The T Cell Receptor

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The primary structure of T cell receptor proteins and genes is well understood. Immunologists are now trying to understand the properties of these interesting molecules. Evidence suggests that T cell αβ receptors recognize a complex of an antigen-derived peptide bound to one of the cell-surface products of the major histocompatibility complex (MHC) genes. It is likely that αβ receptors and MHC proteins have coevolved to have some affinity for each other. During T cell development in the thymus, cells bearing self-reactive receptors are deleted by the mechanisms of tolerance, and cells are preferentially allowed to mature if they bear receptors that will be able to recognize antigen plus self-MHC after they have become full-fledged T cells. Some explanations for these phenomena have been tested, but no satisfactory theory can yet be proposed to account for them.

TWO SYSTEMS THAT SPECIFICALLY RECOGNIZE THE ARRIVAL of foreign materials in the body are the immunoglobulin molecules expressed by B cells and antigen-specific, major histocompatibility complex (MHC)–restricted receptors expressed by T cells. The T cell receptors and the cells that bear them have some unusual properties which make them of particular interest to immunologists. The family of T cell receptor polypeptides has apparently coevolved to interact with another gene family, the proteins of the MHC. Furthermore, as T cells mature they undergo stringent selection, allowing only a small percentage of precursor cells to develop fully. T cells bearing receptors that could react with the host's own molecules are deleted, a process known as tolerance induction, and cells are selected, by a process known as self-MHC restriction, if their receptors are particularly likely to be useful to the host.

The ability of immunoglobulins—either on the surfaces of B cells or secreted into serum—to bind antigen has long been recognized, and the amino acid sequences of many antibodies have been known for some time. Moreover, since the pioneering work of Tonegawa (*1*) and others in the 70's, the construction of functional genes for antibody heavy and light chains from multiple variable (V), diversity (D), and joining (J) genes, with all the economy and flexibility this allows, has been well established. The structures of antibody-antigen complexes, solved by x-ray crystallography, have revealed the strategies by which immunoglobulins bind their targets. Small molecules seem to be bound in clefts within the antibody molecules; the clefts are lined by amino acids of the most variable stretches of immunoglobulin light and heavy chains. Larger protein antigens, such as lysozyme and influenza virus neuraminidase, are bound at almost flat surfaces of the antibody molecule, and again the contact residues are the most variable amino acid residues of the protein. Because of folding of the chains of immunoglobulin and protein antigens, the contact residues on the two interacting moieties are not linearly arranged but are derived from different parts of the molecules (*2*).

One of the goals of those of us who study the antigen-specific MHC-restricted T cell receptor (which we will refer to simply as the T cell receptor) is to arrive at similar levels of sophisticated knowledge about the structure of the complex between this molecule and its antigenic targets. Although great strides have been made in this direction in the last few years, much remains to be done. Difficulties have been largely due to the properties of the complex itself.

Receptors on most T cells are made up of two polypeptides, α and β, disulfide-linked to each other and associated on the plasma membrane with a collection of invariant proteins called CD3 (*3*). CD3 is thought to have a role in transmitting—from the outside of the cell to the inside—the information that the T cell receptor is occupied. T cell receptor α and β chains are encoded by genes constructed by rearrangement of several germline genes, similar to the rearrangement of immunoglobulin genes. The α-chain genes in both mice and man appear to include at least 50 different V-region genes and 50 different J-region genes. Their random associations therefore allow the production of at least 2500 different mature α genes. Actually there are more possibilities because nucleotides not encoded in the germline can be introduced, or germline-encoded nucleotides can be deleted at the point of V gene–J gene joining. These so-called N regions can also occur in immunoglobulin genes (*4*).

In man, functional β genes are constructed from the rearrangement of any of about 50 V regions to one of two D regions and one of 13 J regions. Additional diversity is again allowed by the presence of N regions at the V-to-D and D-to-J joining points and by the fact that the two D-region genes can be read in any frame. Thus at least 4000 different β sequences are possible. In most mice this number is somewhat smaller because their genomes encode only about 21 V- and 12 J-region genes (*5*).

Overall, therefore, T cell receptors have more than 10^7 different sequences, a number that is comparable to that estimated for immunoglobulin molecules, and so even though αβ receptor genes, unlike immunoglobulin genes, do not seem to have the capacity to mutate somatically, αβ receptors might be expected to recognize a considerable spectrum of antigenic structures. That they do not in practice have this capacity seems to be at least in part intrinsic to the amino acid sequences of the receptors themselves but may also be due to features of T cell development and stimulation peculiar to this cell type (see below).

