μ heavy chains. These pre-B cells lack immunoglobulin receptors on their surface until they express low levels of μ chains together with a surrogate light chain complex encoded by the V pre-B and λ5-like (14.1) genes. Pre-B cells may subsequently undergo a productive light chain gene rearrangement to become IgM-bearing B cells. Immature membrane immunoglobulin bearing (mIg) B lymphocytes, committed with regard to the specificity of the antibodies which they and their plasma cell progeny will subsequently synthesize and secrete, are easily rendered immunologicalally tolerant, or killed by antigen contact. On leaving the bone-marrow B cells acquire mIgD and may respond to contact with a complementary protein antigen and helper CD4 T cells by undergoing plasma cell differentiation.

In the germinal centre, B cells interact with antigen on follicular dendritic cells and with helper T cells to undergo proliferation, and somatic diversification by Ig gene mutation and class switching. Thus germinal centre B cells are selected to give rise to mature plasma cells that make high affinity antibodies of diverse Ig isotypes (IgM, IgG, IgA or IgE) or to become recirculating memory B lymphocytes. Cell interaction molecules in this process include CD40 on B cells and dendritic cells and the CD40 ligand on activated T cells. In turn, CD80 molecules expressed by activated B cells interact with CD28 and CTLA on T cells to enhance the immune response. A variety of

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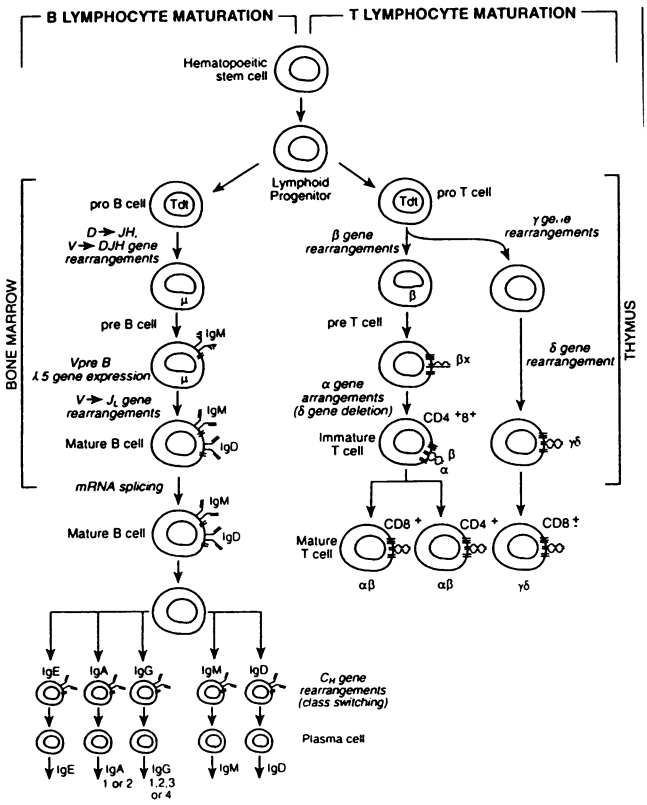

Fig. 1. Development of T and B lymphocytes.

soluble cytokines (IL-2, 4, 6, 10, 14, etc.) and their complementary cell surface receptors are also important elements in specific immune responses, which involve genetically restricted interactions between antigen presenting cells and T-cell subpopulations for cell-mediated immunity (CMI), and between the CD4+ T cells and B cells for antibody response.

T cells can be directed along two major functional tracks that are distinguished by the cytokines they produce. Th1 cells predominantly produce Il-2 and IFN γ whereas Th2 cells produce IL-4, IL-5 and IL-10. Cytokines (see section 4) themselves may direct the track taken by T cells. IFN γ facilitates Th1 differentiation and IL-4 facilitates Th2 differentiation.

The basic elements of the immune system are fully established by week 15 of human gestation. However, the system is immature and requires antigen selection and experience to achieve full maturation during infancy.

3 GENETIC BASIS OF THE IMMUNE RESPONSE

Immunoglobulins, which are tetramers of two heavy and two light chains, serve as antigen receptors on B cells, and the secreted antibodies are the effectors of the humoral immune system. Heavy chains of immunoglobulins are encoded by genes on chromosome l4 at band q32, whereas the genetic locus of kappa light chain genes is on chromosome 2p11 and of lambda on chromosome 22q11 (Fig. 2). The variable domains of immunoglobulins are not encoded by continuous stretches of DNA but rather by discontinuous gene segments that are separated from each other in the germline state (Fig. 2). The heavy-chain gene family consists of several hundred variable-region (V_H) genes that encode the first 95 amino acids of the variable portion of this peptide, more than 20 diversity-region (D) genes that encode a small number of amino acids, six joining-region (J_H) genes that encode the remaining 13 amino acids of the variable region, and nine functional constant-region (C_H) genes. The κ and λ gene families also contain a series of variable-region and joining genes located upstream from the constant-region gene or genes. As the pluripotent stem cell with its immunoglobulin genes in the separated germ-line configuration develops into an immunoglobulin-producing plasma cell, a process of DNA rearrangement occurs. This initiates with a heavy-chain gene that is activated by a rearrangement that combines a single D segment with a single J_H segment, and then a single V_H segment is combined with this DJ juncture. This rearrangement of a V gene segment with a D gene segment brings a promoter controlling sequence, which is present upstream from each V gene segment close to a tissue-specific enhancer sequence that is between the J and C regions. This activates the gene complex, increasing transcription of mRNA for the heavy-chain gene, and leads to the production of cytoplasmic μ chain and, thus, the appearance of the pre-B cell. Surrogate light chains, V pre-B and λ-5 genes are expressed without rearrangement from the earliest stages of B lineage differentiation. The products of these genes become associated with the μ heavy-chain gene product and appear on the surface of pre-B cells. Following effective heavy-chain gene rearrangement, there is a rearrangement of light-chain genes, initiating with rearrangements of the κ immunoglobulin locus with a recombination that juxtaposes one of many V_κ regions with one of the five J_κ segments to generate the complete transcriptionally active V_κ region. If efforts at generating a κ gene are non-productive, activation of λ light-chain genes occurs. Following effective rearrangement of light-chain genes, the mature mRNA is translated, and IgM molecules can be produced and expressed on the cell surface, thus producing the immature B cell.

Although a B cell and its progeny will produce only a single form of light chain, κ or λ, but not both, a B cell is capable of simultaneously producing IgM and IgD membrane forms of immunoglobulin and of switching, subsequently, to the production of other immunoglobulin isotypes. Establishing the order and structure of the heavy-chain constant region genes has helped elucidate the mechanisms by which different classes are produced. The human immunoglobulin heavy-chain constant-region genes located on the long arm of chromosome 14 at band q32 are in the order

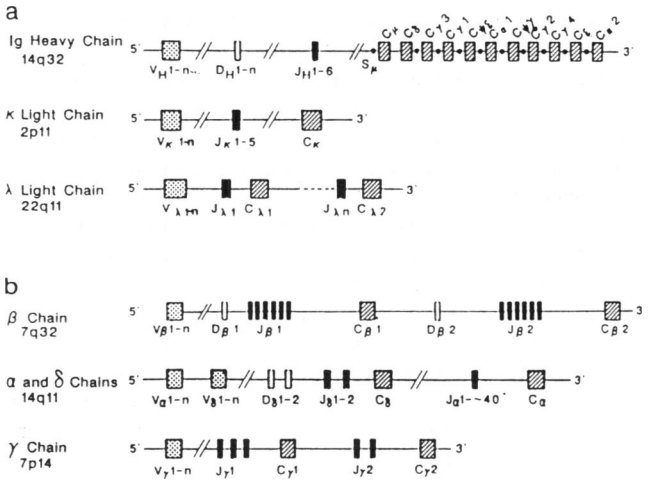

Fig. 2. (a) Genes of immunoglobulin chains and (b) T-cell receptor chains.

shown in Figure 2. The simultaneous production of IgM and IgD membrane forms, as well as the transition from membrane-bound receptors to a secreted form, involves alternative mRNA splicing. In contrast, the transition from a $C_{\mu\delta}$-expressing B cell to one expressing another isotype occurs by a phenomenon known as heavy-chain class switching. Such isotypical switch is accomplished by the splicing of an area termed a switch region upstream from the μ heavy-chain gene, with the switch region 5' to the downstream heavy-chain gene to be expressed. Such recombination would result in a DNA rearrangement that is accompanied by deletion of the DNA between the switch region 5' from the C_μ gene and the switch region immediately 5' from the constant region to be used. This process of switching allows a new constant region to be transcribed with the preexisting $V_H/D/J_H$ recombined gene. In addition, both membrane and secreted forms of the immunoglobulins may be produced by the same cell at different stages of differentiation. At a molecular level, the transition from the membrane to the secreted form involves alternative splicing of mRNA resulting in different mRNAs containing the secreted (Cμs) or membrane (Cμm) carboxy-terminal tail. Terminal differentiation of a B lymphocyte to a plasma cell forecloses these options so that a single plasma cell synthesizes and secretes an immunoglobulin of a single isotype and specificity (i.e. allelic exclusion). The mature B-cell antigen complex is composed of an antigen-binding membrane immunoglobulin and associated proteins serving transducer/transporter functions. The transducer/transporter substructure is composed of disulfide-linked dimers of immunoglobulin Igα and Igβ subunits that are products of the mb-1α and B29β genes. Thus, the receptor complex involves a minimum of eight chains: two Ig heavy chains, two Ig light chains, and two Ig α–β dimers.

The T-cell receptors for antigen are also heterodimers composed of either α and β or γ and δ subunits. The T-cell antigen receptor is associated with a cell surface complex of different nonpolymorphic chains (CD3γ, CD3δ, CD3ε, CD3ζ). The arrangement of T-cell receptor genes is similar to that of Ig genes (Fig. 2). The T-cell receptor TCRβ chain locus is on chromosome 7q32-34, and the TCRγ chain on chromosome 7p14-15. The TCRα and δ genes are on chromosome 14q11. The TCRβ chain gene is comprised of discontinuous germ-line variable region genes (Vβ) and duplicate sets of diversity (Dβ1, Dβ2), joining (Jβ1, Jβ2) and constant (Cβ1, Cβ2) gene segments. The TCRα gene consists of multiple variable (Vα) genes arranged in families, at least 40 joining (Jα) genes in a tandem array and a single 5' constant Cα gene. The TCRδ gene system composed of Vδ, Dδ, Jδ and Cδ segments is nested within the TCRα locus between the TCRα variable and TCRα joining region genes. The fourth gene family, the TCRγ family encoded by genes on the short arm of chromosome 7 (7p15), has many properties in common with other TCR genes, including assembly from diverse variable, joining and constant regions and rearrangement in T cells.

As with Ig genes, there appears to be a hierarchy in the rearrangement and expression of T-cell receptor genes. Rearrangement of the TCRγ and δ genes occurs first. If the rearrangements are effective, the γδ-T-cell receptor subunits together with the CD3 complex of proteins are expressed on the cell surface of Tγδ cells. Precursor cells that fail to make a productive TCRγδ gene rearrangement may initiate rearrangements of the TCRβ genes. Finally, TCRα genes are rearranged and expressed, permitting the production and cell-surface expression of the α/β heterodimer. The T-cell receptor heterodimers become associated with the CD3 complex and the whole unit is then expressed on the surface of T cells.

4 CYTOKINES

Immune responses as well as the effector phase of immune reactions are regulated by soluble mediators called interleukins or cytokines. Many cytokines and their receptors have been characterized in molecular form. Characteristic features of cytokines are their functional pleiotropy and redundancy, i.e. one cytokine shows multiple functions on a wide variety of tissues and cells and many different cytokines exert similar effects in the same cells. Cytokine producers are also multiple, i.e. many cytokines are produced by several different cells, and the production of cytokines is influenced by other cytokines, thus forming a 'cytokine network'. Major producers of cytokines in the immune system are monocytes and T cells.

Many cytokine receptors belong to the 'cytokine receptor family'. They have four conserved cysteine residues in their N-terminal region and 'Try-Ser-X-Try-Ser' motif external to the plasma membrane. These conserved residues are essential for maintaining the tertiary structure of the receptor molecules. Cytokine receptors do not have any unique sequences for signal transduction, such as tyrosine kinase, in their intracytoplasmic domain. Several cytokine receptors, such as IL-6R, IL-5R, GM-CSFR have very short intracytoplasmic domains, suggesting the presence of other chains for signal transduction. The IL-6 receptor system was shown to be composed of two polypeptide chains, an 80 kd IL-6R and a 130 kd signal transducer (gp130). Binding of IL-6 to IL-6R triggers an association with the 130 kd sub-unit which transduces the signal. gp130 has been shown to function as a signal transducer not only for IL-6 but also for LIF, Oncostatin M, IL-11 and ciliary neurotropic factor (CNTF). Thus, this cytokine receptor system consists of two polypeptide chains, a ligand specific receptor and a common signal transducer. Recently, this concept is shown to be applied to most other cytokine receptor systems. In the haemopoietic system, the receptors for IL-3, IL-5 and GM-CSF utilize a common β chain (βc) as a signal transducer. In the lymphoid system, a common γ chain (γc) is a shared element of the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15. This is a reason why mutation in the gene encoding IL-2Rγ chain results in X-linked SCID in humans, whereas a disruption of the IL-2 gene in mice did not lead to a major effect on the development of T and B lymphocytes.

At present,15 (IL-1 to IL-15) interleukins have been cloned and their biological activities in immune regulation are under intense scrutiny. Assays are available for estimation of cytokine levels.

4.1 IL-1

IL-1 is one of the typical examples of multifunctional cytokines. It is produced mainly by monocytes. IL-1α and IL-1β, which show only 24% (human) homology in their amino acid sequences, utilize the same IL-1 receptor, which belongs to the Ig-superfamily. A naturally occurring IL-1 inhibitor (IL-1ra) also shows a certain sequence homology with IL-1α and β and binds to the IL-1 receptor, but it cannot activate the signal pathway. Therefore, IL-1ra functions as a competitive inhibitor of IL-1. IL-1 is important for the early activation of T cells as a co-stimulatory factor. IL-1 is a strong inducer of IL-6 and several activities of IL-1 in immune regulation can be exerted through IL-6. IL-1 is one of the typical inflammatory cytokines and is involved in the generation of prostaglandins. Fever is generated by IL-1 action in the brain.

4.2 IL-2

IL-2 is the major T cell growth factor. Activated T cells produce IL-2 and express high affinity IL-2 receptors and T cells proliferate in

an autocrine or paracrine fashion. The high affinity IL-2 receptor is formed by three polypeptide receptor components, IL-2Rα (Tac, CD25), IL-2Rβ, and IL-2Rγ and signals can be transduced through IL-2Rβ and γ chains. Activated B cells can express IL-2R and IL-2 induces growth and antibody production in such activated B cells. Resting NK cells express IL-2Rβ and γ.

4.3 IL-3

IL-3 is a multi-colony stimulatory factor (multi-CSF) and is involved in proliferation of early progenitors of haematopoietic cells. IL-3 exerts a synergistic effect with IL-6 on the expansion of early haematopoietic progenitors. IL-3 is produced by activated T cells. The IL-3 receptor is comprised of two polypeptide chains like the IL-6 receptor. A signal transducer of the IL-3 receptor (βc) is common to IL-5R and GM-CSFR.

