Perspective

New Insights into Antigen Recognition

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IMMUNOLOGISTS STRUGGLE WITH THE PROBLEM OF HOW THE immune system manages to recognize and respond to an almost infinite number of foreign organisms and yet fails to turn on components of self. Since the finding of the structure of antibody molecules in the 1960's much progress has been made: for example, the discovery of the rearranging immunoglobulin genes and the identification of the T cell receptor. Many of these advances were discussed at a Whitehead Symposium (October 1986), "Immune Recognition," organized by David Baltimore, Herman Eisen, and David Raulet.

One major topic concerned the specificities for antigens of B and T cells. These two types of cells recognize antigen in different ways. B cells and antibodies often distinguish very effectively between different forms of the same protein antigen. Antibodies to native proteins do not always bind denatured versions of the same molecule, and vice versa. There are exceptions to this rule as manifest by the frequent use of antibodies to native proteins to pick up the same protein, after SDS treatment and boiling, in Western blot procedures. By contrast, T cells do not usually distinguish between native, denatured, and even partially degraded forms of the same protein.

Solutions to the problems of antibody specificity can now be provided from x-ray crystallographic studies. Roberto Poljak (Institut Pasteur) summarized the work from his laboratory on the structure of the complex of a monoclonal antibody bound to a protein target, hen egg lysozyme. In the example most closely studied, the two proteins interact at two almost completely flat surfaces with small indentations at which amino acids from one protein penetrate slightly the structure of the other. This interaction is unlike those previously demonstrated for the binding of antibodies to small hapten molecules, in which antigens bind to marked clefts in antibody molecules.

Antibody binding to lysozyme is achieved with very little distortion of the native structures of either the antibody or its ligand. The two proteins contact each other with many amino acids, which are by no means continuous on either protein; for example, lysozyme amino acids between 18 and 27 and 116 and 129 are involved in the binding. These are brought together on the same surface by folding of the native molecules.

These studies illustrate the importance of protein folding to antibody binding and indicate that framework residues on the surface of the immunoglobulin molecule, as well as those in the so-called complementarity determining or hypervariable regions, may participate in antigen binding.

T cells bind antigen in ways that differ from those used by B cells. Cytotoxic and helper T cells cannot bind free antigen, and only recognize antigen when it is associated with a cell surface glycoprotein coded by the major histocompatibility complex (MHC) of the host. Two major types of protein are produced by the MHC for this purpose, class I molecules, usually recognized in association with antigen by cytotoxic T cells, and class II proteins, recognized with antigen by helper T cells. Data accumulated over the last 7 years have suggested that the antigen recognized by helper T cells is a fragment of the native protein; recent studies with virus products have indicated that this may be true for cytotoxic T cells too. These fragments are thought to bind in a trimolecular complex with the T cell receptor and MHC product concerned.

The nature of this ternary complex has long been a mystery to immunologists and a source of confusion to nonimmunologists since it is difficult to understand how the few class II molecules (for example) produced by any given individual can "bind" the myriad of protein antigens to which the host could be exposed and thus lead to T cell receptor binding and a productive T cell response. Nonetheless, strong evidence in support of this model has recently come from B. P. Babbitt, in Emil Unanue's laboratory (Washington University, St. Louis), who showed by equilibrium dialysis that an isolated peptide from hen egg lysozyme binds to an MHC molecule. In this case, it was known that T cells could recognize the peptide in question in association with a particular class II MHC molecule, I-A^k, but not in association with a different allele of I-A, I-A^d. The work of Babbitt *et al.* provided an explanation for this difference, since their equilibrium dialysis experiments showed that the lysozyme peptide bound I-A^k but not I-A^d.

These findings have been extended by Howard Grey, Soren Buus, and their colleagues (National Jewish Center, Denver), and were discussed at the Whitehead Symposium and are described in this issue of *Science* (page 1353). They demonstrated that a number of peptides bind various class II molecules in patterns consistent with their ability to stimulate T cells. In most cases, the binding constants are perhaps surprisingly high, averaging about $10^{-6}M$. Inhibition experiments indicated that all the peptides tested bind to the same site on a given class II molecule.

MHC binding to antigen fragments does have some properties that are strikingly different to those of antibody-antigen interaction. The latter binding has rapid on and off rates; in the former case, antigen fragment binding is slow, but once the peptide is engaged it releases very slowly, with a half-life of perhaps 30 hours. This may indicate some unexpected process, for example, either the antigen fragment or the MHC molecule may have to adopt a rare configuration for binding to occur. A mechanism for such binding has been suggested recently in a paper by Guillet *et al.* (*Science*, 20 February, page 865) who showed that antigenic peptides for T cells have amino acid homology with the class II molecules presenting them. They therefore suggested that the peptide binds to MHC molecules by displacing the homologous region of the protein, a process that might well have slow kinetics.