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Rearrangement of a particular Vα to a particular Jα, and of a particular Vβ to Dβ to Jβ, allows each T cell to express only one of all possible αβ combinations—that is, αβ receptors are clonally distributed in such a way that each T cell expresses a different receptor. Engagement of the receptors on a particular T cell by ligand (see below) raises intracellular Ca^{2+} levels, stimulates phosphatidylinositol breakdown, and leads ultimately to T cell proliferation and activation. This process of clonal expansion allows the derivation, from a single T cell, of large numbers of cells specific for a particular ligand and enables these cells to help in the immune destruction of the invasive organism bearing that ligand.

T Cell Specificity

Building on earlier work by several groups, Zinkernagel and Doherty (*6*) and others, in the mid-70's, made the seminal observation that T cells respond to antigen only when the antigen is presented in association with products of the MHC. It was subsequently shown that most cytotoxic T cells recognize antigen in association with class I products of the MHC, called A, B, and C in man and K, D, or L in mouse; helper T cells recognize antigens in association with class II proteins, called DR, DP, and DQ in man and IA and IE in mouse. These discoveries led inevitably to two different interpretations: (i) that T cells have a single receptor, which must be occupied by a combination of antigen and MHC or (ii) that T cells have two different receptors, one specific for antigen and a second specific for MHC, both of which have to be occupied to stimulate the T cell bearing them.

Much later work has indicated that the first theory is correct: T cells bear single receptors with specificity for a combination of antigen and MHC. For example, recently several groups have shown that transfer of functional α and β genes from one T cell to another is enough to transfer specificity for both antigen and MHC from the first cell to the second (*7*). Moreover, the single-receptor theory predicts some interaction between antigen and MHC, a prediction that has been borne out by new and exciting work demonstrating the direct binding of antigen to MHC (*8*).

When the issue of a single receptor was first brought up, one of the objections that was raised was that this implied that MHC molecules would be able to bind antigen. Although MHC genes are polymorphic and vary in sequence from haplotype to haplotype, a given heterozygous individual does not synthesize many different MHC sequences, expressing on the order of 6 different class I and 20 different class II molecules. For immunologists accustomed to the tremendous variability of antibody and T cell receptor sequences, these small numbers of MHC molecules did not seem adequate to cope with the universe of antigens. In fact, it now appears that MHC molecules and antigens bind to each other by using (perhaps) unexpected strategies. T cell responses, unlike antibody responses, do not distinguish between native and denatured protein antigens. This is because antigen for T cells is usually not the intact protein molecule but rather a proteolytic peptide product. Zeigler and Unanue (*9*), for example, showed some years ago that inhibition of lysosomal function interfered with the ability of cells to present antigen to T cells (*9*). Later it was shown that inhibited cells could present protein antigens if the antigen had been appropriately "processed," by prior treatment with trypsin, for example (*10*). Isolated MHC molecules also present processed protein fragments but not intact protein to T cells in the complete absence of any antigen-presenting cells (*11*).

Binding studies are beginning to provide explanations for these phenomena. It appears that MHC molecules are able to bind selected peptides and are quite promiscuous in that a single MHC molecule is able to bind a variety of different peptides. Any given foreign protein—for example ovalbumin—can be broken up into many different fragments by proteolytic enzymes inside cells, and in most cases at least one of these peptides is able to bind to at least one of the MHC molecules on the surface of the cell (*8*). The pathways that lead to such binding have not yet been worked out, and it is not clear that binding to class I and class II molecules involves the same routing. However, it is now evident that these processes are usually a prerequisite for T cell recognition. In order for a protein to stimulate T cells in a particular individual, it must give rise to peptides able to bind MHC in that individual (self-MHC), and the host must have T cells bearing receptors able to recognize that combination of antigenic peptide and MHC.

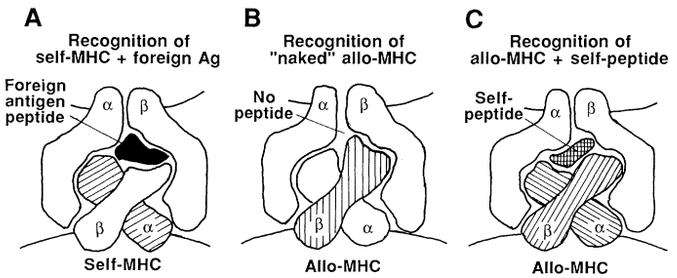

Fig. 1. Are there general rules for recognition of antigen plus MHC? (**A**) A current view of the binding of a T cell receptor to antigen plus a class II MHC molecule is shown. Protein antigens are fragmented by antigen-presenting cells, and some of the peptides so generated associate with class I or class II MHC proteins (or both) expressed on the surfaces of these cells. There the T cell receptor engages the complex. (**B**) A high percentage of T cell receptors is able to recognize foreign MHC molecules. One view is that this occurs by engagement of the receptor with "naked" allogenic MHC as shown here. (**C**) It is likely that MHC molecules must always be bound by peptides. In the absence of foreign antigens, these may be generated by normal catabolism of self-proteins. Thus during turnover of endogenous self-proteins, peptides that can bind to class I and class II molecules can be generated. Recognition of allogenic MHC by T cell receptors may therefore entail recognition of MHC plus a shared self-peptide, as illustrated here.