4.4 IL-4

IL-4 was originally identified as B cell stimulatory factor (BSF-1) and was involved in the early activation of resting B cells together with antigen. IL-4 induces isotype switching of B cells into IgE producing cells. Anti-IL-4 inhibits IgE production in parasite-infested mice, indicating an essential role for IL-4 in IgE production. IL-4 is shown to be a potent growth factor for mast cells and to induce FcεRII (CD23) on B cells and monocytes. These results strongly suggest the involvement of IL-4 in immediate-type hypersensitivity. IL-4 is produced not only by activated T cells but also by mast cells and basophils. IL-4 functions as a growth factor for T cells and is involved in autocrine and paracrine growth of activated T cells. The IL-4 receptor utilizes the γ chain of IL-2R (γc) as a signal transducer. IL-4 knock-out mice do not produce any IgE, confirming an essential role of IL-4 in isotype switching to IgE.

4.5 IL-5

IL-5 may enhance B cell differentiation and also acts as an eosinophil differentiation factor. The IL-5 is mainly produced by activated T cells. The IL-5 receptor consists of two polypeptide chains like the IL-6R, one of which is a signal transducer of IL-5R that is common to GM-CSFR and IL-3R.

4.6 IL-6

IL-6 is one of the most typical examples of a multifunctional cytokine. It was originally identified as a B-cell differentiation factor and is involved in the final maturation of B cells into antibody producing cells. IL-6 is one of the essential factors in antibody production. It also acts on T cells and haematopoietic progenitors for their activation. IL-6 induces maturation of megakaryocytes and functions as a thrombopoietin. IL-6 is a major inducer of the acute phase reaction in inflammation. Excessive production of IL-6 has been shown in several autoimmune diseases. IL-6 is a potent growth factor for myeloma and plasmacytoma cells and appears to be involved in multiple myelomas and plasmacytomas. IL-6 is produced by a wide variety of cells, but mainly by monocytes. IL-1 and TNFα are strong inducers of IL-6 in monocytes. Anti-viral antibody response was 5–10-fold reduced in IL-6 knock-out mice. Furthermore, IL-6$^{-/-}$ mice were defective in their mucosal IgA response. The inflammatory acute phase reaction is severely compromised in IL-6$^{-/-}$ mice. The data show that optimal responses to trauma and infection can only be mediated in the presence of IL-6.

4.7 IL-7

IL-7 is a major B cell lymphopoietin and is involved in the growth and differentiation of pro- and pre-B cells. It also acts as a growth factor for thymocytes and mature CD4+ and CD8+ cells. IL-7 is produced by stromal cells in the marrow, thymus and spleen. IL-7R utilizes γc in signal transduction. Lymphoid development is severely impaired in IL-7R$^{-/-}$ mice.

4.8 IL-8

IL-8 is an inflammatory cytokine produced by monocytes and involved in neutrophil chemotaxis. Several other cytokines, such as Platelet Basic Protein (PBP), Platelet Factor 4 (PF4), γ-Interferon Inducible Protein (IP10) and Growth Related Gene (Gro), show sequence homology with IL-8. These proteins may belong to a large supergene family derived from a single ancestor gene. Pre B-cell stimulatory factor (PBSF) which shows a synergy with IL-7 for B-cell development belongs to this family.

4.9 IL-9

IL-9 is identified as a T-cell growth factor distinct from IL-2 or IL-4. It is produced by CD4+ helper T cells and acts on helper T cells but not on CD8+ cytotoxic T cells. IL-9 was shown to act on mast cells stimulating their growth in a manner similar to IL-4. IL-9R utilizes γc in signal transduction.

4.10 IL-10

IL-10 was originally called CSIF (Cytokine Synthesis Inhibitory Factor), which is produced by monocytes and Th2 cells. It inhibits the production of cytokines by Th1 cells. As with other cytokines, IL-10 also exerts pleiotropic functions and induces growth of T cells and mast cells. IL-10 is produced not only by Th2 cells but also by B lymphoma cells, macrophages and mast cells.

4.11 IL-11

IL-11 is identified as a plasmacytoma growth factor and has the pleiotropic functions of IL-6 and its receptor shares the gp130 of the IL-6R.

4.12 IL-12

IL-12 is a heterodimer of glycoproteins, p35 and p40, which acts on B cells, NK cells and monocytes to induce proliferation and cytokine synthesis, especially of interferon-γ. Its receptor shows strong homology with gp130.

4.13 IL-13

IL-13 is produced by Th2 and mimics the effects of IL-4 on IgE production.

4.14 IL-14

IL-14, formerly called high molecular weight B-cell growth factor, enhances growth and differentiation of B cells.

4.15 IL-15

IL-15 acts on activated T cells, B cells, and on NK cells to induce proliferation and differentiation. Its receptor includes the β and γ chains of the IL-2R.

4.16 Other cytokines

In addition to the interleukins and their receptors, other cytokines and monokines affect the immune system. Interferon-γ, secreted by activated T cells, is the most important cytokine in the induction of MHC class II molecule expression. TNF-α is a prominent monokine that shares many functions of IL-1, except for the induction of IL-2.

The colony stimulating factors, in addition to IL-3, such as GM-CSF, G-CSF and M-CSF act as growth factors for immunologically relevant cells. Immunity to infection with *Listeria monocytogenes* is severely compromised in $TNFR^{-/-}$ as well as interferon-γ-/- and $IL\text{-}6^{-/-}$ mice, indicating an important role of these cytokines in innate immunity.

5 ANTIGEN PRESENTATION, CELL ADHESION AND SIGNAL TRANSDUCTION

Antigens are taken into antigen presenting cells (APC) by receptor mediated endocytosis (via C3 or Ig receptors) or by fluid phase endocytosis. Protein antigens, once in the phagolysosome, are digested by proteolytic enzymes. Antigenic fragments are then shunted to a specialized compartment for peptide loading. In this compartment, antigenic peptides bind to class II histocompatibility molecules and the complex thus formed is transported to the cell surface of the APC. For the most part, cells of monocyte-macrophage lineage and mature B cells serve as APC.

For antigens within the cell including many viral antigens, there is degradation of the proteins in part by proteosomes followed by transport of the resultant peptides into the endoplasmic reticulum where they bind to class I MHC. This transport across the Golgi membrane is mediated by a member of the ABC superfamily of transporters (e.g. TAP-1 or TAP-2), which are encoded by genes within the MHC.

The interaction of APC with T cells as well as T–B-cell collaboration are facilitated by adhesion molecules. The ligands and counter-ligands of many of these adhesion molecules have now been well defined (see Fig. 3). ICAM-1 and LFA-1 are reciprocally interacting; both molecules are found on T cells as well as on APC. LFA-1 is defective in leukocyte adhesion deficiency (see 12.2) and the CD40 ligand is defective in the hyper IgM Syndrome (see 9.3.2).

The interaction of the T-cell receptor (TCR)-CD3 complex with presented antigen results in signal transduction that leads to phosphorylation of CD3 and the activation of T cells (see Fig. 4). Upon TCR cross linking, the src type protein tyrosine kinases (PTK), $p56^{lck}$ and $p59^{fyn}$, respectively associated via their N terminal domains with CD4 and CD3ζ, phosphorylate CD3ζ in tyrosine residues of the Antigen Recognition Activation Motif (ARAM),Y-X-X-L-X(7-8)-Y-X-X-L/I. Three ARAMs are present in CD3ζ. This activation of lck and fyn, may be mediated by the PTK, syk, which is associated with the TCR CD3 complex on resting cells. Activation of lck and fyn also critically depends on the transmembrane phosphatase CD45, which dephosphorylates the carboxy-terminal autoinhibitory tyrosine residue of these src kinases. The phosphorylation of CD3ζ initiates an activation cascade by enabling the TCR to recruit downstream molecules. Thus, phosphorylated CD3ζ is now able to bind the PTK,ZAP-70. Receptor-bound ZAP-70 can phosphorylate substrates such as phospholipase C-γ (PLC-γ) and MAP-2 kinase and recruit them to the receptor complex.

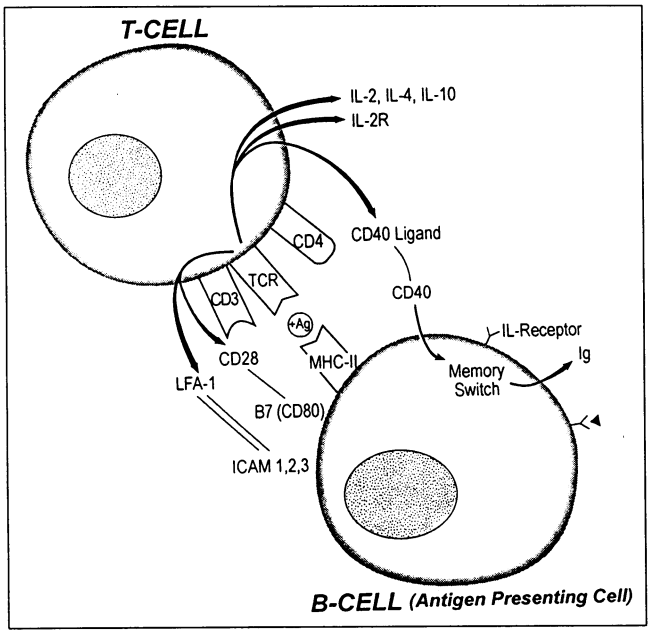

Fig. 3. Adhesion molecules and their ligands.

Phosphorylated PLC-γ recruited to the membrane breaks down membrane inositol phosphatides, mainly PI4,5-P2, to generate the second messengers diacylglycerol (DAG) and inositol triphosphate (IP3), which respectively activate protein kinase C (PKC) and mobilize Ca^{++} from intracellular stores. Released Ca^{++} plays a critical role in the activation of downstream enzymes, which include the Ca^{++}/calmodulin dependent phosphatase, calcineurin. Activated calcineurin dephosphorylates nuclear factor of activated T cells (NFAT) allowing its translocation to the nucleus.

Another activation pathway recruited by aggregation of the TCR is the ras pathway. Phosphorylated CD3ζ has been reported to bind to the SH2 domain-containing adaptor proteins. Shc protein activates p21 *ras* by means of the intermediate adaptor protein Grb-2, consisting of two SH3 and one SH2 domains, and the guanine nucleotide releasing protein SOS, which is capable of exchanging GDP with GTP leading to the conversion of p21 *ras* to an activated GTP bound state. This in turn activates the raf, MEK, MAP kinase pathway, which culminates in nuclear fos/jun expression. The other CD3 components, each of which contains one ARAM motif, may also serve as substrates for $p56^{lck}$ and $p59^{fyn}$ and, as demonstrated for CD3ε, may bind ZAP-70 and thus may function as additional independent signalling units.

In addition to the tyrosine phosphorylation cascade, the lipid kinases, PI3 kinase and PI4 kinase, are recruited to the activated TCR. PI3 kinase is recruited by association of its p85 noncatalytic subunit with the SH3 domain of lck, fyn or ZAP-70. Activated PI3 kinase phosphorylates the D-3 position of the inositol ring of phosphatidylinositol leading to the generation of PI3,4-P2 and PI3,4,5-P3.

Activation of PKC, calcineurin and *ras* result in the activation and expression of a number of transcription factors that include NFκB, AP-1 (*fos/jun*) and NFAT that are critical for the transcription of *IL-2* and *CD40L*. The expression of these genes leads to T-cell activation and proliferation.

Optimal *IL-2* gene expression requires, in addition to TCR cross-linking, engagement of the costimulatory molecule CD28 by its counterreceptor B7 (CD80) on APCs. CD28 contains a YMXM motif, which is a potential target for phosphorylation by PTK that has been activated following TCR cross-linking. The phosphorylated motif recruits PI-3 kinase via the SH2 domain of its non-catalytic subunit. PI-3 kinase activated via CD28 synergizes with enzymes activated via the TCR to enhance IL-2 production.

The B-cell antigen receptor signals in ways similar to the TCR. Surface Ig is associated with Igα and Igβ subunits each of which has an ARAM motif. The src kinases lyn, blk and the ZAP-70 homologue syk associate with the sIg receptor similarly to their association with the TCR. The B-cell specific molecule CD19, which asso-

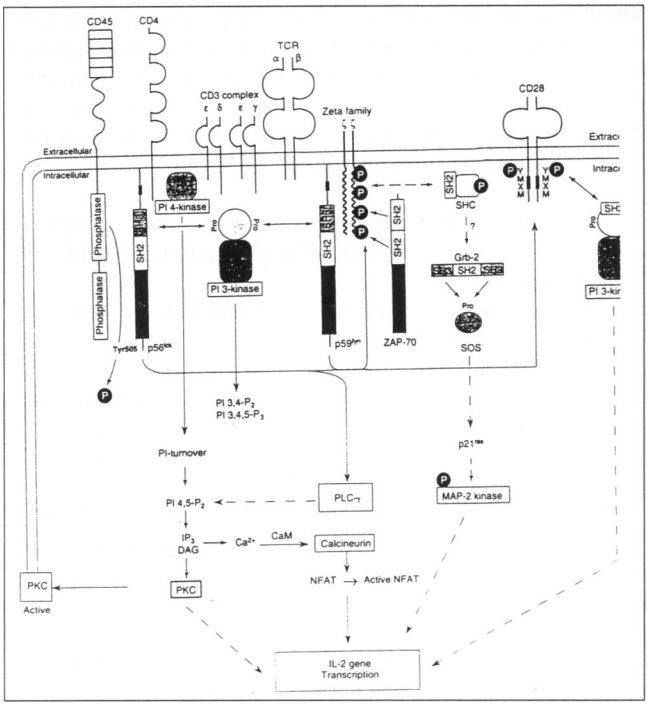

Fig. 4. Signal transduction pathway in T cells.

ciates with the Ig receptor, contains, in its cytoplasmic tail, the YXXM motif. This mediates recruitment of PI-3 kinase.

The role of various enzymes and pathways is illustrated by several ID diseases, and by knock-out mice. Disruption of *lck* leads to abnormal thymic maturation. Disruption of *fyn* affects only the function of peripheral T cells. ZAP-70 deficiency in humans results in CD8 deficiency and in deficient CD4+ T-cell function. CD3γ and ε chain deficiency results in severely impaired T-cell receptor expression and variable impairment in T-cell function. NFAT abnormality leads to deficiency in the production of IL-2 and other cytokines. IL2Rγ chain deficiency causes X-linked SCID.

6 TESTS FOR ASSESSING IMMUNITY

6.1 Evaluation of patients with immunodeficiencies

Whenever infections occur, which are persistent or recurrent and attributable to unusual or opportunistic organisms, primary or secondary ID must be considered. This is particularly the case if family members of the same or prior generations have died in infancy or have similar susceptibility to infections. When such patients are encountered, studies should be carried out that permit identification and preliminary definition of ID diseases.

Screening for ID disease requires analyses of the ability to develop and express B-cell, T-cell or combined cellular immunological functions, the biological amplification processes, e.g. complement system, lymphokines and other cytokines, and the basic effector mechanisms, including phagocytosis and the integrity of inflammation. The screening evaluation should begin with enumeration of the crucial cell populations, T cells, B cells, granulocytes, monocytes. Immunoglobulins, including IgM, IgG, IgA and IgE, can be quantified. Total haemolytic complement and individual complement components of both the classical and alternative activation pathways can be measured immunochemically and functionally. Responsive immunological functions can include quantitation of antibody responses to ubiquitous antigens as well as to specific immunizations with well tolerated antigens such as commercially available vaccines, e.g. tetanus and diphtheria toxoids, killed polio antigens, haemophilus conjugates etc. Polysaccharide vaccines are also quite useful for evaluating antibody responses, but in general, should not be given to children under 2 years. Evaluation of T-cell mediated immunity can be accomplished by skin testing for DTH with a battery of antigens, which in the aggregate yield cell mediated immune responses in a high proportion of healthy individuals. This may be a problem in young infants. In addition T-cell immunity can also be assessed by evaluation of *in vitro* responses of peripheral blood lymphocytes to phytomitogens and common antigens. Phagocytic functions can be assessed by evaluating nitro blue tetrazolium dye reduction after exposure of blood cells to a phagocytic stimulus or alternatively by evaluating killing of micro-organisms or generation of oxygen radicals by using chemiluminescence. To assess the integrity of the inflammatory response, testing by Rebuck skin window techniques has been of value. *In vitro* analysis of the integrity of inflammatory function may also employ measurements of chemotaxis, chemokinesis, and the capacity to produce and release selected inflammatory cytokines.

