Conference Reports

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Application of immunological techniques to disease

‘Application of immunological techniques to disease’ was the title of a conference held at the Royal College of Physicians on 27 September 1991. The conference planned by Dr Carol Seymour FRCP, dealt specifically with the application of immunological techniques to understanding and controlling disease.

An Overview

Professor N. A. Mitchison (University College, London) gave an overview of immunology in much the same sense that an experienced alpinist atop a savage peak might make sense of the tumbled landscape for a party of terrified novices. His review recalled Peter Medawar’s well-known dictum that more knowledge ultimately leads to simplicity and clarity. Immune recognition and the resulting immune reactions are all about eliminating invading microbes and minimising immune-mediated damage to the infected host. The important point is that these reactions take place in a tightly controlled micro-environment. The boundaries of the reaction are defined by many now-well-understood mechanisms.

Chief among these are methods for confining interactions between immunogenic peptides and T cells. Peptides are processed by antigen-presenting cells and presented in the strict confines of class II HLA molecules, geometrically spaced on the cell surface. Only those T cells with the correct antigen receptor can bind a given peptide, and there is an array of adhesion molecules to ensure that the reacting T cells only engage relevant cells. Selective V gene usage in T and B cells governs the specificity of the response. The resulting limitation of the reaction ensures that cytokines are only generated locally or, at least, that systemic leakage is contained. Indeed, the idea that immune regulation depends on the creation of barriers to immune reactions is, at least in man, more attractive than earlier concepts of suppressor T cells.

This thesis has obvious implications for clinicians faced with immune-mediated inflammatory diseases in which there is prominent local and systemic generation of an array of cytokines. A disease such as rheumatoid arthritis is an obvious example of a process which might result from the successive breakdown of constraints on cytokine generation.

A fundamentally important problem in immunology is to understand peptide recognition by T cells. Among the most dramatic recent advances in immunology has been the elucidation of the structure of peptide binding sites on class I, and to a lesser extent class II, HLA molecules. It is now clear that T lymphocytes recognise processed oligopeptides in the binding grooves of these molecules. In addition to the physiological interest in solving this aspect of immune recognition, variations in peptide binding may help to explain disease associations with the inheritance of certain class I and II HLA molecules.

What the immune system sees

This subject was discussed by Dr J. J. Skehel (National Institute for Medical Research, London). Our knowledge of antigen recognition by antibodies has stressed the importance of quaternary structure and not just peptide sequences in determining the location of antibody-binding sites on complex protein molecules. Information is increasingly about the sequences of the oligopeptides which occupy the peptide binding groove of the HLA B27 and other class I molecules. These are usually oligopeptides of nine amino acids with apparent restrictions on the residues in certain positions which permit occupancy. Many points about the peptides engaging the binding groove are still
unsettled. For example, it is not yet clear whether binding resembles the exact fit of a lock-and-key mechanism or whether peptide occupancy induces changes in the configuration of the binding groove. Information is accruing on the equivalent peptide binding by class II molecules. The oligopeptides so far recovered from binding grooves on cell lines contained 14–15 residues, with a preponderance of sequences with homology for heat-shock proteins and histones. However, information is still relatively limited on structural aspects of peptide binding to class II molecules.

Obviously, this kind of structural information does not establish the basis for genetically determined susceptibility to organ-specific autoimmune and related disorders. However, it does provide the experimental basis for testing current ideas that this susceptibility stems from factors such as the association of self or crossreactive these generate, and the repertoire of T-cell responses that is elicited.

**Human T-cell cloning**

The science of human T-cell cloning has now reached a level of sophistication where it is possible to generate lines with virtually any desired specificity and function. As Professor J. R. Lamb (St Mary’s Hospital Medical School, London) explained, this progress can be attributed to elucidating the structure and function of key surface molecules on T cells, improved techniques for propagating T-cell lines in vitro, and transfection methods for introducing novel genes into T cells. The medical applications of these techniques are considerable. It is already practicable to characterise the infiltrating T cells in inflammatory lesions and to probe the phenotypes and behaviour of T cells mediating atopic and immune disorders in experimental and clinical situations. It is still speculative whether T-cell clones of their products can be used to restore defined immune defects or to counter the activities of deranged T-cell populations.