Our current vision of the binding of antigenic peptides and class II MHC molecules by the T cell receptor is shown in Fig. 1A. An antigenic peptide is bound by the α and β chains of a self–class II molecule. The complex can then be bound by the α and β chains of a T cell receptor. T cell receptors also recognize foreign MHC with high frequency, and there is now some question as to whether this occurs because of binding to a free foreign MHC molecule (Fig. 1B) or to foreign MHC complexed with a, possibly self, peptide as shown in Fig. 1C and previously suggested by Matzinger and Bevan (*12*).

This odd system does have some advantages. T cell recognition of antigen only in association with MHC protein forces T cells to interact only with cell-associated antigen. Since T cell functions involve interaction with other cells, including killing of virus-infected cells and helping B cells to divide and differentiate into antibody-secreting plasma cells, the system allows efficient T cell function even in the presence of soluble native antigen that might otherwise occupy T cell receptors and prevent the cell from interacting with its effective target.

Structure of the T Cell Receptor–Antigen–MHC Complex

One of the problems now facing immunologists is that of discovering the rules by which the binding of receptors to the antigen-MHC complex is governed (Fig. 1). Ultimately, of course, the rules for binding will be established from x-ray crystallographic

analyses, but the appropriate crystals may not be easy to make or analyze. Another problem is that two of the proteins in the complex (the T cell receptor and MHC protein) are membrane-bound and insoluble in water without detergents. Unlike immunoglobulin, these proteins are not made in large quantities by cells, and so far it has not been possible to overcome these two difficulties by producing the two proteins in large quantities and in soluble form by molecular biological engineering. Unforeseen difficulties may also arise from the fact that in its native state the T cell receptor is found in association with CD3 proteins, and although these proteins do not seem to affect the ability of the T cell receptor to be recognized by antibody molecules, they may control its ability to bind antigen and MHC in some subtle way.

In lieu of high resolution data, immunologists have had to fall back on other methods in attempts to understand the structure of the ternary complex made up of αβ T cell receptors, antigen, and MHC. The simplest of these was to find out whether particular α or β chain sequences were associated with particular receptor specificities. For example, at first glance, class I and class II MHC molecules are structurally quite distinct, and therefore one might have expected that some Vα or Vβ sequences would preferentially recognize antigen plus one or the other of these molecules. In fact that does not seem to be the case. Any given V region studied so far seems to be able to form part of a receptor restricted by either class I or class II molecules. In one instance, one α chain can form part of a receptor for either D^d (a class I molecule) or IA^b (class II) (*13*). These findings indicate that the two different types of MHC molecule may look more alike to T cell receptors than they do to the human observer and also suggest that superficial examinations of T cell receptor specificity are not likely to be very revealing.

Some investigators have therefore taken these kinds of studies to greater depths. For example Fink and her colleagues (*14*) have studied T cell receptors specific for a pigeon cytochrome c peptide and the class II molecule IE. Their data suggest that β chains may control much of the specificity of the receptor for MHC (*14*). Saito *et al.* (*15*) have made similar observations, since they find that transfection of a T cell specific for a cytochrome c peptide plus IE^k with the β-chain gene from a cell specific for the same peptide plus IE^k or IE^b transfers MHC specificity. These data indicate that in the T cell receptor–antigen–MHC complex, some, and perhaps most, contact points between the receptor and MHC are made by the β chain of the T cell receptor.

Our own recent data support this idea. We have made an antibody against a mouse Vβ chain, Vβ17a. T cells bearing this Vβ react with unexpectedly high frequency with foreign IE molecules (*16*). For example (Table 1), more than 90% of Vβ17a-bearing cells from SWR mice react with an allele of IE known as IE^s, and although reactions of $V\beta17a^+$ T cells from other strains or with other IE alleles are not so marked, they are still at extraordinarily high frequency. As far as we can tell, Vβ17a can impose the property of IE reactivity on the receptor it is in regardless of other Jβ or Vα sequences in the same receptor, although obviously the rest of the receptors must have some effect on IE binding because not all $V\beta17a^+$ have this property. Overall, however, it seems that Vβ17a can make many contact points with IE and forms the major contributor to IE binding of receptors of which it is a part. These results are therefore in line with those mentioned above, which suggest that receptor Vβ contributes a significant portion of binding sites with MHC to the ternary complex.

Table 1. $V\beta17a^+$ T cell receptors bind IE very frequently. T cell hybridomas were produced by fusing T cell blasts from the indicated strain to BW5147, or to BW5147 lacking a functional α-chain gene ($BW\alpha^-$). The hybrids were then screened for reactivity with different alleles of the mouse class II molecules, IA and IE, using various cell lines or spleen cells bearing these molecules. In some cases monoclonal antibodies were used to inhibit reactions against particular MHC molecules on the presenting cells and thus allow precise identification of the target for T cell reactivity. Data for SWR T cells are derived from 57 hybridomas, for C57L from 42 hybridomas, and for SJL from 22 hybridomas.