6.2 Immunoglobulins and antibodies

6.2.1 Measurement of immunoglobulin concentration. Serum immunoglobulins are commonly measured by single radial diffusion, or automated immunoturbidometric methods. Other techniques such as immunoelectrodiffusion, radioimmunoassay, and ELISA are also available but less often used. Electrophoresis and immunoelectrophoresis are not satisfactory techniques for the quantitation of immunoglobulins. Immunoelectrophoresis and immunofixation are useful in the detection of M-components. Immunoglobulins can also be measured in body secretions, e.g. saliva, tears and milk. Monomers of IgM are present in serum of some ID patients such as CVID and hyper IgM and may give spuriously high IgM levels. The subclasses of IgG can be measured by simple radial diffusion or ELISA methods. However, standards and normal values are not yet well enough established for recommendation. IgG subclass determination is of limited value in assessing patients with clinical immunodeficiency, since functional antibody deficiency may be present despite normal IgG subclass levels, and conversely deficient levels of a single subclass of IgG may be found in individuals who have effective antibody production and are clinically normal. Methods for IgA subclass determinations are not yet readily available and their measurement is not yet of value. Serum immunoglobulin concentrations vary with age and environment. Thus appropriate local age-related norms must be used.

Concentrations of immunoglobulins cannot be used as the sole criteria for the diagnosis of primary ID. Diminished immunoglobulin levels may be due to loss as well as decreased synthesis. An indirect indication of loss may be obtained by measuring serum albumin, which is usually lost concomitantly (e.g. through the gastrointestinal tract). Limited heterogeneity of immunoglobulins and abnormal kappa-lambda light chain ratios have been observed in ID syndromes.

6.2.2 Assessment of antibody formation following immunization. Antibody mediated immunity (humoral immunity) may be assessed by antibody responses to antigens to which the population is commonly exposed, or following active immunization. Protein or polysaccharide antigen may be used; the latter are particularly relevant in patients with sinopulmonary infections. Live vaccines (BCG and vaccines for poliomyelitis, measles, rubella, mumps) should never

be given when primary ID is present or suspected. BCG, or any live vector for immunization are strongly contraindicated in any person in whom primary or secondary ID is present or suspected.

The following tests are recommended:

1.'Natural' antibodies: A and B isohaemagglutinins, heteroagglutinins and heterolysins (e.g. against sheep or rabbit red cells), antistreptolysin and bactericidal antibodies against *Escherichia coli.*

2. Antibody response to usual immunization.

(a) In unimmunized children, commercial diphtheria/tetanus (DT) vaccine may be given in recommended doses. Blood is taken 2 weeks after immunization and tetanus and/or diphtheria antibodies determined. A Schick test may be performed. Three doses of killed poliomyelitis vaccine (1.0 ml intramuscularly, at intervals of 2 weeks) can also be used; blood is taken 2 weeks after the last injection and antibody determined, usually by virus neutralization.

(b) In patients who have been immunized with diphtheria/tetanus (DT) or diphtheria/pertussis/tetanus (DPT) vaccine, one booster injection is given, followed by determination of antibodies and/or a Schick test.

3.Additional active immunizations that may be recommended:

(a) Bacteriophage ϕX 174, a bacterial virus which is not infective for humans, has been shown to be a potent, safe and useful antigen; it allows measurement of antigen clearance and primary and secondary immune responses[a].

(b) To measure antibody responses purely to carbohydrate antigens[b], pneumococcal or meningococcal polysaccharides, or Hemophilus b polysaccharide free of carrier proteins, should be used as well as typhoid-Vi antigen. Blood is drawn after 2 weeks and antibody is determined. These and other pure polysaccharide antigens are not useful (and may be contraindicated) in infants under 2 years of age, particularly when ID is suspected unless they are conjugated to proteins. Interpretation of results in children under 5 years old is difficult.

(c) Other useful antigens to measure primary response include:
- (i) Tick borne encephalitis (killed) vaccine[c]
- (ii) Hepatitis B vaccine[d]

*6.2.3 B lymphocytes.*B lymphocytes are counted by detection of membrane-bound immunoglobulin or by monoclonal antibodies to B-cell antigens (CDl9 and CD20) using immunofluorescence. Monocytes can be counted and distinguished from B lymphocytes by peroxidase or esterase staining or ingestion of IgG coated latex particles or by monoclonal antibodies specific for monocytes such as to CD14.

Pre-B cells may be identified among bone marrow cells with purified fluorochrome labelled antibodies to detect cytoplasmic μ heavy chains in cells without demonstrable surface immunoglobulins and cytoplasmic light chains.

6.3 Cell-mediated immunity (CMI)

A number of tests are commonly employed for assessing CMI, including: delayed-type skin reactions; enumeration of T cells and T-cell subsets; *in vitro* stimulation of lymphocytes by mitogens, antigens or allogenic cells; other *in vitro* tests of T-cell function.

6.3.1 Skin testing. Delayed cutaneous hypersensitivity (DCH) is a localized immunological skin response: the prototype is the tuberculin skin test. Because DCH is dependent on functional thymus-derived lymphocytes (T lymphocytes) it is used in screening for T-cell mediated immunodeficiency. Antigens generally used are: mumps, trichophyton, purified protein derivative (PPD), candida or monilia, tetanus and diphtheria toxoid. To ascertain defective CMI several antigens must be used. All skin tests are done by intradermal injection of 0.1 ml of antigen and should be read in 48–72 hours for the maximal diameter of induration, which indicates intact cell mediated immunity. Erythema is not an indication of DCH. A negative test is not informative in young infants.

1. Tuberculin: 0.1 ml containing 2 to 10 international units (IU) of Tween stabilized soluble PPD. If negative, the test should be repeated using 50 IU.
2. Candida or monilia[e]: Initially test at 1:100 dilution. If no reaction, test at 1:10 dilution.
3. Trichophyton[f]: use at 1:30 dilution.
4. Mumps[g]: use undiluted; read at 6–8 hours for early Arthus reaction (antibody mediated) and then at 48 hours for DCH.
5. Tetanus and diphtheria fluid toxoids[h]:use at 1:100 dilution.

We do not recommend the use of dinitrochlorobenzene (DNCB) for skin testing because it is both mutagenic and causes necrosis. We also do not recommend the use of any multitest system for assessing CMI.

6.3.2 T lymphocytes. Because of the reliance on the phenotypic designation of T-cell subsets in evaluating patients with ID, it is essential to understand the normal differentiation and functions of these cells (see Section 2).

T cells can be enumerated by immunofluorescence with the use of monoclonal antibodies to CD3. Monoclonals to CD3 enumerate NK as well as T cells. Flow cytometry techniques are more reliable, reproducible and sensitive than visual microscopic enumeration. Similarly CD4 and CD8 monoclonal antibodies recognize important subsets of T cells.

CD4 cells recognize antigen in association with the class II MHC (HLA-D) molecules, and CD8 cells recognize antigens in association with class I MHC (HLA-A, HLA-B, and HLA-C) molecules. Antigen specific T-cell responses are MHC restricted. Abnormalities in the number of CD4 or CD8 cells can be associated with abnormalities in cognitive as well as regulatory functions of T cells and may lead to immuno-incompetence or auto-immunity.

In suspected cases of hyper IgM immunodeficiency, T cells activated by PMA and ionomycin should be analysed for expression of the CD40 ligand. Monoclonal antibodies against CDl6, CD56 and CD57 even though they are not lineage-specific, may be useful for the detection and enumeration of natural killer (NK) cells.

6.3.3 In vitro stimulation of lymphocytes. Lymphocytes can be activated *in vitro* by (a) mitogens such as phytohaemagglutinin (PHA) pokeweed mitogen (PWM) or concanavalin A (Con A); (b) antigens such as PPD, candidin, streptokinase, tetanus and diphtheria, if the patient has had prior encounter with the antigen, or with superantigens such as toxic shock syndrome toxin (TSST1); (c) allogeneic cells; and (d) antibodies to T-cell surface molecules involved in signal transduction such as to CD3, CD2, CD28 and CD43.

T-lymphocyte activation can be assessed directly by (1) measuring blastogenesis and/or proliferation of cells; (2) expression of activation antigens; and (3) release of mediators. The blastogenic response is assayed after 3 to 7 days by ^{3}H- or ^{14}C-labelled thymidine incorporation for 16–24 hours, followed by DNA extraction techniques or cell precipitation on filter paper and subsequent liquid scintillation counting. Control values of unstimulated cultures vary from person to person and from day to day. Data on unstimulated and

stimulated cultures should always be given. Soluble PHA or Con A require the presence of monocytes for stimulation of T cells; under certain conditions, however, such as when bound to particulate matter, they may also stimulate B cells. PWM stimulates a response in both T and B cells, although T cells must be present for the B cells to be stimulated. The mixed lymphocyte reaction (MLC) results from T-cell reactivity to MHC antigens displayed on B cells and monocytes. It should be noted that, when normal irradiated or mitomycin C-treated lymphocytes are the stimulators of an MLC, the normal T cells in the culture may secrete factors which induce blastogenesis in the patient's lymphocytes. Therefore it is preferable to use B-cell lines or T-cell depleted normal cells as the stimulators.

Activated T cells express IL-2Rα (CD25), transferrin receptors (CD71) and MHC class II molecules not present or present in low numbers on resting T cells. T cell populations to be assessed for their capacity to express these receptors are stimulated with a soluble lectin such as PHA and examined 3 days following stimulation by direct or indirect immunofluorescence using monoclonal antibodies to the interleukin-2 (CD25) or transferrin (CD71) receptors or MHC class II molecules. For indirect immunofluorescence, an irrelevant mouse monoclonal and a fluorochrome labelled anti-mouse immunoglobulin are used as a control for potential Fc binding of mouse monoclonals.

Activated T cells and monocytes synthesize and secrete interleukins-2,4,5 and 6, interferon-γ and other cytokines. The supernatants of peripheral blood mononuclear cells stimulated by soluble PHA can be assessed for IL-2 by an ELISA technique or by determining their capacity to stimulate ^{3}H-thymidine uptake by mouse IL-2 dependent cultured T-cell lines (e.g. CTLL2). The bioassay should be confirmed with blocking antibodies to IL-2. Specific *in vitro* systems exist to assay the other cytokines. Not all assays are reliable.

7 OTHER TESTS

Examination of blood is always useful, and biopsies of bone marrow, rectum and intestine, skin and lymphoid tissue may also be recommended for the diagnosis or classification of ID. In addition, postmortem examination may permit diagnosis of familial defects, important for genetic counselling and for understanding the pathogenesis of ID.

7.1 Blood

A total lymphocyte count is essential in the diagnosis of primary ID. Most patients with severe combined ID (SCID) and thymic hypoplasia have persistently low total lymphocyte counts (less than 1 x 10^9/l or 1000/mm^3). Lymphopenia can also be secondary to viral infections, malnutrition, cell loss, autoimmune diseases and myelophthisis as in hematopoietic malignancy. Normal lymphocyte counts do not exclude the diagnosis of SCID. Lymphocyte counts are variable in other forms of ID. Patients with reticular dysgenesis have pancytopenia. Thrombocytopenia and small platelets in a male infant suggest the Wiskott–Aldrich syndrome. Some patients with ID are anaemic; this may include Coombs-positive haemolytic anaemia.

7.2 Bone marrow

Bone marrow aspiration or biopsy is important for exclusion of other diseases, for identification of plasma cells and pre-B cells and for diagnosis of obscure infections.

7.3 Lymph nodes

Lymph node biopsy is not necessary for the diagnosis of ID, but may help in classification. For standardization, lymph node biopsies should be done 5–7 days after local antigenic stimulation by diphtheria or tetanus toxoids. Lymph node biopsies are potentially hazardous in SCID; they heal poorly and may produce a portal of entry for infection. Rapidly enlarging lymph nodes should be biopsied; infection, malignancy or follicular hyperplasia may be the cause.

7.4 Rectal and intestinal biopsy

Examination of rectal tissue for plasma cells and lymphoid cells by histological and immunohistological methods is useful in patients with common variable ID and selective IgA deficiency. Lymphoid cells are found in rectal and intestinal biopsies in normal infants aged more than 15–20 days of age. Jejunal biopsy may show villous atrophy and may demonstrate *Giardia lamblia* and cryptosporidial infections.

7.5 Skin biopsy

Biopsy of skin is useful to establish a diagnosis of graft-versus-host (GVH) reaction in patients with ID after blood transfusion, bone marrow and fetal tissue transplantation or from maternal/fetal transfer of lymphocytes *in utero*.

7.6 Thymus

Thymic biopsy should be performed only by skilled surgeons. It should be performed only when thymoma is suspected.

7.7 Chimerism

Chimerism (the occurrence in one individual of two genetically different cell lines) when observed in ID can be congenital or acquired. The former is due to intrauterine implantation of maternal cells; the latter can occur after blood transfusion, bone marrow transplantation or fetal tissue implants. The presence and origin of lymphoid chimeric cells can be ascertained by karyotype, human leukocyte (HLA) or other antigenic typing, and restriction fragment length polymorphism.

7.8 Special studies

Adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) levels should be determined in all patients with possible SCID and T cell deficiency. Serum alpha fetoprotein levels may be of value in separating patients with ataxia–telangiectasia (AT) from those with other neurological disorders; it is increased (40–2,000 μg/l) in at least 95% of persons with AT. In patients with SCID, blood mononuclear cells should be examined for the presence of class II histocompatibility molecules to rule out the diagnosis of MHC class II deficiency. Chromosomal studies are useful in AT and in the DiGeorge anomaly.

7.9 Studies for infectious agents

The diagnosis of infection in ID is complex and beyond the scope of this report. In patients with ID, diagnosis of viral infection by antibody determinations are of little or no value. Direct viral isolation and/or identification of the viral genome (e.g. by PCR) are necessary to prove infection. In the presence of CNS symptoms, CSF cultures are important and brain biopsy may be required. HIV can be detected in peripheral blood lymphocytes and plasma by PCR analysis. Lung biopsy may be useful for the diagnosis of *Pneumocystis carinii* and other pulmonary pathogens.

8 GENETICS CARRIER DETECTION AND PRENATAL DIAGNOSIS

Inheritance patterns are known for most of the primary immunodeficiency diseases and are given in Tables 1, 2, 3, 4 and 5. The known chromosomal map locations of several immunodeficiencies are given in Table 6. Table 4 contains the presently known chromosomal map locations of the complement genes. These recent advances in the precise mapping of the various immunodeficiency diseases and the availability of restriction fragment length polymorphisms (RFLP) has created opportunities for carrier detection and prenatal diagnosis (Table 6).

Carrier detection is now possible in several of these diseases. Where the location of the gene has been reasonably established (see Table 6), RFLP may, in informative families, identify carriers with reasonable certainty. In those instances where a specific enzyme or complement component defect is present, heterozygote carriers can be ascertained from reduced levels of the enzyme or component in question. In some X-linked diseases, preferential lyonization of cells carrying the abnormal X chromosome during cell proliferation permits determination of the carrier status. Preferential lyonization does not occur in carriers of the hyper IgM syndrome or chronic granulomatous disease.