These findings do leave immunologists with several problems. First, they confirm that the paradox of MHC-antigen binding does exist, but they do not explain how MHC molecules have evolved to be so promiscuous in their binding properties. Second, the results do not explain how the system avoids being permanently saturated. Results from Unanue's group indicate that peptides derived from self proteins can also bind to self MHC molecules. If the binding constants for these interactions are similar to those found for foreign peptides, and there is every reason to suppose that this is true, how do mouse MHC molecules avoid being permanently saturated with peptides from mouse proteins, those of mouse serum albumin, for example? Perhaps the answer lies in continuous MHC protein recycling, and clearance of bound peptides in some intracellular, acidic, lysosome-related compartment.

Although many mysteries remain, these studies do serve as useful

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lessons for others interested in practical applications of immunology. The model of antibody interaction with hen egg lysozyme indicates the dangers of assuming that antibodies will cross-react between native and denatured proteins. The MHC-peptide binding studies illustrate the dangers of using peptide vaccines as inducers of immunity. This was indicated at the Whitehead symposium by Louis Miller (National Institutes of Health) who reported on resistance to malaria. The malarial sporozoite bears a protein with extensive peptide repeats. Antibody against this peptide is known to protect against infection. The repeating peptide is, however, immunogenic only in mice that express I-A^b, indicating that other mouse class II proteins may be unable to bind the peptide, and hence unable to stimulate helper T cell and consequent antibody responses in other strains of mice. Presumably work with peptide vaccines in man will have to take into account the variability of human class II molecules, and their consequent likely variation in antigen peptide binding and ability to present these peptides to helper T cells.

If antibody molecules and T cell receptors use different methods to bind antigen, what is the source of this difference? Both antibody molecules and T cell receptors are made up of two varying polypeptides, light and heavy chains for immunoglobulins, and α and β chains for T cell receptors. All these chains use similar strategies and probably, as indicated by studies from Fred Alt's laboratory (Columbia University, College of Physicians and Surgeons, New York), the same enzymes, to assemble functional genes by rearrangement from their component genetic parts of V (variable), J (joining) and constant regions, and D (diversity) regions as well for heavy chains and β chains. During construction of functional, rearranged genes both immunoglobulin and T cell receptor genes are subject to insertion, at the V, D, and J joining points, of random nucleotides, called N regions, which are not encoded in the genome. Some years ago David Baltimore and his colleagues suggested that N region insertions might be catalyzed by terminal deoxynucleotide transferase, an enzyme found at highest concentrations in the thymus and bone marrow. Recent experiments from Baltimore's and, independently, Alt's laboratories have indicated that this is true.

In spite of these similarities, however, it is clear that immunoglobulins and T cell receptors each have their own sets of V, D, and J region genes, and it is therefore likely that even though there are very many possible sequences for each type of molecule, these sequences may obey some general rules that impose consistent differences on the ways in which the two types of molecule recognize antigen. Such a suggestion may apply particularly to T cell receptors, which must always bind MHC proteins as well as antigenic peptides. Binding of the MHC proteins may demand some common geometrical or structural feature of the receptor molecules. This is, indeed, a possibility that was suggested by Neils Jerne 15 years ago.

Several types of experiment indicate that T cell receptors have indeed evolved to have a particular affinity for MHC proteins. For example, my colleagues and I reported the identification of a mouse V_β sequence that reacts with a monoclonal antibody, KJ23, and which seems to have a particularly high affinity for mouse class II I-E molecules. T cell receptors that use this V_β region recognize I-E molecules with unexpectedly high frequency regardless of the other components of the receptor, such as J_β or the α chain. This V_β is therefore a striking illustration of the idea that germline T cell receptor genes have coevolved with the class I and class II protein products of the MHC so that the two have some affinity for each other.

The data obtained with KJ23 and its V_β target are surprising since other investigations of T cell receptor α and β chain usage (illustrated at the meeting by the work of David Raulet) had failed to show that either α or β chain V regions impose a predictable specificity on the T cell receptor that uses them. Rather, the specificity for antigen and MHC of a particular receptor seems to be controlled usually by all its variable sequences, that is, V, N, and J regions of α chains and V, D, J, and N sequences of β chains.

It is possible that germline immunoglobulin genes code for antibody molecules that are also of particular significance to the species that carries them. A well-recognized example is one of the two germline λ chain genes in mice, which codes for light chains used in recognition of α-(1 → 3)-dextran, a common component of bacterial cell walls. Other germline immunoglobulins may have surprising properties; in particular, several investigations have indicated that they bind self antigens with unexpectedly high frequency, for example, 10 to 15 percent of normal mouse spleen cells produce rheumatoid factors, a phenomenon discussed at the Whitehead meeting by Martin Weigert (Institute for Cancer Research, Philadelphia). The significance of such autoantibodies has yet to be understood, but they do serve to illustrate the different biases in T and B cell reactivity, controlled by their different germline genes.