Source of $V\beta17a^+$ normal T cell	MHC type	Fusion partner	Percentage of T cell hybridomas recognizing IE			IA		
			b	k	s	b	k	s
SWR	($H\text{-}2^q$)	BW5147	60	68	91	33	7	14
C57L	($H\text{-}2^b$)	$BW\alpha^-$	2	33	35	4	6	0
SJL	($H\text{-}2^s$)	$BW\alpha^-$	23	55	32	0	0	5

Our findings with Vβ17a may reflect another important immunological phenomenon. Neither Vβ17a nor IE are found as proteins in all mouse strains (*16, 17*). One could imagine that mutations preventing failure to express either of these products have been allowed to accumulate during the evolution of *Mus musculus* because the two proteins have evolved to recognize each other too effectively. Animals that produce both proteins have to delete $V\beta17a^+$ T cells (see below) or pay the price of autoimmune diseases and probable death. In an extension of these thoughts, we suggest that other potentially lethal T cell receptor or MHC genes may lie buried and unexpressed in the genomes of mouse and man.

Little else is known so far about the organization of the T cell receptor–antigen–MHC trimolecular complex. Some experiments have indicated that D-, J-, and N-region sequences may control receptor specificity for antigen (*18*). A current best guess for the structure of the complex is that it will resemble the binding of antibody molecules to other proteins. That is, we expect many contact residues between the three components, with perhaps T cell receptor and MHC molecules interacting over large more or less flat surfaces with the antigenic peptide sandwiched between them like a hot dog in a hot dog roll. The recent x-ray crystallographic solution of the structure of a human class I molecule supports such a model, since two polymorphic regions of the molecule line a long cleft that contains unknown material, probably an exogenous peptide (*18a*).

Germline T Cell Receptors Have Affinity for MHC Molecules

Some years ago, Jerne (*19*) raised the possibility that (T cell) receptors might have some intrinsic affinity for MHC. That is, evolutionarily, α- and β-chain polypeptides might have been selected to bind MHC-like structures. Alternatively, we can account for the fact that receptors on mature T cells recognize antigenic peptides only when bound to MHC proteins by suggesting that T cell receptors have as many different types of binding sites as immunoglobulins but that during T cell development, the only T cells that are allowed to mature are those that bear receptors likely to bind MHC-associated antigen. Although this latter suggestion seems unlikely it is in fact possible, since it is known that tremendous selection goes on during T cell development in the thymus, and only a small percentage of all thymocytes actually go on to mature (see below).

There is evidence that Jerne's suggestion is correct, although selection in the thymus certainly also plays a role in determining which T cell receptors appear on mature T cells. Support for the suggestion stems from the following reasoning. As mentioned earlier, an individual's T cell receptors, which normally recognize antigen bound to MHC expressed in the same individual, also tend, with high frequency, to bind MHC proteins from other individuals

in the apparent absence of antigen. At first sight this result in itself might be thought to provide evidence for the theory, but it is subject to the objection that all of these cells that are reactive with foreign MHC have already gone through the selective processes that operate during T cell development.

Obviously, the most straightforward method of testing the theory is to sample the ability of unselected T cell receptors to recognize MHC. This is not as easy as it seems because immunologists are not yet agreed about what constitutes a pre-T cell population that has begun to express receptors but that has not yet been selected. Even if this population could be identified, the guess is that it would not be easy to grow these cells as clones or lines, as all the likely candidates cannot be grown in vitro. Other methods have therefore been tried.

We have done several experiments which suggest that recognition of foreign MHC is inherent to germline nonselected T cell receptors. The most striking of these was our discovery of the Vβ17a sequence with its ability to impose IE reactivity on many of the receptors of which it is a part. Vβ17a is expressed by four mouse strains that have undergone a large deletion of their Vβ genes (*20*). These animals consequently have only about 11 functional Vβ genes instead of the usual 21 or more. The fact that one of these 11, almost regardless of other receptor components, has affinity for an MHC molecule, IE, can hardly be coincidence. On the contrary we interpret our results with Vβ17a to be, admittedly dramatic, illustrations of an inherent affinity between T cell receptor polypeptides and those of the MHC. Evolutionary studies and studies of wild mice will be needed to bear these ideas out. Sophisticated population genetics will be needed to explain the ways in which two multigene families can coevolve to retain some affinity for each other.