At the present time, prenatal diagnosis can be made by obtaining fetal blood samples, amnion cells or chorionic villus biopsy. In some ID RFLP can be used to establish diagnosis prenatally. The absence of B or T lymphocytes from umbilical cord blood can be used for the prenatal diagnosis of X-linked agammaglobulinaemia and SCID respectively. Amnion cells or fetal blood samples can be used to ascertain adenosine deaminase or purine nucleoside phosphorylase. Absence of cell membrane components such as MHC class II molecules and CD18 can identify MHC Class II deficiency and the leukocyte adhesion defect 1.

9 PRIMARY SPECIFIC IMMUNODEFICIENCY

9.1 Introduction

Nomenclature and characteristics of currently recognized Primary Immunodeficiency Diseases are given in Tables 1, 2, 3 and 8. The columns provide:

9.1.1 Designated nomenclature. Nomenclature that defines the presumed cause or the most characteristic expression of the disease is generally used. Eponyms have been avoided where possible because original descriptions often preceded modern immunological techniques and may result in misleading classification. Precise nomenclature and standardization of diagnostic criteria are crucial for case documentation and comparison, and compilation of registries.

9.1.2 Serum immunoglobulin levels. Defective antibody formation is the primary abnormality in the majority of Primary Immunodeficiency Diseases. It is generally reflected closely by decreased serum Ig. Thus serum antibody and serum Ig concentrations are combined under a single heading.

9.1.3 Circulating B and T lymphocytes. Enumeration and characterization of circulating lymphocytes is essential for the diagnosis of Primary Immunodeficiency Diseases. Methods for T and B lymphocyte enumeration and differentiation markers are given in sections 6.2.3 and 6.3.2 and for function in 6.3.1 and 6.3.3. Skin tests for cell-mediated immunity (CMI) generally reflect T-lymphocyte numbers and *in vitro* functional assays; CMI is thus omitted from the itemized characteristics.

9.1.4 Presumed pathogenesis. Many Primary Immunodeficiency Diseases result from impeded B- or T-lymphocyte development and differentiation. The normal ontogeny described in Section 2 and in Figure 1 should be consulted for details. In the few instances where the defect can be more precisely identified, greater specification is provided.

9.1.5 Inheritance. The inheritance of those Primary Immunodeficiency Diseases that have been characterized is noted. Approximate chromosomal gene map location is given in Table 6.

9.1.6 Associated features. Commonly associated, characteristic and often diagnostic non-immunological features for some of the Primary Immunodeficiency Diseases are listed.

9.2 Combined immunodeficiencies (CID)

This group of diseases (Table 1) is characterized clinically and immunologically by defects in both T and B lymphocytes. Criteria for diagnosis generally include presentation in infancy with severe, potentially lethal infections, profound abnormalities of CMI and antibody deficiency, and lymphopenia, particularly of T lymphocytes. The clinical presentation usually includes failure to thrive and unusually persistent infections with low virulence opportunistic organisms (for example, Candida, *Pneumocystis carinii*, cytomegalovirus). These findings require differentiation from infants with AIDS. Antibody may be absent in infants with AIDS; HIV studies should include viral isolation or PCR studies for viral genome. SCID is further distinguished on the basis of pathogenesis where known (e.g. enzyme defects), mode of inheritance and level of faulty differentiation.

9.2.1 Severe combined immunodeficiency (SCID). Both X-linked and autosomal recessive forms of SCID have been identified. Infants with SCID who have normal B lymphocyte numbers usually have the X-linked form. The X-linked form of SCID is due to mutations in the γ chain of the IL-2 receptor (IL-2R). This chain of the IL-2R is also shared by the receptors for IL-4, IL-7, IL-9 and IL-15. Some patients with SCID have symptoms similar to graft-versus-host disease (GVH) in the neonatal period. This has been termed 'Omenn's Syndrome'; the disease is not, however, due to engraftment of maternal cells. The genetic and molecular bases of several forms of SCID have been determined and are listed in Table 8 and described in Section 9.5.

9.2.2 Adenosine deaminase (ADA) deficiency. There is a group of distinctive patients whose CID results from defects in the enzyme ADA. This group of phenotypically similar genetic defects include point mutations and deletions within the gene encoding ADA on chromosome 20q13-ter. In the absence of ADA, toxic metabolites of the purine pathway (dATP) and the methylation pathway (S-adenosyl homocysteine) accumulate within the cell and impair proliferation; as a result both T- and B-lymphocyte functions are defective. Inheritance of the defect is autosomal recessive.

9.2.3 Purine nucleoside phosphorylase (PNP) deficiency. This autosomal recessive disease results from defects in the gene encoding the enzyme PNP located on chromosome 9. In the absence of PNP, toxic metabolites, in this case dGTP, accumulate within the cell

Table 1. Combined immunodeficiencies

Designation	Serum Ig	Circulating B cells	Circulating T cells	Presumed Pathogenesis	Inheritance	Associated features
1. Severe combined immunodeficiency (SCID):						
(a) X-linked	Decreased	Normal or increased	Markedly decreased	Mutations in γ chain of IL2,4,7,9,15 receptors	XL	
(b) Autosomal recessive	Decreased	Markedly decreased or normal	Markedly decreased	Maturation defect of both T and B cells	AR	
2. Adenosine deaminase (ADA) deficiency	Decreased	Progressive decrease	Progressive decrease	T-cell and B-cell defects from toxic metabolites (e.g. dATP, S-adenosyl homocysteine) due to enzyme deficiency	AR	Cartilage abnormalities
3. Purine nucleoside phosphorylase (PNP) deficiency	Normal or decreased	Normal	Progressive decrease	T-cell defect from toxic metabolites (e.g. dGTP) due to enzyme deficiency	AR	Autoimmune haemolytic anaemia: neurological symptoms
4. MHC class II deficiency	Normal or decreased	Normal	Normal, decreased CD4 numbers	Mutation in transcription factors (CIITA or RFX-5 genes) for MHC class II molecules	AR	Failure to thrive protracted diarrhoea
5. Reticular dysgenesis	Decreased (maternal)	Markedly decreased	Markedly decreased	Defective maturation of T and B cells and myeloid cells (stem cell defect)	AR	Granulocytopenia and thrombocytopenia
6. CD3 γ or CD3ε deficiency	Normal	Normal	Normal	Defective transcription of CD3γ or CD3ε chain	AR	
7. CD8 deficiency	Normal	Normal	Decreased CD8, normal CD4 numbers	Mutations in Zap-70 kinase gene	AR	

and impair proliferation. T lymphocytes are particularly sensitive to the accumulation of dGTP and they are affected to a greater degree than B lymphocytes. There are thus immunological differences between ADA and PNP deficiency.

9.2.4 MHC class II deficiency. The disease is due to a defect in proteins that promote transcription of class II molecules. The disease is heterogeneous and at least three complementation groups are presently known. Complementation group A results from mutations in the gene encoding class II transcription activation (CIITA). Complementation group C results from mutation in the genes for the heterodimer RFX5. In the absence of class II MHC molecules, cognitive functions, particularly those involving CD4+ T lymphocytes, are impaired. Circulating lymphocyte numbers are normal, but CD4+ T cells are decreased. Antibody synthesis and serum immunoglobulins are decreased and CMI is defective. Several of these children have been recipients of successful bone-marrow transplants.

9.2.5 Reticular dysgenesis. This rare hereditary autosomal recessive disease is generally lethal shortly after birth. It results from failure in the maturation of both lymphoid and myeloid precursors. It is characterized not only by striking lymphopenia, but also by severe granulocytopenia and thrombocytopenia, and overwhelming infections with early death. These newborns often exhibit engraftment of maternal cells.

9.2.6 CD3 deficiency. The phenotype of CD3 deficiency may be variable, even within a family, due to variable expression of CD3 on the T-cell membrane. Deficiencies or abnormalities of CD3 γ and ε have been described.

9.2.7 CD8 deficiency. This rare deficiency is inherited as an autosomal recessive and is due to mutations in the gene for ZAP-70 (see Fig. 4), a tyrosine kinase involved in TCR signalling. CD4+ T cells are present in normal or elevated numbers but are not functional. Some of these children have been recipients of successful bone marrow transplants.

9.3 Predominantly antibody defects

The defect in several of the Primary Immunodeficiency Diseases is restricted to antibody formation, either from impeded intrinsic B lymphocyte development or failure of effective B lymphocyte responses to T lymphocyte signals. This group of diseases, summarized in Table 2, presents clinically with recurrent pyogenic infections.

9.3.1 X-linked agammaglobulinaemia. This is the prototypic antibody deficiency. Affected males present in infancy or early childhood with recurrent pyogenic infections. The tonsils are small and lymph nodes are usually not palpable. Criteria for diagnosis include profound inability to make antibody and resultant low concentrations of all immunoglobulin isotypes. There is a decrease in circulat-

ing B lymphocytes (usually less than 5/1000 lymphocytes); plasma cells are absent from lymph nodes and bone marrow. Even following repeated immunizations regional to the nodes, germinal centres are absent. The number and function of T lymphocytes (including cell-mediated immunity) are unaffected. Pre-B cells are found in the bone marrow. The gene defect has been localized to the long arm of the X chromosome (Xq2l.3- 22). The genetic defect in XLA has recently been found to be due to mutations in a hitherto unknown cytoplasmic tyrosine kinase that has been designated btk or B-cell tyrosine kinase. It consists of an N-terminal plecstrin-like domain, an SH3 domain, an SH2 domain and a C-terminal SH1 or tyrosine kinase domain. Mutations in all four domains have been found in XLA. The xid mutation in mice is due to a missense mutation in which an arginine at residue 28 of the N-terminal domain is transverted to a cysteine. In female carriers of XLA the defective chromosome is preferentially lyonized during B lymphocyte proliferation, permitting carrier detection. The clinical phenotype may be very variable, even within the same family. Since the identification of the gene defect, it has been appreciated that the clinical phenotype is broader than originally conceived and all young males with a predominant antibody defect should be examined for mutations in *btk*.

9.3.1.1 X-linked hypogammaglobulinaemia with growth hormone deficiency. X-linked hypogammaglobulinaemia and isolated growth hormone deficiency has been described. Affected males in different families may or may not have mutations in the *btk* gene. The genes encoding growth hormone and its receptors do not map to the X chromosome.

9.3.2 Immunoglobulin deficiency with increased or normal IgM (the hyper-IgM syndrome). This syndrome apparently represents a group of distinct entities with similar clinical (and phenotypic) expression. Seventy per cent of the cases are X-linked in inheritance; others have been autosomal recessive. Diagnostic criteria include impeded antibody formation. Patients may have an intact IgM antibody response. There is no switch to IgG antibody formation. Thus serum IgM (and sometimes IgD) levels are elevated while IgG and IgA levels are diminished. Circulating B lymphocytes bear only IgM and IgD. The defect is a failure of isotype switch but there is no defect in the switch region DNA of B lymphocytes. Most patients have recurrent or persistent neutropenia and thrombocytopenia. Defects in CMI have been noted in some patients.

Table 2. Predominantly antibody deficiencies

Associated designation	Serum Ig	Circulating B cells	Presumed Pathogenesis	Inheritance	Associated features
1. X-linked agammaglobulinaemia	All isotypes decreased	Profoundly decreased	Mutations in btk gene	XL	-
2. Hyper IgM syndrome (a) X-linked	IgM and IgD increased or normal other isotopes decreased	IgM and IgD bearing cells present others absent	Mutations in CD40 ligand gene	XL	Neutropenia Thrombocytopenia Haemolytic anaemia Opportunistic infections
(b) other	"	"	Unknown isotope switch defect	AR, unknown	"
3. Ig heavy-chain gene deletions	IgG1 or IgG2, IgG4 absent and in some cases IgE and IgA2absent	Normal	Chromosomal deletion at 14q32	AR	-
4. Chain deficiency mutations at AR	Ig(K) decreased: antibody response normal or decreased	Normal or decreased κ-bearing cells	Point mutations at chromosome 2p11 in some patients	AR	-
5. Selective deficiency of IgG subclasses with or without IgA deficiency	Decrease in one or more IgG isotypes	Normal or immature	Defects of isotype differentiation	Unknown	-
6. Antibody deficiency with normal Ig's	Normal	Normal	Unknown	Unknown	-
7. Common variable immunodeficiency	Various decreases of multiple isotypes	Normal or immature or decreased	Variable; undetermined	Varied: AR, AD, or unknown	See text section 9.3.6
8. IgA deficiency	IgA1 and IgA2 decreased	Normal or immature	Failure of terminal differentiation in IgA+ B cells	Various: AR, unknown	Autoimmune and allergic disorders
9. Transient hypogammaglobulinaemia of infancy	IgG and IgA decreased	Normal	Differentiation defect: delayed maturation of helper function	Unknown	Frequent in families with other IDs

Table 3. Other well-defined immunodeficiency syndromes

Designation	Serum Ig and antibodies	Circulating B cells	Circulating T cells	Presumed pathogenesis	Inheritance	Associated features
1.Wiskott-Aldrich syndrome	Decreased IgM: antibody to polysaccharides particularly decreased	Normal	Progressive decrease	Cytoskeletal defect affecting haematopoietic stem cell derivatives; mutations in *WASP* gene	XL	Thrombocytopenia; small defective platelets; eczema; lymphoreticular malignancies; autoimmune disease
2.Ataxia–telangiectasia	Often decreased IgA, IgE and IgG subclasses; increased IgM monomers; antibodies variably decreased	Normal	Decreased	Disorder of cell cycle checkpoint pathway leading to chromosomal instability	AR	Ataxia; telangiectasia; increased alpha fetoprotein; lympho-reticular malignancies
3.DiGeorge anomaly	Normal or decreased	Normal	Decreased or normal	Polytypic embryonic field defect affecting thymic development	Unknown	Hypoparathyroidism: cardiac outflow tract malformation; abnormal facies; partial monosomy of 22q11.pter or 10p in some patients

In the X-linked form, the genetic defect has been identified in mutation of the gene for the CD40 ligand, which is expressed on activated T lymphocytes. The interaction of the CD40 ligand with CD40 on B lymphocytes is requisite for productive isotype switching. The gene for the CD40 ligand maps to Xq26, where the hyper-IgM syndrome had previously been mapped. The CD40 ligand is a type 2 glycoprotein that is homologous to tumour necrosis factor. In most cases no CD40 ligand is expressed on the T cells of these patients. In others a mutant non-functional protein is expressed and these patients may have a less severe phenotype.

9.3.3 Ig heavy-chain deletion. Deletions and duplications in chromosome l4q32 of the heavy chain constant region genes occur in 5 – 10 % of the Caucasoid population and are probably common in all groups. There also is frequent unequal crossover of the heavy-chain locus. Individuals who are homozygous for such deletions lack the relevant isotypes and subclasses. Heterozygotes are unaffected. Most such families were found during the screening of entirely well, normal blood donors and had neither a history of, nor the findings of, recurrent infections. A few individuals homozygous for these defects have presented with recurrent pyogenic infections.

9.3.4 Kappa chain deficiency. Two families have been described whose immunoglobulin chains have lambda light chains only. No kappa chain was found. Antibody formation was variable; circulating B lymphocytes were normal except that they did not carry kappa light chain. Point mutations in the kappa chain gene located at chromosome 2p11 were reported in one family.

9.3.5 Selective lgG subclass deficiencies with or without IgA deficiency. Criteria for diagnosis should include normal total serum IgG levels with sub-normal levels of one or more IgG subclasses. It is difficult to be certain of normal subclass levels. As noted in Section 6.2.1, the assays for subclasses are not well standardized; age-related and population-related norms are not available; genetic variation exists among individuals. Since IgGl is the predominant serum IgG subclass, deficiency of IgGl cannot generally occur without a decrease in total serum IgG, in which instance the defect should be considered as 'Common Variable Immunodeficiency'. IgA levels are frequently, but not invariably, decreased. Low levels of IgG3 are the most common IgG subclass abnormality in adults, whereas low levels of IgG2 are more common in children, particularly in association with poor responses to polysaccharide antigens. IgG4 levels vary widely in normal persons, and many entirely normal persons have no demonstrable IgG4 by standard techniques; selective deficiency of IgG4 alone is difficult to interpret. Patients with IgG2 deficiency, which is often associated with low or undetectable IgG4 levels and an inability to respond to polysaccharide antigens, may be confused with 'Antibody Deficiency with Normal Immunoglobulins'.