Other factors also allow for differences between the specificities of T cells and B cells; for example, T cells undergo extensive selection in the thymus as they develop. The result of such selection is that only about 1 percent of the cells that develop in the organ actually escape to the periphery to become functional, mature T cells. Two processes that are related to T cell specificity occur in the thymus: (i) the inexplicable phenomenon of self MHC restriction; that is, T cell precursors are selected in the thymus such that their receptors will bind antigen in association with MHC proteins which were expressed in that thymus; (ii) tolerance, as indicated by experiments showing that T cell precursors which recognize self MHC proteins too well are eliminated or suppressed in the organ.

These two phenomena have led to interest in the pathways of T cell development in the thymus, and in the role of the receptor and receptor-like proteins in this process. Many investigations have focused on γ chains, polypeptides that share many features with receptor α and β chains, but that do not form part of the receptor for antigen and MHC on conventional T cells. Although γ chain genes were discovered a couple of years ago by Susumu Tonegawa and his colleagues (Massachusetts Institute of Technology), the protein for which they code has only recently been isolated by Michael Brenner and co-workers at Harvard. This protein was found on the surface of a subpopulation of human lymphocytes in association with another, possibly receptor-like polypeptide called δ, and also loosely bound to the collection of anchoring and signaling proteins, T3, which can also be found bound to the α-β T cell receptor. Several groups at NIH have now collaborated and identified a γ protein in mouse, again associated with δ and T3. Their findings were discussed at the Whitehead meeting by Ada Kruisbeek. Cells expressing γ messenger RNA and protein develop relatively early in the thymus, before those bearing conventional α-β receptors. Whether this reflects any role of γ-δ–bearing cells in determining T cell MHC restriction or tolerance remains to be seen.

Whatever the function of γ^+ cells, something is now known about tolerance induction. Although it has been suspected for many years that tolerance occurs by elimination of self-reactive cells, an alternative point of view, that suppressor cells inhibit self-reactivity by existing clones of cells, has also been suggested. However, experimental proof in unmanipulated animals for either is lacking. My colleagues and I now have data that support the clonal elimination theory. We have examined the expression of the I-E-recognizing, KJ-23–reactive, T cell receptor V_β chain in mice that do or do not express I-E molecules themselves. Peripheral T cells bearing this V_β are reduced in numbers in mice that have I-E proteins. These cells were also deleted from the most mature thymocyte population of such mice, but were present in normal numbers in immature

thymocyte populations. Thus T cell tolerance can indeed occur by elimination and destruction of self-reactive cells, and the stage of thymocyte development at which this might happen is indicated.

Somatic mutations occur frequently in immunoglobulin (Ig) genes, but only rarely in genes for T cell receptors. At first it was thought that IgM antibodies contained no mutations, whereas changes were frequent in IgG antibodies, leading Patricia Gearhart and Leroy Hood (Caltech) to suggest that mutation occurs during and after gene rearrangements associated with switching from IgM to IgG production. Later work has shown that IgM antibodies may indeed have some variability. At the Whitehead meeting Klaus Rajewsky (Koln) described experiments in which the time of variation was analyzed more accurately. He and his colleagues immunized animals with a hapten, NP, and then transferred small numbers of B cells from these animals into recipients, which were then challenged with an anti-idiotypic antibody that binds to the major class of antibodies to NP in these animals. Such a challenge allowed the detection, not only of B cells making antibody which could react with NP, but also of B cells with mutated antibody, which could still react with the anti-idiotypic antibody, but which had varied so that it could no longer react with NP itself. Antibody-secreting hybridomas were made from the animals and analyzed for reactivity, and for variation. Two important findings were made. First, some antibodies did react with the anti-idiotypic antibody, but not with NP, indicating that somatic mutation does not always lead to a "better" antibody; indeed it may give rise to a molecule with no function in the response to the original antigen. Second, antibodies from a single mouse tended to have the same mutations, indicating that somatic mutation had occurred before transfer and not after challenge in the secondary host. This suggests that somatic mutation happens at a particular stage in the primary response, IgM, and, since primary antibodies do not contain many variations, probably not in antibody-secreting cells themselves. More likely mutation occurs in B cells preparing to be memory cells.

In summary, therefore, the meeting served to emphasize the differences between antigen recognition by T cells and B cells and thus to underline the fact that a single line of defense against potential pathogens is not enough.