**Major histocompatibility complex and disease**

Nothing in immunology more charms its devotees or more irritates its critics than the ephemeral nature of immunological dogma. Dr R. Duncan Campbell (MRC Immunochemistry Unit, Oxford) stressed that there are more things in the human major histocompatibility complex (MHC) which might account for disease associations than are addressed solely by elucidating the structure and contents of peptide binding grooves in class I and II molecules. The MHC complex spans some 3500 kb of DNA in the 6p21.3 band of the short arm of chromosome 6. The organisation of these genes has been established by a combination of techniques, including recombination analysis, cosmid walking, and pulsed-field gel electrophoresis. The polymorphic histocompatibility antigens involved in the genetic regulation of immune responsiveness are located in discrete regions. Class I coding genes are found in a 1500 kb region at the telomeric end of the MHC complex and the 800 kb class II multigene family is at the centromeric end of the complex. The class III region spans some 1100 kb between the class I and II regions. Many of the proteins encoded by genes in this region contribute to the immune system, notably the complement components C2, C4, and factor B, the cytokines tumour necrosis factors (TNF) α and β, and the major heat-shock protein HSP70. However, there are at least 20 further genes in the class III region encoding proteins whose functions are mainly unknown; they may be in linkage disequilibrium with HLA alleles associated with diseases such as type I diabetes mellitus and rheumatoid arthritis. Indeed, an association of some C4 and HSP70 loci with type I diabetes has been detected. However, the main features of class III DNA structure are marked stability and multiple ‘housekeeping’ sequences with RNA transcripts in all tissues so far examined. To date, a cosmid library of 38 mainly discrete single copy genes and their mRNA transcripts has been examined. There is evidence for marked polymorphism in the HSP70 coding genes. These proteins, and others coded by class III genes, may be involved in the transport of immunogenic peptides to the surface of antigen-presenting cells and their presentation by HLA molecules. The data now emerging from these recent studies offer the prospect of discovering susceptibility genes for immune diseases other than those encoding class I and II HLA molecules.

**Heat-shock proteins in rheumatoid arthritis**

Dr P. M. Lydard (Middlesex Hospital, London) reviewed the evidence for implicating an immune response to heat-shock proteins (HSP) in the pathogenesis of rheumatoid arthritis. HSP are ubiquitous and abundant and have been identified in species as diverse as bacteria, fungi, and humans. Moreover, HSP are highly conserved, human and bacterial proteins showing 60% sequence homology. HSP have an immense capacity to interact with a large array of intra-cellular proteins. Many 'chaperone' functions have been attributed to HSP, including protein folding and unfolding, assembly and disassembly, and sorting and translocation. Thus their role in oligopeptide presentation to T cells is just one of a number of suggested functions. Almost inevitably, HSP have been implicated in the pathogenesis of autoimmune disease, whether determined by molecular mimicry, whereby bacterial HSP and tissue auto-antigens present similar epitopes to T cells, or by sequence homology due to sequence similarities in bacterial and self antigens. Patients with rheumatoid arthritis mount T and B immune responses to HSP both systemically and locally in the affected joints. Moreover, HSP-reactive T-cell clones have been established from rheumatoid syn-
ovial tissues. However, such tissue is a rich source of immunological memory cells in general, no auto-reactive specificity has been detected in these clones, and there is no HLA DR4 restriction despite the association of this antigen with rheumatoid arthritis.

As Dr Lydyard's presentation was as objective as it was well informed and illuminating, it is not surprising that the audience was no more persuaded by the force of the argument than is the rheumatological community at large.

Auto-antigens in autoimmune disease

Autoimmune thyroiditis is a pertinent example of a general problem in autoimmunity, namely to characterise the epitopes which are the targets for the abnormal immune response. Dr J. P. Banga (King's College Hospital Medical School, London) described an elegant experimental system for defining and binding sites of one group of thyroid auto-antibodies. Cloned cDNA templates of thyroid peroxidase were used in conjunction with the polymerase chain reaction to express selected segments of the thyroid microsomal/peroxidase antigen as recombinant protein in E. coli. Six small fragments containing 120 residues on average and one large fragment of 269 residues were obtained, which together comprised 80% of the extracellular portion of the molecule. The auto-antibody binding sites of sera from different patients were analysed by immunoblotting. Six antigenic binding sites were identified on overlapping fragments, with marked variation in different sera.

It will also be necessary to define the equivalent epitopes on tissue antigens which are recognised by T cells. This will in turn open up the prospect of directing T-cell responses by first characterising the epitope-specific receptors on the reactive cells and then applying the emerging techniques for epitope-based immunotherapy.

Chronic granulomatous disease

Professor A. W. Segal (University College Hospital, London) described the molecular basis of this disease in which neutrophils are unable to kill ingested microorganisms. It lies in a defect of a multicomponent microbiocidal oxidase that normally yields the low midpoint potential cytochrome b245. The defect is X-linked in the majority of patients but in 30% it is autosomal recessive. In more than 90% of patients with autosomally recessive disease there is defective synthesis of a 47 kDA cytosolic component of the oxidase. In three unrelated patients with this mode of inheritance, a dinucleotide deletion at a GTGT tandem repeat has been discovered, corresponding to the acceptor site of the first intron-exon junction. Deletions and mutations at this site may frequently give rise to genetic defects in this enzyme system in the granulocytes of affected individuals. These rare inherited disorders are of great immunological interest for two reasons: the defects are not confined to granulocytes but also affect antigen presentation through proteolytic defects in macrophages and other antigen-processing cells; second, inherited deficiencies in the integrin system produce other forms of immunodeficiency which may mimic classical chronic granulomatous disease. It is also possible that, as with the complement system, acquired disorders may affect adhesion molecules in ways which are as yet unrecognised.