9.3.6 Selective antibody deficiency with normal immunoglobulins. It has been known for decades that some individuals selectively fail to respond to certain antigens. The characteristic defect is failure to respond to polysaccharide antigens. While most such persons are normal, some have recurrent sino-pulmonary infections. Criteria for diagnosis should include demonstrated failure to response to specific antigens, a normal response to other antigens and normal total serum IgG and IgM levels. In some of these persons diminished serum IgG2 levels have been found. This appears to be an associative not causative relationship; IgG2 levels are not predictive of antibody responses. Antibody responses to polysaccharide antigens are often found to be diminished in persons with sickle cell anaemia, asplenia (see Section 10), the Wiskott–Aldrich syndrome (Section 9.5.1) and the DiGeorge syndrome. In uncontrolled case studies, patients non-responsive to polysaccharide antigens with normal immunoglobulins and chronic sinopulmonary disease benefited from IgG replacement. Non-responders to polysaccharide antigens produce antibody well with conjugate vaccines. Some individuals who are not responsive to hepatitis vaccine may fall into this category.

Table 4. Complement deficiencies

Deficiency	Inheritance	Chromosomal location	Symptom
C1q	AR	1	SLE-like syndrome
C1r*	AR	12	SLE-like syndrome
C4	AR	6	SLE-like syndrome
C2**	AR	6	SLE-like syndrome, vasculitis, polymyositis
C3	AR	19	Recurrent pyogenic infections
C5	AR	9	Neisserial infection, SLE
C6	AR	5	Neisserial infection, SLE
C7	AR	5	Neisserial infection, SLE vasculitis
C8α***	AR	1	Neisserial infection, SLE
C8β	AR	1	Neisserial infection, SLE
C9	AR	5	Neisserial infection
C1 inhibitor	AD	11	Hereditary angioedema
Factor I	AR	4	Recurrent pyogenic infections
Factor H	AR	1	Recurrent pyogenic infections
Factor D	AR	?	Neisserial infection
Properdin	XL	X	Neisserial infection

* C1r deficiency in most cases is associated with C1s deficiency. The gene for C1s also maps to chromosome 12 p ter.

** C2 deficiency is in linkage disequilibrium with HLA-A25, -B18 and -DR2 and complotype, S042 (slow variant of Factor B, absent C2, type 4 C4A, type 2 C4B)

***C8α deficiency is always associated with C8γ deficiency. The gene encoding C8γ maps to chromosome 9 and is normal but C8γ covalently binds to C8α.

9.3.7 Common variable immunodeficiency (CVID). The term 'common variable immunodeficiency' (CVID) is used to describe a conglomeration of as yet undifferentiated syndromes. All are characterized by defective antibody formation, which is the *sine qua non* for diagnosis. The diagnosis is otherwise based on exclusion of other known causes of humoral immune defects. The term 'acquired immunodeficiency syndrome' (AIDS) should be reserved for patients in whom the diagnosis of HIV infection has been established.

Perhaps because it has not yet been differentiated into its many probably distinct component syndromes, CVID is one of the most frequent of the primary specific immunodeficiency diseases. The incidence has been estimated at 1:50,000 to 1:200,000. It affects males and females equally. The usual age of presentation is the second or third decade of life.

In common with all primary immunodeficiencies affecting humoral immunity, the clinical presentation of CVID is generally that of recurrent pyogenic sinopulmonary infections. Early diagnosis is important; some patients are only discovered when they have significant chronic lung disease, including bronchiectasis.

As with XLA, some patients develop unusual enteroviral infections with a chronic meningo-encephalitis, and other manifestations including a dermatomyositis-like syndrome. Patients with CVID are also highly prone to gastrointestinal diseases, often secondary to chronic *Giardia lamblia* infection.

There is an unusually high incidence of lymphoreticular and gastrointestinal malignancies in CVID. Lymphoproliferative disorders are often apparent from physical examination, where in contrast to XLA, a third of CVID patients have splenomegaly and/or diffuse lymphadenopathy. The lymph nodes show a striking reactive follicular hyperplasia. Non-caseating granulomas resembling sarcoidosis and striking non-malignant lymphoproliferation occur. The gastrointestinal tract is also commonly involved in this process with a characteristic nodular lymphoid hyperplasia. Malabsorption with weight loss and diarrhoea and associated changes such as hypoalbuminaemia, vitamin deficiencies and other findings resembling celiac sprue are seen. This may not respond to gluten-free diets. Chronic inflammatory bowel diseases occur with increased frequency. Patients with CVID are prone to a variety of other autoimmune disorders (e.g. pernicious anaemia, haemolytic anaemia, thrombocytopenia and neutropenia).

As noted above, the *sine qua non* for the diagnosis of CVID is defective antibody formation. These are usually accompanied by decreased serum IgG levels and generally but not invariably decreased serum IgA and IgM. Because CVID is a diagnosis of exclusion, those patients with elevated or high normal levels of serum IgM should be evaluated for the Hyper-IgM syndrome (see Section 9.3.2). Male patients with very low or undemonstrable IgG, especially if they have markedly diminished numbers of circulating B cells, should be evaluated for XLA (see Section 9.3.1). In some patients cell mediated immunity (CMI) may be impaired with diminished T cell function, and absent DTH; the immunodeficiency under these circumstances involves both cellular and humoral immunity and the disease could be considered as a 'Combined Immunodeficiency' although the clinical expression is primarily defective antibody production.

In CVID the relative deficits of the IgG and IgA subclasses generally follows the order of the IgG and IgA heavy chain constant region gene segments of chromosome 14, the immunoglobulins of the down-stream gene segments being progressively more affected; the order of the appearance during ontogeny.

As noted in Section 9.3.8, IgA deficiency is common in the general population. In CVID, IgA levels are undetectable or markedly below the normal range in almost all patients. Family members may also have an unusually high incidence of IgA deficiency. In addition families of patients with CVID have an increased incidence of autoimmune disorders, auto antibodies (including anti-lymphocyte antibodies) and malignancies, suggesting a wide expression of immune dysregulation. As would be expected in a heterogeneous group of undifferentiated diseases, various inheritance patterns for CVID (autosomal recessive, autosomal dominant, X-linked) have been noted. Sporadic cases with no obvious inheritance pattern are, however, the most common.

In multiplex families containing several persons with CVID and IgA deficiency, involved individuals inherit characteristic MHC alleles often featuring deletion or duplication of C4 genes in association with a selected group of MHC class II and III genes (including HLA-DAQ1*0201, HLA-DR3, C4B-Sf, C4A-deleted, G11-15, Bf-0.4, C2-α, HSP70-7.5, TNFα-5, HLA-B8 and HLA-A1). The MHC super-gene complex, which contains many elements vital to antigen presentation as well as several genes of unknown function, is vital to precise T/B-cell interaction through the TCR pathway.

Many studies to identify the nature of the immunological defect(s) as a means of differentiation have been published. None to date has provided patterns sufficiently consistent for classification. While B cells (defined as CD19+) may be reduced in number, with appropriate stimulation they produce and secrete immunoglobulins. There is no convincing evidence for any intrinsic B-cell defect of immunoglobulin genes, synthesis or secretion.

Table 5. Defects of phagocytic function

Disease	Affected cells	Functional defects	Inheritance	Features
Chronic granulomatous disease				
(a) X-linked CGD (deficiency of 91kD binding chain of cytochrome b)	N + M	Killing (faulty production of superoxide metabolites)	XL	McLeod phenotype*
(b) Autosomal recessive (See Table 7)	N	Killing — as above	AR	—
Leukocyte adhesion defect 1 (deficiency of beta chain (CD18) of LFA-1, Mac 1, p150,95)	N + M + L + NK	Mobility, chemotaxis, adherence, endocytosis	AR	Delayed wound healing; chronic skin ulcers, periodontitis, leukocytosis
Leukocyte adhesion defect 2 (failure to convert GDP mannose to fucose)	N + M + L + NK	Mobility, chemotaxis adherence, endocytosis	AR	Delayed wound healing chronic skin ulcers, periodontitis, mental retardation, leukocytosis
Neutrophil G6PD deficiency	N	Killing	XL	Anaemia
Myeloperoxidase deficiency	N	Killing	AR	—
Secondary granule deficiency	N	Killing	AR	—
Schwachman syndrome	N	Chemotaxis	AR	Anaemia, thrombocytopenia, pancreatic insufficiency hypogammaglobulinaemia

*Some patients have deletions in the short arm of the X chromosome; in these patients additional features including Duchenne muscular dystrophy, retinitis pigmentosa, delayed separation of umbilical cord may be found.

CVID patients commonly have reduced CD4/CD8 ratios, with a reduction in CD4+CD45RA+ ('unprimed') T cells and this suggests that there has been activation of T cells. The reported increased levels of IL-4 and IL-6, soluble CD8, CD25, β2-microglobulin, HLA-DR, LFA-3 and ICAM-1 are probably the result of viral infection.

About 60% of CVID patients have diminished proliferative responses to T-cell receptor stimulation, and decreased induction of gene expression for IL-2, IL-4, IL-5 and IFNγ. There is no evident abnormality of the T-cell receptors. T-cell receptor gene analyses indicates normal heterogeneity of gene rearrangements. Decreased IL-2 production after T-cell receptor stimulation, which is correlated with diminished CD-40 ligand (gp39) expression, appears to result from an abnormality residing in CD4+ T cells. This abnormality can be overcome by stimulating T cells with PMA and ionomycin — an alternative T-cell activation pathway (see section 5). Thus many CVID patients appear to have defective signal transduction, which could explain the diminished humoral immunity that is present.

9.3.8 IgA deficiency. About 1 in 700 Caucasian persons (in contrast to 1:18,500 Japanese individuals) have no demonstrable serum IgA. Most of these individuals have no apparent disease. Some persons with intercurrent sinopulmonary infections have been reported with entirely normal serum IgM and IgG levels, but absent or extremely reduced serum IgA levels. Whether the IgA deficiency or some other factors are involved in their recurrent illnesses is not clear. IgA deficiency is however more frequent in patients with chronic lung disease than in a normal age-matched population. In complete IgA deficiency, both IgA1 and IgA2 are absent; lymphocytes are normal. The defect is presumed to result from maturational failure of IgA producing lymphocytes. Autosomal recessive inheritance has been shown in some families. Fixed haplotypes of MHC genes are frequently associated with CVID and IgA deficiency.

9.3.9 Transient hypogammaglobulinaemia of infancy. Maternal IgG is actively transferred to the fetus throughout pregnancy. The serum IgG level of full-term infants is equal to or slightly greater than that of the mother. Maternal IgG in the infant disappears after birth with a half-life of 25–30 days and the infants own Ig production is initiated, starting with IgM and followed by IgG and then IgA. The time of initiation and the rate of production of Ig by infants varies considerably. During the first 3 to 12 months of life in premature infants (where the transfer of maternal IgG is often limited) and in some full-term infants (particularly in families with immunodeficiency), the nadir of serum Ig concentration may be very low — within an 'immunodeficient' range. The initiation of antibody production may be delayed for as long as 36 months and ultimately is manifested by increased levels of serum IgG. Unless there is some other underlying defect, the condition corrects itself and requires no treatment. Antibody production by the infants themselves can usually be documented by serial measurement of serum IgG levels and of antibody responses to vaccine antigens.

Table 6. Chromosone map location of IDs listed in Tables*

1.	X-linked severe combined immunodeficiency	Xq13.1-13.3
2.	X-linked agammaglobulinaemia	Xq21.3-22
3.	X-linked immunodeficiency with increased IgM	Xq26-27
4.	Wiskott–Aldrich syndrome	Xp11.22-11.3
5.	X-linked chronic granulomatous disease	Xp21.1
6.	X-linked lymphoproliferative syndrome	Xq26
7.	Adenosine deaminase deficiency	20q13-ter
8.	Purine nucleoside phosphorylase deficiency	14q13.1
9.	CD8 deficiency (ZAP-70 deficiency)	2q12
10.	Kappa chain deficiency	2p11
11.	Ig heavy chain deletion	14q32.3
12.	Ataxia-telangiectasia**	11q23.1
13.	Autosomal recessive chronic granulomatous disease	
	p22 phox	16q24
	p47 phox	7q11.23
	p67 phox	1q25
14.	Leukocyte adhesion deficiency 1	21q22.3

* The complement gene map locations (and hence the deficiencies thereof) are given in Table 4.

** The map location of five (the most common) of the six complementation groups, which includes 97% of AT patients.

9.4 Predominantly T-cell defects

In addition to the ID diseases listed in Tables 1, 2, 3 and 8, other primary defects in the immune system where the genetics and pathogenesis of the ID are not yet completely understood have been described in isolated cases and are listed in Table 8.

9.4.1 Primary CD4 T-cell deficiency. Profound, persistent decrease in circulating CD4+ T lymphocytes, with defective CMI, has been documented in patients not infected by HIV, who present with opportunistic infections, such as cryptococcal meningitis and oral candidiasis. Immunoglobulin levels may be normal or slightly decreased. The pathogenesis and genetics of this abnormality are not yet known. When such patients are identified CD4 enumeration should be carried out in other family members.

9.4.2 Primary CD7 T-cell deficiency. A child with SCID was described who was CD7+ T-cell deficient. No genetic transmission of the defect could be ascertained.

9.4.3 IL-2 deficiency. A child with SCID and normal circulating T-cell numbers was found to be unable to transcribe the IL-2 gene. The inheritance of the defect could not be determined.

9.4.4 Multiple cytokine defect. A child with SCID was deficient in IL-2, IL-4, IL-5 and interferon-γ. T cells lacked the nuclear factor of activated T cells (NFAT) promoter. The genetics of the defect are not yet known.

9.4.5 Signal transduction defect. A few children with SCID or CID fail to show normal calcium flux and diacylglycerol generation after antigenic stimulation of their T cells. The defect can be circumvented by stimulation with PMA or aluminum tetrafluoride (AlF_4). The genetics of this condition are not known and the precise defect(s) is not well characterized.

9.5 Immunodeficiencies associated with other major defects

There are a variety of diseases in which immunodeficiency is an important but not exclusive component. Included in this section (see Table 3) are those diseases where immunodeficiency is the dominant manifestation in syndromes with other defects.

9.5.1 Wiskott–Aldrich syndrome. This X-linked disease presents in infancy or early childhood. Initial clinical manifestations include eczema, recurrent, often unusual or unresponsive infections, and thrombocytopenia. The platelets are small. Surface sialoglycoproteins, CD43 and gpIb, and other sialoglycoproteins are unstable in the membranes of leukocytes and platelets. The lymphocytes have a characteristic 'bald' appearance on scanning electron microscopy. The cytoskeleton in the T cells and platelets is abnormal and the actin in these cells does not bundle normally. The proliferative response of the T cells to anti-CD3 is absent or greatly diminished. Serum immunoglobulins may at first be normal, but a progressive decrease (initially of IgM) develops. Antibody production, especially but not exclusively to polysaccharide antigens, is impaired. Progressive lymphopenia, most marked in the T lymphocyte series with resulting

Table 7. Prenatal diagnosis

Diseases	Informative Restriction Fragment Length Polymorphisms	Findings in fetal cord blood or amnion cells
X-linked agammaglobulinaemia	+	Absence of B cells
X-linked severe combined immunodeficiency	+	Absence of T cells
Autosomal recessive severe combined immunodeficiency	-	Absence of T cells (and B cells)
Wiskott–Aldrich syndrome	+	'Bald' lymphocytes by scanning EM
Ataxia–telangiectasia	+	Radiosensitivity
MHC class II deficiency	-	Absence of MHC class II molecules on cell membranes
Leukocyte adhesion deficiency	(*)	Absence of CD18 on phagocytes
X-linked chronic granulomatous disease	+	Abnormal oxygen radical production
Autosomal recessive chronic granulomatous disease	(*)	Abnormal oxygen radical production
Adenosine deaminase deficiency	(*)	Decreased ADA in red blood cells
Purine nucleoside phosphorylase deficiency	(*)	Decreased PNP in red blood cells

*Potentially possible, but not yet well established.