Selection of the T Cell Repertoire in the Thymus

T cell precursors seed the thymus (in mice) at about day 12 of gestation. There, they rearrange their T cell receptor α and β genes and also those of two other receptor proteins, γ and δ (*21, 22*) (discussed below). The αβ receptors begin to appear in thymocyte surfaces at about day 17 of fetal life. Several processes that involve the receptor occur before these cells or their descendants are allowed to escape from the thymus as mature peripheral T cells. These include the elimination of self-reactive cells by the mechanisms of tolerance induction and the selection of cells that will continue to mature because their receptors are likely to recognize antigen plus self-MHC in the periphery (*23*). Very little is known about these two processes. One problem in studying them has been the complexity and inaccessibility of thymocyte subpopulations themselves.

Thymocyte subpopulations can be defined in several ways. One useful method has been to describe their surface molecules by means of antisera, or more recently, monoclonal antibodies. For example, thymocytes are often defined by their expression of two surface molecules, CD4 and CD8. Most thymocytes bear both of these molecules and are consequently called "double-positive." Cells that are thought by other criteria to be more mature usually bear, like peripheral T cells, only one of the two molecules (*24*). Very early thymocytes, and perhaps some mature cells, including γδ-bearing cells, lack both surface molecules (double-negative cells) (*25, 26*). The functions of CD4 and CD8 are not completely understood. On peripheral T cells they seem to stimulate the interactions of T cells with their targets, perhaps by binding in some non–clone-specific way to MHC molecules on target cells (*27*). They may also transduce signals (*28*). Their functions in thymocyte maturation are completely unknown.

However, so-called double-positive $CD4^+$ $CD8^+$ cells arise ontologically before mature thymocytes, but after seeding of the thymus with early precursors (*25*). About 50 percent of double-positive cells (which comprise the vast majority of cells in a mature thymus) bear αβ receptors (*22*). They are therefore thought by some to be compulsory intermediates between receptor-negative, double-negative thymocyte precursors and mature thymocytes and peripheral T cells. This conclusion is hotly debated, however, and definitive experiments have not been done, largely because it has been very difficult to stimulate such cells to do anything consistent in suspension culture (except die), regardless of the addition of factors or other stimulatory molecules. Intrathymic transfer of these cells has been similarly unrewarding.

Our own view is that precursor double-negative cells enter the thymus and randomly rearrange and express the entire germline repertoire of T cell receptor sequences, probably at the stage when these cells are also double-positive, $CD4^+$ $CD8^+$. The forces of selection and tolerance then act on these cells, most of which die because they do not bear a receptor that can be selected for recognition of antigen plus self-MHC, or because their receptor recognizes self too well, or because the cell has failed to express a receptor at all. During selection, information is somehow passed to the developing thymocyte with the result that if its receptor is destined to recognize antigen plus class I MHC molecules, the cell will mature to express CD8 only on its surface, whereas if its receptor is to recognize antigen plus class II MHC molecules, it will mature to bear CD4 only.

Tolerance Induction

The fact that our lymphocytes do not respond to our own antigens is one of the most important and mysterious characteristics of the immune system. Tolerance to self must be acquired, because, in general, we all contain the same germline sets of immunoglobulin and T cell receptor genes, and our lymphocytes are certainly capable of recognizing antigens (especially MHC antigens) on the tissues of other members of our own species, and yet, when healthy, we do not usually produce an immune response to ourselves. Experimental evidence supports the idea that lymphocytes learn to be tolerant to self as they develop. One explanation to account for this idea is that lymphocytes go through a particular maturational stage during which contact with antigen is lethal to the cells. Developing lymphocytes are constantly exposed to self antigens, and self-reactive cells are eliminated at this stage. Cells destined to recognize foreign antigens (albeit, in the case of T cells, in association with self-MHC molecules) are not eliminated at this stage because the foreign antigen is not continuously present in the host. Such cells therefore pass through the tolerance-sensitive stage and become mature lymphocytes to await the introduction of foreign antigen at a later stage.

An alternative explanation is that the immune response sets up a network of helper and suppressor T cells and that the two types of T cells are in balance for response to self antigens. Because of the action of suppressor cells, helper and cytotoxic T cells cannot mount a full-blown response to self but only to foreign material for which specific suppressor cells are lacking.

Different as these two theories may be, it has in fact been very difficult to distinguish them experimentally. This difficulty occurs largely because, in the past, it has been possible to measure lymphocyte specificity only by measuring lymphocyte function. Thus, the absence of T cells able to recognize self antigens has been inferred by the failure of the cells to respond to self antigens, a result that can be explained by either theory.

Table 2. Tolerance occurs at a particular stage of thymocyte development. Thymocytes from the two strains of mice were isolated and stained for Vβ17a expression by incubation with biotinylated KJ-23 (anti-Vβ17a) followed by phycoerythrin-coupled avidin. Stained cells were identified on an EPICS cytofluorograph as previously described (*16, 29*).