Complement and disease

It is a great tribute to the communicative skills of immunologists that general medical audiences are now prepared to listen to addresses on complement with attention and even pleasure, a trend continued by Dr B. Paul Morgan (University of Wales College of Medicine, Cardiff). Complement comprises a series of proteins that eliminate microbial and other antigens from blood and tissues. This is achieved by complement components operating alone or in collaboration with antibodies and cells that express complement receptors. The primary function of the two pathways of complement activation is to form enzymes termed C3 to C3b. The C3b molecules form cluster round the C3 convertases which with iC3b, a product split from C3b, are strong ligands for complement receptors CR1 and CR3 expressed by phagocytic cells such as granulocytes and macrophages. Further enzymes generated within the C3b clusters activate C5, C6, and the membrane attack complex. This process achieves three main objectives: targeting microbial and other antigens to cells expressing complement receptors, recruiting phagocytic cells to inflammatory lesions, and destroying target membranes on cells and microorganisms by generating pore-forming proteins. As a point of practical importance, measuring plasma concentrations activation fragments such as C3a, C5a, Ba, C5d, and C4d is far more informative than traditional measurements of total haemolytic activity or C3 and C4 concentrations.

While complement deficiency diseases are well recognised, there is equal interest in countering tissue damage induced by complement activation. An indispensable first step is to understand the normal mechanisms for limiting complement activation and its effects. These include regulatory proteins that inhibit spontaneous activation in the fluid phase or protect host cells from the destructive effects of activated complement; membrane-bound complement inhibitors are widely distributed on the surface of cells exposed to complement; decay-accelerating factor (DAF); membrane cofactor protein (MCP); C8 binding protein and CD59 are anchored to the membrane by glycosylphosphatidylinositol linked to their carboxy terminus, allowing these inhibitors to diffuse rapidly along the membrane in order to mop up complement ligands such as the C3 convertases and the membrane attack complex.
Immunopharmacology of asthma

The importance of inflammatory mechanisms in bronchial asthma is well recognised and has practical implications for treating acute asthma and preventing exacerbations of the disease. The immunopharmacological changes in the late phase of asthma were described by Professor S. T. Holgate (Southampton General Hospital, University of Southampton). The interpretation of these events is based on sequential immunohistochemical observations and make it clear that adhesion molecules, interleukin-4, and other cytokines are generated by mast cells and other cells in the bronchial mucosa. Thus the components of an activatable pro-inflammatory cascade are present in bronchial asthma. The intriguing and as yet unsolved problem is to determine whether the potential for these local pro-inflammatory pathways precedes the other pathological events in asthma or is a secondary consequence.

Reprogramming the immune system

The ultimate objective of much current experimental immunotherapy is either to eliminate T-cell clones mediating autoimmune diseases or graft rejection or to tolerance these cells to the relevant antigens. If achieved, this strategy would end an era of non-specific immunosuppression and its attendant risks. Professor H. Waldmann (University of Cambridge) has developed monoclonal antibodies which will tolerate mice to soluble proteins, allogeneic bone marrow, and skin grafts. Moreover, these reagents act by inducing peripheral anergy rather than clonal elimination. The clinical challenge is to induce tolerance by similar methods, preferably with minimum non-specific immunosuppression. That objective was achieved in vaculitis by giving a monoclonal antibody which non-specifically depleted T and B lymphocytes. This was followed by giving an anti-human CD4 monoclonal antibody which maintained the initial remission by putatively inducing tolerance to the hypothetical antigen. Lymphocyte depletion by similar methods has proved most encouraging in rheumatoid arthritis.

If, in contrast to other methods of T-cell depletion, these novel antibodies cure autoimmune diseases such as rheumatoid arthritis, we are approaching not just a chronological millennium. Judgment will depend on results which are self-evidently outstanding or, more soberly, on the outcome of controlled trials. For the present, one has to assume that repeated injections of monoclonal antibodies will be needed. The highly immunogenic Fc portions of unmodified heterologous monoclonal antibodies preclude repeated injections of these reagents and even chimaeric or humanised antibodies induce anti-idiotypic responses to sequences in the antigen-binding of these molecules. The messianic era of immunosuppression has not quite arrived.