Table 8. Other primary immunodeficiency diseases

Primary CD4 Deficiency
Primary CD7 Deficiency
IL-2 Deficiency
Multiple Cytokine Deficiency
Signal Transduction Deficiency

defective CMI, develops. Autoimmune diseases including severe vasculitis and glomerulonephritis may be present. Death occurs in late childhood or early adulthood, often from lymphoreticular malignancy. The defective gene is on the short arm of the X chromosome at Xp11.22 and is non-randomly lyonized during differentiation of all blood cells; thus carrier detection is possible. The gene has been cloned and encodes a protein of 501 amino acids, which has been called the Wiskott–Aldrich syndrome protein (WASP). Its function is not known.

9.5.2 Ataxia–telangiectasia. This autosomal recessive syndrome is characterized by progressive cerebellar ataxia, the appearance of fine telangiectases, especially on ear lobes and conjunctival sclera, and eventually, in most patients, recurrent sinopulmonary infections. Raised levels of serum alpha fetoprotein are regularly present. Immunodeficiency while not invariably demonstrable in the early life of affected persons, develops in at least 70% of cases. There is no consistent pattern; no single abnormality has been found to exist in all patients. Serum Ig is decreased in varying patterns: IgG2, IgG4, IgA and IgE are commonly low or absent. Antibody responses to polysaccharide and protein antigens may be reduced. The numbers and function of circulating T lymphocytes, including DCH, are generally diminished. There is an increased incidence of autoantibodies.

Cells from patients with ataxia–telangiectasia have a disorder of their cell cycle checkpoint pathway that results in an extreme hypersensitivity to ionizing radiation. Lymphocytes show frequent chromosomal breaks, inversions and translocations involving sites of the T-cell receptor genes and immunoglobulin gene complexes. In fibroblasts chromosomal breaks, inversions and translocations are random. AT patients and their families have a strikingly increased susceptibility to malignancies. Breast cancer in women family members is increased 5-fold. The overall risk of cancer in heterozygotes generally is increased 3.5-fold. Death in patients usually occurs in early adult life after years of increasing disability from pulmonary disease or (often lymphoreticular) malignancy.

The disease has a great many genetic variants. At least 6 complementation groups have been identified (groups A,C,D,E,V1 and V2). The chromosome locus of the defective gene(s) in the complementation groups A, C, D, E and V1 but not V2 localizes to 11q22-23. Some families, however, suggest that other loci may also be involved. Although many markers for the locus have been developed, the gene(s) has not yet been isolated.

For several syndromes with immunodeficiency and chromosomal instability see Section 10.1.

9.5.3 The DiGeorge anomaly. The DiGeorge anomaly is one of a series of contiguous gene syndromes that affect multiple organs during early embryogenesis. Almost all (80–90%) patients with the DiGeorge anomaly have deletions (often microdeletions) of 22q11-pter. There are several other syndromes that are located to the same area. Because they all involve deletions of 22q11-pter they have been termed 'CATCH 22', an acronym for the involved organs: Cardiac Abnormalities, Abnormal Facies, Thymic Hypoplasia, Cleft Palate and Hypocalcaemia. This group of syndromes would include the velocardiofacial (Shprintzen) Syndrome, the CHARGE associations, Kallmann syndrome, and the arrhinencephaly/holoporencephaly syndromes. Additional cases of the DiGeorge Anomaly may derive from 10p deletions, from the Fetal Alcohol Syndrome, Retinoic Embryopathy or Maternal Diabetes. The characteristic pathologic manifestations include multiple anomalies of the third and fourth branchial arch derivatives; Type 1 truncus arteriosus, dysmorphic facies with micrognathia, thymic and parathyroid hypoplasia or aplasia. Clinically, neonatal tetany and/or cardiac failure are the presenting manifestations in most affected infants. The facial features then arouse suspicion as to the diagnosis.

Infections are usually not a presenting manifestation. Even though the thymus is frequently involved, only about 20% of those with the anomaly have decreased numbers and function of T lymphocytes. At autopsy the thymus is small, atrophic often containing normal appearing ectopic lobes. Surviving infants over time may naturally acquire functional T cells and the immunodeficiency becomes corrected. It is difficult therefore to assess the value of the various treatment regimens that have been attempted.

9.6 Associated conditions

In addition to the infections of the respiratory and gastrointestinal tracts noted previously, patients with Primary Specific Immunodeficiency are particularly prone to several other conditions.

9.6.1 Malignancies. Age-specific mortality rates for cancer in primary ID groups exceed by 10–200 times the expected rates for the general population. The majority of cancers are observed in patients with ataxia–telangiectasia and the Wiskott–Aldrich syndrome.

In patients with ataxia–telangiectasia, transpositions, inversions and breaks of chromosomes 7 and 14 at the sites of the T-cell receptor are associated with lymphoreticular malignancies. However, these patients develop many malignancies of rapidly replicating cells in organs other than those of the lymphoreticular system.

Full functional correction of the immunodeficiency, as may be accomplished in patients with SCID or Wiskott–Aldrich syndrome, by bone marrow from an MHC matched sibling donor transplantation, has led to impressive correction of the abnormally increased susceptibility to malignancy. By contrast, incomplete correction of the immunodeficiency by bone marrow transplantation has been associated with occurrence of lymphoreticular malignancy in approximately 50% of the patients over a period of years.

The types of tumours in ID patients of all groups is different from those observed in nonselected populations: lymphoreticular malignancies. Some of these malignancies have shown clear evidence of clonal proliferation, some have been associated with Epstein–Barr virus infection. Unlike most lymphoreticular malignancies other than hairy cell leukaemias and some myelomas, treatment of the lethal lymphoreticular malignancies in patients with ID have shown impressive responses to treatment with interferon α. The data on lymphoproliferative disorders in ID are thus sufficient to suggest an association between at least some forms of ID and oncogenesis. Possible mechanisms for the association include defective immunological surveillance; defective immune response to oncogenic viruses; chronic overstimulation or proliferation of responsive cells to antigen; independent effects of the same common cause (i.e. chromosomal instability in ataxia telangiectasia). In CVID there is a 50-fold increase in gastrointestinal malignancies and an increase of several hundred fold (*c.* 300) of lymphomas in female patients.

9.6.2 Autoimmunity. A significant number of autoimmune syndromes have been described in association with ID. These include pernicious anaemia, autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpura, systemic lupus erythematosus, thyroiditis, Sjogren syndrome, chronic active hepatitis and myasthenia gravis. In addition to autoantibodies against blood cells, autoantibodies to immunoglobulins and various tissue antigens have been observed. Inflammatory bowel disease is a frequent complication of ID.

9.6.3 Atopic allergy. ID may play a role in atopic allergy. A small proportion of atopic subjects have low levels of serum IgA at the time of their symptoms; a prospective study of the newborn offspring of atopic parents showed that those who later developed atopic allergy had transiently lower levels of IgA before their illness.

9.6.4 Unusual viral infections. Patients with antibody ID (particularly with X-linked agammaglobulinaemia) are especially susceptible to chronic viral meningo-encephalitis (and a viral dermatomyositis-like syndrome) due to ECHO or other enteroviruses. Such patients may shed ECHO virus for prolonged periods (more than two years). The virus may be isolated from cerebrospinal fluid and post mortem from all viscera. Untreated the infection is fatal. Intravenous and/or intrathecal IgG have controlled these infections in some patients.

Several patients with primary ID have been infected with HIV. Since seroconversion to HIV is of little or no use in the diagnosis of HIV in ID patients, their blood must be examined by PCR techniques to ascertain the presence of HIV genomic material. It is of interest that some patients with CVID infected with HIV or hepatitis C have become immunologicalally competent.

9.7 Treatment of specific immunodeficiency

9.7.1 Bone marrow transplantation. Transplantation of bone marrow cells from HLA genotypically identical donors (i.e. matched sibling donors or other HLA identical members of a family) has led to complete immunological reconstitution of many patients with SCID, including those with ADA and PNP deficiency, and those with reticular dysgenesis. Bone marrow transplantation has also been successful in the Wiskott–Aldrich Syndrome, Leukocyte Adhesion Deficiency and MHC Class II immunodeficiencies. Ideally, donor and recipient should be identical at the HLA-A, B, C and DR loci. Unfortunately, two-thirds of patients do not have a compatible donor. A few successful bone-marrow transplants from matched unrelated donors have been performed; such donors may be found in one of the registries of HLA testing, special attention being paid to the HLA-D match. Great progress has been made in haploidentical bone-marrow transplantation in recent years. Extensive conditioning of the recipient to prevent rejection and the elimination of T cells from the graft (with a lectin column, monoclonal antibodies or depletion by E-rosetting) to avoid graft-versus-host disease are mandatory. Success with T-cell engraftment has been very encouraging, but it has been difficult to establish B-cell engraftment with haploidentical bone marrow. Full reconstitution has been obtained with a longer delay than in HLA identical donors.

Graft-versus-host (GVH) disease, when it occurs, generally appears 8–20 days after a transplant and is usually manifested by fever, Coombs' test-positive haemolytic anaemia, erythematous, maculopapular skin rash, bloody diarrhoea, hepatosplenomegaly, aregenerative pancytopenia and death. The various means proposed to prevent graft-versus-host disease have included the use of cyclosporin A, alone or together with methotrexate. T-cell depletion of donated bone marrow has also been used. Persistent low-grade GVH reactions, characterized by hepatomegaly, jaundice or skin rashes, can continue for many months and become chronic and severely debilitating.

The establishment of immune competence ('take' of the graft) may be indicated by: improvement of clinical status (e.g. weight gain, rapid resolution of moniliasis); appearance of T and B cells in the circulating blood; genetic markers of donors, including enzyme activity in previously deficient patients; increase of immunoglobulin levels (including Ig of donor origin); appearance of humoral antibodies (including those following antigenic stimulation); return of C1q level to normal; and appearance of CMI reactions. Of these, the establishment of chimerism is the most reliable evidence of engraftment. Appropriate tests for mosaicism include sex and other chromosomal studies, RFLP analysis, HLA and red cell antigens, plasma protein or enzyme allotypes.

Tests of immunological competence should be repeated periodically in successful cases, since subsequent gradual decline has been observed in some instances. Children dramatically restored immunologically have also occasionally died of pre-existing pulmonary infections with *Pneumocystis carinii* or other organisms just after immunological capacity has been restored. Prophylactic treatment with sulfamethoxazole-trimethaprim has proven useful in the treatment of these complications. Several deaths from varicella have occurred in successfully transplanted ID patients; such patients should be passively protected with Varicella-Zoster Immune Globulin (VZIG) and acyclovir following exposure, if no circulating antibody can be demonstrated. Other anti-viral agents are being developed and tested at the present time.

The risk of developing EBV-induced B-cell lymphomas in transplant recipients, particularly of haplo-identical bone marrow donations, has been a difficult and as yet unsolved problem.

9.7.2 Replacement of immunoglobulins. The efficacy of immunoglobulin replacement for antibody deficiency syndromes was well established more than 40 years ago. It is now accepted that all patients with primary specific immunodeficiency who have significantly diminished serum IgG levels and/or demonstrated defects in antibody production should receive IgG replacement. Preparations suitable for either intramuscular or intravenous use are available for this purpose; the intramuscular preparations should never be given intravenously, but they can be given subcutaneously. Intravenous immunoglobulin replacement is the preferred treatment. Standards for the preparations are the subject of an IUIS/WHO report (Bull. WHO 60(l), 43, 1982). Viral partition and inactivation during fractionation procedures has recently been described for HIV and proven satisfactory. Thus HIV and other retroviruses are effectively excluded by current fractionation procedures, essentially eliminating the risk for transmission of AIDS. Clusters of hepatitis C have been reported in patients who received certain lots of IVIG.

Experience has shown that replacement therapy with intravenous Ig is life-saving. If replacement is started early, and if sufficient amounts are given with sufficient frequency, the cycle of recurrent infections and progressive lung damage can be arrested. Indeed it has been documented that if large doses of IgG (>400 mg/kg/month) are given, abnormal pulmonary function may improve even if bronchiectasis is present.

Preparations of IgG suitable for intravenous use have now been shown highly effective and safe. Predictable near normal serum IgG

levels can be maintained with ease. General experience suggests that the 'trough' serum IgG level for optimal clinical status should be maintained at levels at least 200–400 mg/dl (2–4 gm/l) above those produced intrinsically by the patient. This will in most instances require an IgG dose of 350–500 mg/kg/month, or 150–250 mg/kg every 2 weeks.

Preparations of IgG for replacement contain predominantly IgGl and IgG2. The amounts of IgG4 in most preparations are small, and in some IgG3 is absent. Patients with selective IgG subclass deficiency (whether or not they have IgA deficiency) may benefit from IgG replacement; but neither the indications for such therapy nor the dosage have been well established.

Immunoglobulin replacement therapy using subcutaneous infusions of gammaglobulin is increasingly used. The total number of patients treated so far is still small but the results indicate that this type of treatment is well tolerated with a very low frequency of adverse systemic reactions. A large scale study of immunoglobulin subcutaneous infusion is now in progress.

Untoward reactions (dyspnea, flank pain, hypotension, collapse, fever, rashes and even death) may occur with a particular IM or IV preparation, probably due to immunoglobulin aggregates. Only very rarely can reactions be attributed to antibodies to IgA. Reactions tend to occur more frequently in severely hypogammaglobulinaemic patients, particularly at the initiation of treatment and in those with intercurrent infections. Many reactions can be traced to excessively fast rates of infusion.

9.7.3 Enzyme replacement. Partial replacement of enzymes in infants with ADA or PNP deficiency has been attempted with frozen irradiated red blood cells. Apparently, the amounts of purine degradation enzymes within the red cells are not sufficient to permit efficient degradation of toxic metabolites within lymphocytes. Partial enzyme replacement in ADA deficiency has also been attempted by the use of bovine ADA modified by conjugation with polyethylene glycol. Repeated weekly administration of the conjugated enzyme resulted in marked clinical improvement in several patients.

Since the gene for ADA has been cloned, it has become possible to express it in T cells with a retroviral vector. This has provided the basis for the attempt at gene therapy in patients with ADA deficiency. This therapeutic approach is at present experimental and as the stable expression cannot yet be achieved in progenitors of the lymphoid cells, the results obtained from the use of this method may be transient.

HLA identical bone-marrow transplantation is at present the treatment of choice.

9.7.4 Blood transfusions. Blood transfusions should never be given to patients with cell-mediated immunodeficiency, unless fully oxygen saturated blood has been irradiated to eliminate viable white blood cells which may inappropriately engraft the patient. Blood transfusion is also safe when processed by freezing and centrifugation. However, lymphocytes are still viable in outdated blood, washed red blood cells, unprocessed plasma, and platelet preparations.