Mouse strain	MHC type	Expression of IE	Percentage of population bearing Vβ17a	
			Double-positive immature thymocytes	Single-positive mature thymocytes
SJL	s	−	4.1	10.4
C57BR	k	+	3.1	0.6

Recently our experiments with Vβ17a have allowed us to circumvent this problem, since, as mentioned above, this Vβ imparts IE reactivity with high frequency to T cells that bear it. The presence of cells bearing Vβ17a can therefore be used to measure the presence of IE-reactive cells in mice. IE is not expressed in all mouse strains because of mutations in the genes for one or both of its component chains. Using an antibody to Vβ17a, we have shown that T cells bearing this Vβ are very low in number in IE^+ animals, whereas they are at the expected 10% levels in mice that lack IE (*29*). This result demonstrates that tolerance, to self-MHC at least, is caused by deletion rather than by suppression of self-reactive cells.

How, where, and at what developmental stage are self-reactive cells eliminated? There is evidence that they die in the thymus at a particular stage of thymocyte maturation and that death occurs when their receptors bind to "self" on cells in the thymus that have originally migrated there from the bone marrow (*30, 31*). Candidates for bone marrow–derived cells include thymic macrophages, dendritic cells, and thymocytes themselves.

Irradiation followed by bone marrow grafting makes it possible to obtain a mouse that bears IE on its bone marrow cells only. In preliminary studies, we found that such mice lacked Vβ17a-bearing cells; that is, bone marrow cells appear to be sufficient to induce tolerance in such animals.

We have also established the stage of thymocyte maturation at which tolerance occurs. This was done by comparing Vβ17a expression on different thymocyte populations in normal animals that were IE^+ or IE^- (*29*). As shown in Table 2, both types of mice contained about the same percentages of immature double-positive cells bearing Vβ17a, but $V\beta17a^+$ cells were depleted in the mature single-positive population of IE^+ mice. These results suggest that tolerance to self occurs during interaction of developing thymocytes with bone marrow–derived cells at some stage before they differentiate into mature cells. Teleologically speaking, this is not a surprising result. Since immature thymocytes are not able to respond to antigen plus MHC by division and differentiation into, for example, effector cytotoxic cells, their presence is not potentially harmful to the host that harbors them. Mature thymocytes are functional—that is, they can respond to antigen plus MHC and can become effector cells. Hence, even though they are (temporarily) found in the thymus, they are a potential threat to their host.

How self-reactive cells are eliminated remains a complete mystery. Perhaps thymocytes are consumed by thymic macrophages with which they interact. Perhaps triggering of their receptors at a particular maturational stage is a lethal event for developing thymocytes. So far we have little experimental evidence for either theory, although preliminary data from our own and others' work do suggest that receptor-induced Ca^{2+} fluxes, and the consequences of Ca^{2+} fluxes, may be different in immature thymocytes and mature T cells.

Restriction in the Thymus

Restriction for self-MHC is one of the most confusing and contested features of the T cell life history. This phenomenon was first demonstrated about 10 years ago by Bevan and Zinkernagel (*23*) and their co-workers. Their studies showed that if T cells are derived from a thymus-bearing MHC^a, then those cells will be able to recognize antigenic peptides plus MHC^a very well but will only rarely react with antigenic peptides plus MHC^b. This is true even if the T cells themselves contain the genes both for MHC^a and MHC^b.

It has been very difficult to account for these results, especially now that it is known that a single T cell receptor is able to bind both antigen and MHC. Apparently, somehow T cells are picked out to mature in the thymus if their receptors will be able to recognize antigen plus self-MHC but not self-MHC alone in the periphery, and they accomplish this even though antigen is not overtly present in the thymus.

There are lots of theories to account for these findings—none, unfortunately, very convincing. Many of the theories hinge on the fact that selection is supposed to occur because of interaction between receptors on developing thymocytes and MHC molecules on thymus cortical epithelial cells (*30, 32*). For example, one could imagine that cortical epithelial cells are "sticky" and that interaction between cell adhesion molecules on thymocytes and epithelial cells promotes interaction between T cell receptors and self-MHC. Hence, selection picks out cells bearing receptors with a huge range of different affinities for self-MHC, from very high to very low. Subsequently, the processes of tolerance (on bone marrow–derived cells) delete only those cells bearing receptors with moderate to high affinities for self-MHC. Thymocytes with low affinity for self are