9.7.5 Treatment of opportunistic infections. Individual infections should be treated early with full doses of antimicrobial agents. Where possible narrow spectrum drugs selected on the basis of microbial sensitivity testing should be used. Prophylactic antibiotics are not generally recommended; they increase the hazard of infection with fungi or other resistant organisms. Long-term treatment with combination sulfa drugs (co-trimoxazole, sulfamethoxazole-trimethoprim) is believed to be of some benefit, but this has not been critically evaluated.

Antiviral agents such as acyclovir have proven valuable in the treatment of some patients with persistent or severe viral infections.

9.7.6 Gastrointestinal disorders. Intestinal disease is frequent in ID patients and, in addition to treatment of infection or infestation, disaccharide or gluten-free diets may be of benefit in patients with sprue-like symptoms. In some instances intravenous hyperalimentation of limited duration may be justified. *Giardia lamblia* and Campylobacter are frequent causes of diarrhoea, steatorrhoea or weight loss in ID. Treatment with atabrine or metronidazole is effective for giardiasis.

10 IMMUNODEFICIENCY ASSOCIATED WITH OR SECONDARY TO OTHER DISEASES

Table 9 lists some of the many congenital and hereditary diseases which may be associated with immunodeficiency.

10.1 Chromosomal instability and defective repair

The immune system is dependent upon rapid and accurate lymphocyte differentiation and replication. Any syndrome associated with chromosomal instability such as ataxia telangiectasia (9.4.2) can be expected to have associated immunological defects.

10.1.1 Bloom syndrome. Low birth weight, retarded growth, rashes from light sensitivity, molar hypoplasia and facial telangiectasia, characterize this autosomal recessive chromosomal instability syndrome which has been mapped to 11q23. Immunodeficiency with frequent infections, increased susceptibility to malignancies and reduced T-cell function and decreased serum IgM are found. At times serum IgG and IgA may also be diminished. IgM+ B cells are normal in number; the defect appears to be in B-cell maturation to IgM secretion. NK cell defects have been described. In one family with a child resembling Bloom Syndrome, a DNA ligase 1 defect was described.

10.1.2 Fanconi anaemia. The autosomal recessive syndrome is characterized by multiple organ defects including bone marrow failure, hyperpigmentation and cafe au lait spots, limb defects (radial hypoplasia), genitourinary anomalies, abnormal facies (microphthalmia, micrognathia, broad nasal base, epicanthal folds) and chromosomal breaks. There is an increased incidence of leukaemia. Decreased T lymphocyte and NK cell function and serum IgA concentrations have been described.

10.1.3 ICF - syndrome. This syndrome is characterized by immunodeficiency, centromeric instability, usually of chromosomes 1,9, and 16, and abnormal facies (ocular hypertelorism, flat nasal bridge and tongue protrusion). Mental retardation, and recurrent sinopulmonary, gastrointestinal and skin infections occur. Generally, but not uniformly, serum IgM, IgG and IgA are decreased. The diagnostic finding is abnormal condensation of heterochromatin in chromosomes 1, 9, and 16 with increased frequency of mitotic recombination and the formation of multibranched chromosomes. The inheritance is presumed to be AR.

10.1.4 Nijmegen breakage syndrome. This condition presents with microcephaly, mental retardation, short stature, a 'bird-like' facies and recurrent infections. Chromosomal instability with increased

sensitivity to ionizing radiation and X-rays is found. Immunoglobulin levels and T-cell function are decreased. An associated syndrome, the Seemanova syndrome, appears to be identical to the Nijmegen syndrome except that the patients have normal intelligence.

10.1.5 Seckel ('bird-headed' dwarfism) syndrome. This striking syndrome results in dwarfism (intra-uterine in onset), a 'bird-head' facies, severe brain dysplasia, mental retardation and many skeletal anomalies. Increased chromosomal breakage has been described. Some of the affected individuals develop a hypoplastic anaemia, pancytopenia and decreased serum immunoglobulins. The inheritance appears to be AR.

10.1.6 Xeroderma pigmentosum. Patients with this rare autosomal recessive condition have a marked sensitivity to sunlight from infancy and develop striking skin lesions — erythema, bullae, telangiectasia, keratoses, basal and squamous cell carcinomas. They are unable to repair UV damage to their DNA. A small number (>5%) of the affected children have recurrent infections and a demonstrable immunodeficiency with a decrease in CD4+ cells. Their sera appear to contain antibodies which suppress T-cell (and possibly NK cell) function. Serum IgG levels may be diminished.

10.2 Chromosomal defects

Of the many syndromes known to be associated with chromosomal abnormalities, several are accompanied by immunodeficiency.

10.2.1 Down syndrome. Trisomy 21 (Down Syndrome) is characterized by up slanting palpebral fissures, flat facies, hypotonia and mental retardation (which may be very mild) and recurrent infections. There is a progressive decrease in serum IgM. The thymus may be dysplastic. CD8 T cells may be increased due to cells with an NK phenotype; NK cell activity however, is low. Abnormal DTH, antibody formation and cytokine production have been reported. Chromosome 21 carries the gene encoding the interferon receptor; Trisomy 21 lymphocytes are more sensitive to interferon than normals.

10.2.2 Turner syndrome. These patients, who have generally an XO karyotype, present clinically with short stature, ovarian dysgenesis, transient lymphoedema, a webbed neck and broad chest. They often have recurrent infections, autoimmune diseases and increased numbers of malignancies. About half have immunodeficiency, with decreased serum IgG and IgM. T- and B-cell numbers and responses are usually within normal limits. Patients with variants of the Turner Syndrome, including mosaics, may show the same features.

10.2.3 Deletions or rings of chromosome 18 (18p- and 18q-). Individuals with rings and/or deletions of the short or long arms of chromosome 18 may present with mid-facial hypoplasia or ptosis, mental retardation, growth deficiency. Some have been found to have markedly decreased serum IgA.

10.3 Skeletal abnormalities

The known inter-relationship between new bone formation, lymphocytes and cytokines leads to the expectation of some forms of skeletal dysplasia in patients with immunodeficiency. Short-limbed skeletal dysplasia (dwarfism) has, for example, been described in patients with ADA deficiency (9.2.2). In the syndromes listed below, immunodeficiency is frequently although not universally present.

10.3.1 Short-limbed skeletal dysplasia (short-limbed dwarfism). The preferred nomenclature is short-limbed skeletal dysplasia (SLSD). The term is used to describe a group of patients in which stature is disproportionately reduced, with greater involvement of the limbs than the trunk. It has been reported (9.2.1) in patients with ADA deficiency and in SCID with normal ADA.

10.3.2 Cartilage-hair hypoplasia (metaphyseal chondrodysplasia). These patients present with short-limbed skeletal dysplasia and usually, though not always fine, sparse (hypoplastic) hair and cellular immunodeficiency. The inheritance is AR. In Finland, the incidence is approximately 1:23,000 births. Multiple organ systems may be involved: Ligamentous laxity, macrocytic anaemia, neutropenia, megacolon (including Hirschsprung's intestinal stenosis) have all been described. Most patients have frequent infections and demonstrably defective cellular immunity. The defects in cellular immunity probably relate to lymphopenia, particularly of T cells. B-cell numbers and functions are normal. The gene defect and map location are unknown.

10.4 Immunodeficiency with generalized growth retardation

Generalized growth retardation is common in children with recurrent infections, malnutrition and chronic pulmonary disease. It is prominent in syndromes involving the endocrine system (e.g. X-linked hypogammaglobulinemia with growth hormone deficiency (Section 9.3.1.1) and the gastrointestinal tract.

10.4.1 Schimke immuno-osseous dysplasia. Several patients have been described with skeletal dysplasia, pigment abnormalities (lentigenes) and nephropathy. The inheritance is AR. Most patients have recurrent infections with striking lymphopenia, especially of T (CD4+) cells. Mitogen responses and DTH were diminished. B-cell numbers and function were normal. The nephropathy is associated with circulating immune complexes.

10.4.2 Immunodeficiency with absent thumbs, anosmia and icthyosis. Several syndromes are characterized by radial dysplasia and/or absent thumbs, e.g. Fanconi Syndrome (10.1.2). Three sibships have been reported with short stature, absent thumbs, anosmia, icthyosis (with chronic mucocutaneous candidiasis) and recurrent infections - prominently viral and fungal as well as bacterial. Serum IgA was absent; IgG and IgM was variably decreased. Mitogen responses were diminished.

10.4.3 Dubowitz syndrome. This is a rare anomaly associated with pre- and post-natal dwarfism, distinctive facial dysmorphism and eczema. Bone-marrow failure, with pancytopenia has been reported. Inheritance is AR.

10.4.4 Growth retardation, facial anomalies and immunodeficiency. A variety of other small case reports suggest that the combination of facial anomalies and growth retardation may be associated with recurrent infection. In some instances there are decreased immunoglobulins; in some neutropenia. The reports are insufficient at this time to categorize the clusters more clearly. But the finding of facial anomalies with growth retardation warrants immunological investigation.

10.4.5 Progeria (Hutchinson–Gilford syndrome). Alopecia, short stature and loss of subcutaneous fat are the hallmarks of this rare syndrome. Skin fibroblasts have reduced ability to replicate. A

Table 9. Immunodeficiency associated with or secondary to other diseases

Chromosomal instability or defective repair
- Bloom syndrome
- Fanconi anaemia
- ICF syndrome
- Nijmegen breakage syndrome
- Seckel syndrome
- Xeroderma pigmentosa

Chromosomal defects
- Down syndrome
- Turner syndrome
- Chromosome 18 rings and deletions

Skeletal abnormalities
- Short-limbed skeletal dysplasia
- Cartilage-hair hypoplasia

Immunodeficiency with generalized growth retardation
- Schimke immuno-osseous dysplasia
- Immunodeficiency with absent thumbs
- Dubowitz syndrome
- Growth retardation, facial anomalies and immunodeficiency
- Progeria (Hutchinson-Gilford syndrome)

Immunodeficiency with dermatological defects
- Partial albinism
- Dyskeratosis congenita
- Netherton syndrome
- Acrodermatitis enteropathica
- Anhidrotic ectodermal dysplasia
- Papillon–Lefevre syndrome

Hereditary metabolic defects
- Transcobalamin 2 deficiency
- Methylmalonic acidaemia
- Type 1 hereditary orotic aciduria
- Biotin dependent carboxylase deficiency
- Mannosidosis
- Glycogen storage disease, Type 1b
- Chediak–Higashi syndrome

Hypercatabolism of immunoglobulin
- Familial hypercatabolism
- Intestinal lymphangiectasia

Other
- Hyper IgE syndrome
- Chronic mucocutaneous candidiasis
- Hereditary or congenital hypo- or asplenia
- Ivermark syndrome

described reduction in T (CD4+) cells and reduced IgG levels may relate to the rapidly accelerated aging process. A somewhat similar and very rare condition, the Smith–Mulvihill syndrome, presents with short stature, progeria, microcephaly with ocular and dental anomalies, and pigmented naevi. In some instances recurrent infections and diminished IgG levels and in one patient lymphopenia with diminished T and B cells were found.

10.5 Immunodeficiency with dermatological defects

10.5.1 Immunodeficiency and partial albinism (Griscelli syndrome). This disease is characterized by albinism due to abnormal migration of melanosomes from melanocytes (where pigment is clumped) to keratinocytes. It is distinguished from the Chediak–Higashi syndrome by the absence of giant granules. Patients have a propensity for fungal, viral and bacterial infections. Immunoglobulins and DTH may be decreased. Abnormal T-cell cytotoxicity and diminished NK cell activity have been described. These patients have in addition to increased susceptibility to infection, a lymphoproliferative reaction similar to that seen in the Chediak–Higashi syndrome which leads to early death. The defect has been corrected by bone-marrow transplantation. Inheritance is AR.

10.5.2 Dyskeratosis congenita. This disease is characterized by cutaneous pigmentation, nail dystrophy and oral leukoplakia. Inheritance can be X-linked, AR or AD. There is an increased risk of malignancy. Bone-marrow failure frequently occurs in childhood with resultant increased infections, but variable immunological defects. Hypogammaglobulinaemia, is found in many patients, along with diminished DTH.

10.5.3 Netherton syndrome. A large group of patients with trichorrhexis, icthyosis and atopy have been described. Some have had abnormally low or high IgG levels.

10.5.4 Acrodermatitis enteropathica. This autosomal recessive disease characterized by eczema, diarrhoea, and malabsorption has been reported in association with recurrent sinopulmonary infections, decreased serum Ig, intermittently reduced numbers and function of T cells and abnormal cell mediated immunity. In some patients abnormal chemotaxis was found. The syndrome is attributable to zinc deficiency due to defective zinc absorption from the gastrointestinal tract. Symptomatology responds dramatically to the administration of increased amounts of zinc given by mouth.

10.5.5 Anhidrotic ectodermal dysplasia. This syndrome is characterized by hypohidrosis, faulty dentition and hypotrichosis. Most cases are X-linked recessive; a few are AR. Heterozygotic females may have partial symptomatology. Recurrent upper respiratory infection are a frequent problem. While abnormalities of immunoglobulin levels and DTH have been described, no consistent T- or B-cell abnormality has been found. Diminished chemotactic activity has also been reported in a possibly related condition, Congenital Icthyosis.

10.5.6 Papillon–Lefevre syndrome. Hyperkeratosis of the hands and feet with periodontal disease leading to premature loss of teeth is in some cases associated with pyoderma. Neutrophil chemotaxis is often diminished. This syndrome needs to be distinguished from the Leukocyte Adhesion Defects (12.2) and the Hyper-IgE (Job's) syndrome (10.8.1).

10.6 Hereditary metabolic defects

Several hereditary metabolic defects other than adenosine deaminase and purine nucleoside phosphorylase deficiency can also impair immune function. In the instances listed below the impairment of immune function may be only a minor component of the manifestations of the disease.

10.6.1 Transcobalamin 2 deficiency. Autosomal recessive defects in the vitamin B_{12} transport protein, transcobalamin 2, have been described. These defects impair the normally rapid cell proliferation required for haematopoiesis, lymphocyte proliferation and gastrointetinal tract epithelial cell regeneration. Affected infants present with diarrhoea, failure to thrive, megaloblastic anaemia, defective granulocyte function and immunodeficiency involving primarily B lymphocyte function. Administration of vitamin B_{12} in pharmacological doses rapidly reverses the signs and symptoms. Folinic acid may also be required.

10.6.2 Methylmalonic acidaemia. Methylmalonic acidaemia is similar to Transcobalamin II deficiency; it represents a series of several distinct enzymatic defects that affect cobalamin (B_{12}) metabolism and result in the accumulation of excess levels of methylmalonic acid which inhibits bone marrow stem cell growth. Leukopenia is common; B-cell numbers and serum IgG may be reduced. Folic Acid treatment may reverse the problem.

10.6.3 Type I hereditary orotic aciduria. An autosomal recessive disease which presents with retarded growth, recurrent diarrhoea, megaloblastic anaemia, increased numbers of infections (including fatal meningitis and varicella), and lymphopenia with decreased numbers of T lymphocytes and impaired cell mediated immunity.

10.6.4 Biotin-dependent carboxylase deficiency. Infants affected with this autosomal recessive condition present with convulsions, ataxia, alopecia, Candida dermatitis, keratoconjunctivitis and increased urinary excretion of beta-hydroxyproprionic acid. Isolated IgA deficiency and reduced numbers of peripheral T and or B lymphocytes have been reported. Biotin administration results in biochemical and clinical improvement.

10.6.5 Mannosidosis. This lysosomal storage disease resembles Hurler syndrome with abnormal facies, dysostosis, hepatosplenomegally and recurrent infections. The accumulation of the mannose rich lysozomes may interfere with both neutrophil and lymphocyte function.

10.6.6 Glycogen storage disease, Type 1b. Patients with this variant of glycogen storage disease may have neutropenia and neutrophil dysfunction, presumably due to defective glucose metabolism. They have recurrent infections.

10.6.7 Chediak–Higashi syndrome. This autosomal recessive disease is characterized by partial oculo-cutaneous albinism due to dysmaturation of melanosomes. Giant granules are found in all nucleated cells: abnormalities of granulocyte and monocyte mobility and chemotaxis, and defective NK cell activity are demonstrable. There is commonly an associated acute lymphoproliferative phenomenon resembling familial lymphohistiocytosis (FLH) which leads to cytopenia and hypofibrinogenaemia.

10.7 Hypercatabolism of immunoglobulin

Many diseases are associated with hypercatabolism of Ig. These can be distinguished from failure of Ig production by metabolic studies. The following are some of the conditions in which hypercatabolism of Ig may lead to immunodeficiency.

10.7.1 Familial hypercatabolism. A kindred has been described with recurrent infections, bone abnormalities, abnormal glucose metabolism and diminished levels of serum albumin and immunoglobulin which could not be explained by increased gastrointestinal or urinary losses.

10.7.2 Intestinal lymphangiectasis. Losses of lymphocytes and immunoglobulins into the gut can result in significant lymphopenia, diminished cell mediated immunity and decreased serum Ig levels.

10.8 Other

10.8.1 Hyper-IgE syndrome. Recurrent (usually staphylococcal) abscesses which are often 'cold', lung abscesses which result in pneumatocoeles, skeletal anomalies, coarse facies, eosinophilia and very high serum levels of IgE are characteristic of the Job or hyper-IgE syndrome. The B lymphocytes from these patients spontaneously produce large amounts of IgE *in vitro*. Several kindred involving both males and females, and affected mothers or fathers with affected children have been reported, suggesting that in some instances the disease is autosomal dominant. Sporadic cases also occur. The immunological defect is not yet fully understood.

10.8.2 Chronic muco-cutaneous candidiasis. These patients have severe persistent candidal infections of skin and mucosa. They have markedly impaired cell mediated immunity to *Candida* antigens. The relationship, if any, between the specifically diminished T-lymphocyte responses and infection is not understood. A mannose deficiency has been noted in the monocytes of some patients. This condition is frequently associated with familial polyendocrinopathy.

10.8.3 Hereditary or congenital hyposplenia or asplenia. Persons with hyposplenia or asplenia (whether post-traumatic, surgical, congenital or hereditary) are at increased risk for sepsis. Infants with congenital or hereditary asplenia are particularly prone to such infections. The responsible organisms are usually pyogenic, pneumococci being the most common. Increased susceptibility to intracellular parasites (for example, malaria) and some viral agents has also been reported.

10.8.4 Ivemark syndrome. This syndrome probably represents disturbed laterality including partial *situs inversus* during very early embryogenesis resulting in major defects in organ formation. In addition to cardiac defects, asplenia is found. The problem of infection are as described in 10.8.3.

10.8.5 Familial intestinal polyatresia. Multiple areas of atresia throughout the gastrointestinal tract characterize this AR syndrome. Three male siblings in one affected family were described to have an associated severe combined immunodeficiency with markedly decreased T-cell number and function, and reduced immunoglobulins.

11 COMPLEMENT DEFICIENCY

11.1 Complement system

The classic complement system consists of nine numbered components (Cl to C9) and five regulatory proteins (Cl inhibitor, C4 binding protein, properdin, Factors H and I). The first component (Cl) is comprised of three subcomponents, Clq, Clr, and Cls. It is the molecular interaction between Clq and aggregated IgG or IgM (as in an antigen-antibody complex) that initiates activation of the classic complement sequence. The fixation of Clq activates Clr and Cls. Cls

cleaves C4 and C2, whose active fragments C4b and C2a form the classical pathway C3 convertase.

An alternative pathway to C3 activation consists of C3b, Factor B and Factor D. Factor B binds to a cleavage fragment of C3, C3b, to form C3bB. Factor D cleaves the bound Factor B to form the alternative pathway C3 convertase (C3bBb). It activates C3 in a fashion similar to the C3 convertase of the classical pathway, C4b2a. Properdin appears to stabilize this alternative pathway C3 convertase.

A large number of biological activities important in the inflammatory response and in host resistance to infection take place at various steps in complement activation. The lytic property for bacterial or animal cells, however, requires the activation of C5 to C9 by the classical or alternative pathway. The enhancement of phagocytosis by complement is probably of great biological significance and requires the deposition of C3b or iC3b on the particle to be ingested. Certain viruses are neutralized after interaction with antibody and only the first two complement components (Cl and C4); other viruses require C2 and C3 in addition. Immune adherence, a property whereby antigen-antibody complexes adhere to complement receptor 1 (CR1), occurs with complement activation through the C4 and C3 steps. The ligands for CRl are C4b and C3b. Histamine release from mast cells, smooth muscle contraction and increased vascular permeability caused by anaphylatoxin activity are properties of each of the two small fragments (C3a and C5a), which are released when C3 or C5 are cleaved by their respective convertases. These fragments are also chemotactic for polymorphonuclear leukocytes, particularly C5a, which also causes exocytosis of neutrophils. The classic complement pathway appears to be important in the dissolution of immune complexes.

11.2 Genetic defects in human complement

Genetic defects have been described for almost all the complement components in humans, including Clq, Clr (and Cls), C4, C2, C3, C5, C6, C7, C8 and C9 deficiency (Table 4). In all these instances defects are transmitted as phenotypically autosomal-recessive traits, and the heterozygotes can usually be detected because their sera contain approximately half the normal level of the deficient component as determined by functional and/or immunochemical tests. Nonfunctional variants of Clq have been described. C8 deficiency is unusual in that the β chain is not covalently associated with the α and γ chains. Thus affected Caucasian C8 deficients lack the β chain and black C8 deficients lack the α, γ chains. Both forms have nonfunctional, incomplete C8 molecules in their serum. C9 deficiency has a very high incidence in Japanese. Genetic deficiencies in the alternative pathway are very rare. Deficiency of properdin is X-linked. The mode of inheritance of Factor D deficiency is not entirely clear.

Genetic defects have also been recognized for three inhibitors of the complement system: Cl inhibitor, Factor I and Factor H. Deficiency of the Cl inhibitor is inherited as an autosomal dominant. This deficiency is associated with hereditary angioedema (HAE), or Quincke's disease. In 15% of affected kindred the sera contain normal or elevated amounts of an immunologically cross-reacting (CRM+), non-functional protein due to missense point mutations in the C1 inhibitor gene in the exon encoding the active site. In the majority of affected kindred the defects are due to nonsense mutations or unequal crossovers in the Alu sequences of introns 4, 5, 6, 7 and 8.

The genes for factor B, C2 and C4 are located on the short arm of chromosome 6 between HLA-D and HLA-B. The C4 gene is duplicated and the two genes are designated *C4A* and *C4B*; C4A molecules usually bear the Rodgers blood group substance and C4B the Chido blood group substance. Complete C4 deficiency is very rare and occurs only when all four alleles (the 2 of C4A and the 2 of C4B) are not expressed. In one case, this was due to isodisomy of a paternal chromosome 6p that was deficient in *C4A* and *C4B* (C4AQO and C4BQO). Thirty-five per cent of individuals in all racial groups lack one to three C4 alleles. Those with C4AQO have a high incidence of SLE and juvenile rheumatoid arthritis. The genes for factor B and C2 are so tightly linked that no crossover has yet been observed between them, but unequal crossover in the MHC may result in the expression of three C4A alleles and one C4B allele, or vice versa.

Genetic polymorphisms are known for C4A, C4B, C2, Factor B, C3, C6, C8α, and C8β. Polymorphic variants of C5, C7, Factor D, Factor H, Factor I and C1 inhibitor are rare.

All patients with complement deficiency are more or less unduly susceptible to infection and to development of immune complex disease. For example, patients with Cl inhibitor deficiency (HAE) have prominent angioedema but are also prone to develop immune complex disease.

Impeded androgens have proved extremely effective in the treatment of hereditary angioedema. Purified Cl inhibitor preparations are available for intravenous administration and should be used in the treatment of acute attacks of angioedema. There is no satisfactory replacement therapy for the other complement deficiencies, largely because the catabolic rate of these proteins is very high. Sometimes patients with late component deficiencies require anti-microbial prophylaxis or immunizations because of recurrent neisserial infections.

12 DEFECTS OF PHAGOCYTIC FUNCTION

Apart from neutropenia, which has many causes, some of which are genetically determined and are not considered here, there may be genetically determined defects of phagocyte function, affecting polymorphonuclear and/or mononuclear phagocytes. Neutrophil function depends on movement in response to chemotactic stimulus, adherence, endocytosis, and killing or destruction of the ingested particles. Mobility depends on the integrity of the cytoskeleton and the contractile system; directional mobility can be receptor mediated. Endocytosis depends on the expression of certain membrane receptors, for example, for IgG, C3b and iC3b, and on the fluidity of the membrane. Defects of phagocytic function and their associated features are listed in Table5.

The measurement of nitroblue tetrazolium (NBT) dye reduction by actively phagocytosing leukocytes has been accepted as a standard measure for the adequacy of super oxide production. More sensitive assays include chemiluminescence and the direct measurement of superoxide. Assays for bacterial killing yield highly variable results depending on the bacterial species used in the assay. Assays for chemotaxis can be performed by the use of Boyden chambers or migration in agarose. Defects of contractibility can only be assessed with Boyden chambers.

12.1 Chronic granulomatous disease (CGD)

Defects in intracellular killing of ingested micro-organisms usually result from failure of production of superoxide radicals, oxygen singlets, and hydrogen peroxide. This failure results in chronic granulomatous disease (CGD). The organisms cultured from lesions of patients with CGD are generally catalase-producing and include Staphylococci, *E. coli*, *Serratia marcescens*, fungi, such as *Nocardia*

and aspergillus, and other organisms with formation of chronic infected granulomas, especially of lymph nodes, liver and lung. The reaction NADPH + $2O_2^{\cdot}$—› NADP + $2O_2^{-}$ + H^+ requires a phagocyte-specific cytochrome b and NADPH oxidase. This cytochrome b558 is a heterodimer in the phagocyte membrane and is composed of a 91 kd chain and a 22 kd chain, which bears the cytochrome heme. When phagocytes are activated, the components of NADPH oxidase in the cytosol are phosphorylated and they migrate to the membrane and bind to cytochrome b558. X-linked CGD results from a defect in the 91 kd protein. In some cases, CGD is associated with a defined deletion in the short arm of the X chromosome at Xp21. In some cases of autosomal recessive CGD, the 22 kd protein whose gene is encoded on chromosome 16 is defective or one of the two components of NADPH oxidase, p67phox or p47phox, are defective.

12.2 Leukocyte adhesion defects (LAD)

Recently, a large number of cases have been described with a defect in the iC3b receptor of phagocytes (CD11b), the C3dg receptor of phagocytes called p150,95 (CD11c) and the LFA-1 (CD11a) adhesion molecule of T lymphocytes and phagocytes. This deficiency results from abnormal biosynthesis of a 95 kd β chain (CD18), which is common to the iC3b receptor, p150/95 and LFA-1; the gene encoding the beta chain maps to chromosome 21. This defect has been called leukocyte adhesion defect (LAD1). It is inherited as an autosomal recessive disorder. Two entities formerly described as 'actin dysfunction' and 'GP110 deficiency' are now known to be due to this deficiency. The phenotypic expression of the leukocyte adhesion defect is variable. In the severe phenotype <1% of normal adhesion molecules are expressed whereas in the moderate phenotype approximately 10% of these molecules are expressed. Patients with defects in mobility and adherence and endocytosis (see Table 5) usually present with infections of skin, periodontitis and intestinal or perianal fistulae.

A second form of leukocyte adhesion deficiency (LAD2) has been described in unrelated Palestinian children. These infants are unable to synthesize fucose from GDP mannose so that they cannot form the Lewis x ligand for the selectin molecules. The phenotype in this form of LAD is similar to the common form of LAD except that mental retardation has been noted in the former. It is inherited as an autosomal recessive. The enzyme defect has not been precisely defined and its chromosome map location is therefore not known.

12.3 Neutrophil G6-PD deficiency

Glucose-6-phosphate dehydrogenase (G6-PD) is a necessary component of the hexose monophosphate shunt. The G6-PD gene, located at Xq28, is prone to frequent mutations; over 200 variants have been recorded. In Neutrophil G6-PD deficiency the variant leads to a severely defective enzyme and, because of its function in the NADPH system, results in reduced intracellular H_2O_2 production on leukocyte activation. As in CGD, there is failure in the killing of catalase positive intracellular organisms. The clinical presentation is the same as in CGD except that it occurs at a later age. Since NBT cannot be reduced, the NBT test can be used for ascertainment. Reduced G6-PD in red blood cells causes concomitant anaemia.

12.4 Myeloperoxidase deficiency

Myeloperoxidase is one of the more abundant enzymes in polymorphonuclear leukocytes. The gene is located at 17q21.3-q23. Deficiency is not uncommon (1:2000 to 4000 in the US) and is generally without adverse effects. Granulocytes lacking the enzyme fail to kill *Candida*; some affected persons (presumably having a more defective mutation and often in association with other diseases) have suffered from severe, recurrent candidal infections.

12.5 Secondary granule deficiency

Neutrophils have two types of granules which contain a variety of enzymes. In a small group of patients described to have abnormal neutrophil structure (bi-lobed nuclei), specific secondary granules (which normally contain lactoferrin) are absent. Defective oxidative mechanisms and diminished bacterial killing have been described. Clinically there are increased numbers of skin infections and progressive pulmonary disease. The precise nature of the defect is unknown. The category may contain more than one entity.

12.6 Schwachman syndrome

Hereditary pancreatic insufficiency associated with neutropenia, defective neutrophil mobility and chemotaxis, thrombocytopenia and anaemia are the principle features of this syndrome. Affected infants have recurrent pyogenic sinopulmonary and skin infections and may have hypogammaglobulinaemia. It is inherited as an AR.

12.7 Other

Phagocyte function may also be defective in a number of generalized diseases, such as diabetes, liver failure, glycogen storage disease type II b, etc. The phagocytic dysfunction does not constitute a characteristic or diagnostic feature of these diseases. Certain syndromes (such as the hyper-IgE syndrome) may be associated with a secondary chemotactic defect.

12.8 Treatment

Infections should be treated with appropriate antibiotics, surgery, and in case of septicaemia, neutrophil transfusion. Sulfamethoxazole-trimethoprim prophylaxis is valuable, especially for CGD. There are reports of improvement in some CGD patients with interferon-γ treatment. Bone marrow transplantation has been successful in some patients with LAD1.

FOOTNOTES

[a]These determinations require special facilities and can be arranged by writing to Dr H.D. Ochs, Department of Pediatrics RD-20, University of Washington, Seattle, WA 98195, USA.

[b]Obtainable from the Institut Pasteur-Merieux, Lyon, France.

[c]Antigen and assay obtainable from Dr M. Eibl, Institute of Immunology, Borschkegasse 8a, 1090 Vienna, Austria.

[d]Obtainable from Merck, Westpoint, PA 19486, USA.

[e]Obtainable from Hollister-Stier Labs, Box 3145, Terminal Annex, Spokane, WA, USA.

[f]Undiluted glycerin-free Dermatophytin (Trichophyton), Hollister-Stier Labs, Box 3145, Terminal Annex, Spokane, WA, USA.

[g]Mumps skin test, Eli Lilly & Co., Indianapolis, IN 46206, USA.

[h]Paediatric diphtheria and tetanus toxoid, Wyeth Laboratories, P.O. Box 8299, Philadelphia, PA 19101, USA.