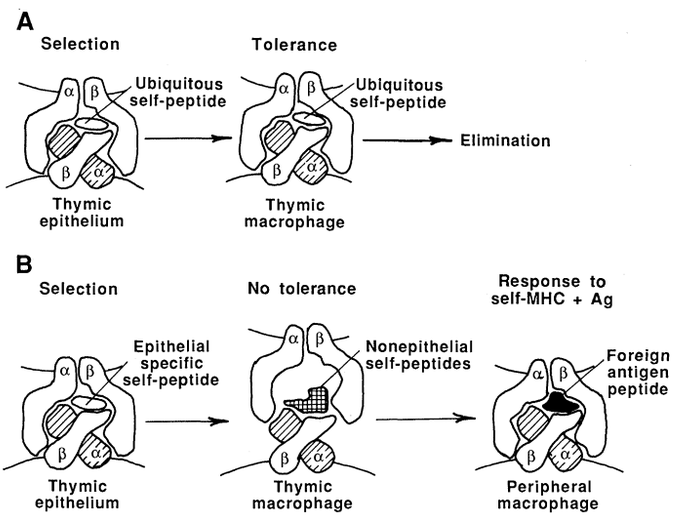

Fig. 2. A "peptide" model for thymus selection. Several theories can account for the paradox that T cells that emerge from the thymus are tolerant to self-MHC, yet the cells that are selected to mature in the thymus have receptors that will be able to recognize antigen in association with MHC alleles expressed on thymus cortical epithelial cells, a selective event that occurs in the apparent absence of antigen. One explanation, illustrated here, is that MHC molecules on thymus cortical epithelial cells are bound by a spectrum of peptides that is not duplicated elsewhere in the animal. (**A**) Some of these peptides will be present on thymus cortical epithelial cells and bone marrow–derived macrophages. Hence thymocytes bearing receptors that engage these combinations of self-peptide and MHC will be both selected and made tolerant. (**B**) Some peptides will be exclusive to thymus cortical epithelial cells. Hence thymocytes bearing receptors that engage these combinations of self-peptide and MHC will be selected but not made tolerant. These cells will mature and form part of the peripheral T cell pool; they will be able to respond to antigenic peptides that mimic the thymic cortical epithelial cell peptide on which they were selected.

allowed to mature fully and later are only able to respond to self when their interaction with self-MHC is boosted by binding to self-MHC of the appropriate foreign peptide.

If this theory is correct we would expect quite dramatic results if $V\beta17a^+$ thymocytes are allowed to mature in thymuses in which the epithelial cells bear IE but the bone marrow–derived cells do not. Such animals can be obtained in several ways—for example, by a combination of thymectomy, irradiation, and bone marrow and thymus grafting, and we have begun some studies in animals of this type. Our preliminary results indicate that $V\beta17a^+$ cells are produced in animals that bear IE on only the stromal cells of their thymuses; thus tolerance does not seem to be induced in response to MHC molecules on thymus epithelium. Surprisingly, however, the presence of IE on thymus epithelium only does not seem to overselect $V\beta17a^+$ T cells (*32*). On the contrary, these T cells are slightly lower in frequency than they are in totally IE^- hosts. Similar results have been obtained in the past by others (*30, 31*). These suggest that the affinity argument laid out above cannot be completely right.

What alternative explanations can be offered? Perhaps partial tolerance occurs on thymic epithelial cells, which may be able to select cells bearing receptors with both high and low affinity for self-MHC, for example, and delete only those with very high affinity for self. Another theory is shown in Fig. 2. Perhaps MHC products on thymic epithelial cells are not exactly the same as those found elsewhere in the body; for example, they may be bound to peptides characteristic of thymic epithelial cells in the thymus, but be bound to different peptides when expressed on B cells and macrophages. As illustrated in Fig. 2, receptors on developing thymocytes may therefore be able to engage a combination of peptide and MHC on thymic epithelial cells that is not expressed anywhere else. Consequently thymocytes bearing these receptors would be selected in the thymus but not subsequently made tolerant. These cells would then mature, only to respond later in the presence of the appropriate foreign antigenic peptide plus MHC.

Such a theory would suggest that $V\beta17a^+$-bearing receptors do not recognize the combination of IE plus thymic epithelial cell peptides very frequently and consequently are not "overselected" in animals containing IE on their thymic epithelial cells, but not elsewhere.

γδ Receptors

The discussion in this article so far concerns T cells bearing αβ receptors—T cells that recognize antigen associated with self-MHC. Recently, however, another set of clonally varying molecules, called γδ, has been found on a small percentage of peripheral T cells and thymocytes (*33*). Like the αβ T cell receptors and immunoglobulins, γδ proteins are made up of two polypeptides encoded by rearranging genes (*34, 35*). Also, like αβ receptors, γδ proteins are expressed on T cell surfaces in association with CD3 (*33*). The αβ and γδ complexes with CD3 seem to activate T cell function and proliferation by similar means.

Current knowledge of γδ proteins indicates that they may have fewer potential sequences than the extremely clonally diverse αβ proteins (*36*). Also, there is some evidence for a shift in the spectrum of γδ polypeptides expressed early in thymocyte development as opposed to those expressed later in life (*37*). There is no evidence that γδ and αβ are ever expressed on the same cell, and indeed the finding that δ genes are encoded between Vα's and Jα's, and would therefore be deleted during α gene rearrangement (*35*), almost precludes the simultaneous expression of αβ and γδ on the same cell. Thus, it is unlikely that γδ can contribute to antigen-plus-MHC recognition by αβ-bearing cells.

There has been much recent interest in γδ proteins, and the function of cells that bear them. The major problem in the field is, however, unsolved, since immunologists have yet to discover the natural ligands for γδ. Will γδ receptors bind native antigen as immunoglobulins do, antigen complexed to MHC as T cell receptors do, or serve some totally different function in the animal?

Hopes for the Future

Many questions about T cells and how they bind antigen remain. In the near future we can probably expect greater understanding of the structure of the ternary complex of antigen, MHC, and the T cell receptor. The use of transgenic mice and other methods may resolve the problems of thymic restriction. The mechanisms whereby self-reactive cells are deleted in tolerance or stimulated to damage the host in autoimmunity may become clearer. We can hope for an understanding of the specificity and function of cells bearing the unexpected γδ receptor. In any event, research on this subject will continue in the future as it has in the past to combine interest in an intriguing and important biological subject with advances in the solution of a problem that has many implications for the treatment and understanding of human and animal diseases.

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Development of the Primary Antibody Repertoire

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The ability to generate a diverse immune response depends on the somatic assembly of genes that encode the antigen-binding portions of immunoglobulin molecules. In this article, we discuss the mechanism and control of these genomic rearrangement events and how aspects of this process are involved in generating the primary antibody repertoire.

HUMORAL IMMUNITY IN VERTEBRATES IS EFFECTED BY antigen-binding antibody [or immunoglobulin (Ig)] molecules that are secreted by cells of the B lymphocyte lineage. The differentiation of B-lineage cells can be divided into two general stages (*1*) (Fig. 1). The first stage is antigen-independent and involves differentiation of stem cells to B lymphocytes, cells defined by the presence of surface Ig that functions as an antigen receptor. In mammals, this process occurs in the liver of the fetus and subsequently is maintained continuously in the bone marrow of adults. Each newly generated B cell (and its progeny) expresses a novel species of Ig molecule on its surface with a unique set of binding specificities. After acquiring antigen receptors, B lymphocytes migrate to peripheral lymphoid organs, such as the spleen and lymph nodes, where contact occurs between lymphocytes and circulating antigens (*1*). In the absence of further stimulation, these peripheral B lymphocytes are nondividing (resting) cells. The second stage of B cell differentiation (antigen-dependent) involves specific binding of antigen to the surface Ig receptor of a resting B cell. This process induces that cell to proliferate; its progeny can then differentiate into plasma cells, the effector cells of the humoral immune system, which secrete into the bloodstream large amounts of an Ig molecule with the same binding specificity as that present on the membrane of the progenitor B cell (*2*).

The membrane-bound form of an Ig molecule consists of two identical heavy and two identical light Ig chains (HC and LC, respectively) (Fig. 2). The carboxyl termini of H and L chains have a constant amino acid sequence (C region) among antibodies of the same class. The amino termini of both HCs and LCs contain regions that vary in amino acid sequence among different clonally derived sets of B lymphocytes; these variable regions are encoded by genes which are assembled from component segments (*3*) in precursor B (pre-B) cells during the antigen-independent stages of B cell development [reviewed in (*4*)]. The variable regions of H and L chains interact to form the antigen-binding site, which determines the particular binding specificities of the Ig molecule. Somatic assembly of variable region genes, together with the combinatorial assortment of different H and L chains, frees the immune system from the limitations of a germline-encoded set of responses and provides the animal with the potential to create an almost infinite number of different antigen-binding specificities. However, the specificity of the immune response depends on antigenic selection of discrete clones of B cells, each restricted to expression of a homogeneous set of Ig receptors [clonal selection; Fig. 1 (*2*)]. Restriction is achieved by limiting a given B cell to functional assembly and expression of only a single HC allele and a single LC allele as Ig chains that associate to form surface antigen receptor. This phenomenon, termed allelic exclusion (*5*), may be a unique property of the family of genes that encode antigen receptors. The total set of antigen-binding specificities (the "repertoire") expressed in newly generated B cells may be different from the repertoire expressed by subsets of peripheral B cells (*6*). Elucidation of molecular and cellular factors that influence development of these repertoires is of fundamental importance to understanding normal and aberrant immune function. This article will focus, primarily, on the aspects of the Ig variable region gene assembly process which influence the "primary" repertoire expressed by newly generated B lymphocytes, but will also consider mechanistic or selective forces that may modify the repertoire in subsets of peripheral B cells.

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Tumor Cell Model Systems

Tumors and cell lines representative of various stages of the B lineage (Fig. 1) have been used to elucidate the mechanism, control, and consequences of the Ig gene assembly process; insights provided by these systems often have been confirmed or extended by studies of corresponding normal cells (*7, 8*). We will briefly introduce