T Cell Reactivity to MHC Molecules: Immunity Versus Tolerance

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The specificity of mature CD8$^+$ and CD4$^+$ T lymphocytes is controlled by major histocompatibility complex (MHC) class I and class II molecules, respectively. The MHC class specificity of T cells is stringent in many assays, but is less evident when cells are supplemented with exogenous lymphokines. The repertoire of T cells is shaped through contact with MHC molecules in the thymus and involves a complex process of positive selection and negative selection (tolerance). Tolerance of immature T cells to MHC molecules can reflect either clonal deletion or anergy and results from intrathymic contact with several cell types, including epithelial cells and cells with antigen-presenting function. Unlike immature T cells, mature T cells are relatively resistant to tolerance induction. In certain situations partial unresponsiveness of mature T cells can be achieved by exposing T cells to foreign MHC molecules expressed on atypical antigen-presenting cells. Tolerance is rarely complete, however, and the precise requirements for tolerizing mature T cells are still unclear.

T CELLS THAT EXPRESS αβ T CELL RECEPTOR (TCR) HETERODIMERS are specific for peptide fragments of antigen bound to MHC glycoproteins (*1, 2*). MHC molecules are highly polymorphic and fall into two classes. Class I molecules are expressed on most cell types and are recognized by the CD8$^+$ subset of mature T cells. Class II molecules are found largely on cells with antigen-presenting cell (APC) function and are recognized by CD4$^+$ T cells.

Because MHC class I and II molecules bind peptidal forms of self antigens as well as foreign antigens, one has to explain how the immune system induces tolerance to a wide variety of self antigens without compromising reactivity to foreign antigens. This dilemma has generated intense interest for many years and is still not fully resolved. Examining the mechanism of tolerance induction to self antigens is not easy, and most investigators have resorted to studying tolerance to foreign antigens, with the hope that tolerances to self and foreign antigens are controlled by similar mechanisms.

Here we discuss tolerance induction to foreign MHC molecules. MHC "alloantigens" are particularly useful for examining tolerance induction for two reasons. First, the precursor frequency of T cells for MHC alloantigens is extraordinarily high (*2*), which makes it easy to examine primary responses to these antigens. Second, the observation that T cell recognition of MHC molecules correlates with the use of particular V-region segments of the TCR β chain has made it possible to detect MHC-reactive T cells with monoclonal antibodies (MAb) to V_β (anti-V_β) (*3*). Before discussing tolerance induction to MHC molecules, one must first consider how T cells mount immunogenic responses to these molecules.

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MHC Specificity of Subsets of T Cells

The specificity of CD8$^+$ and CD4$^+$ cells for class I and class II molecules, respectively, appears to reflect that CD8 and CD4 molecules have binding specificity for conserved regions of MHC molecules (*4*): CD8 binds to class I and CD4 binds to class II. Such binding may be a device to strengthen the interaction of the TCR with polymorphic (immunogenic) regions of MHC molecules. CD8 and CD4 molecules can thus be considered adhesion molecules that augment T cell binding to APCs.

In the case of primary responses to MHC (H-2) alloantigens in mice, the MHC-class specificity of purified T cell subsets is very precise. This is apparent from the response of C57BL/6 (B6) CD4$^+$ and CD8$^+$ T cells to APCs from mutant strains of mice expressing isolated MHC class I differences, bm1, or class II differences, bm12 (*5*). In the absence of added lymphokines, purified B6 CD8$^+$ cells proliferate well to bm1 stimulators, but very poorly to bm12 stimulators. Conversely, B6 CD4$^+$ cells respond well to bm12 but give only marginal responses to bm1. Similar specificity applies in various in vivo assays, including skin allograft rejection and induction of graft-versus-host disease (GVHD).

The stringency of MHC class specificity is unexpected because, at least for V_β expression, the range of TCR molecules on CD4$^+$ and CD8$^+$ cells seems to be quite similar. This is exemplified by studies with T cells expressing V_β17a TCR molecules (*3, 6*). Since V_β17a$^+$ T cells are selectively deleted in mice expressing I-E molecules (a subset of class II molecules), it is argued that V_β17a TCR have innate reactivity to I-E antigens. In support of this idea, it has been found that V_β17a$^+$ T hybridomas can be triggered to release lymphokines [interleukin-2 (IL-2)] in response to I-E$^+$ APCs. Significantly, the I-E reactivity of V_β17a$^+$ T hybridomas applies only to CD4$^+$ and not CD8$^+$ hybrids (*6*). This finding suggests that the affinity of V_β17a TCR for I-E antigens is below the threshold required for cell triggering. Overt stimulation of V_β17a$^+$ T cells appears to depend critically on the extra I-E binding specificity provided by CD4 molecules.

Although this evidence suggests that the MHC class specificity of CD4$^+$ and CD8$^+$ cells is near absolute, the deletion of V_β17a$^+$ T cells in I-E$^+$ mice involves CD8$^+$ cells as well as CD4$^+$ cells (*6*).

Similar findings apply to $V_\beta11^+$ T cells (7). The simplest explanation for these data is that the deletion of $V_\beta17a^+$ and $V_\beta11^+$ cells to I-E antigens occurs at an early stage of thymocyte maturation when T cells are "double-positive" for CD4 and CD8 molecules. However, it is also possible that the deletion occurs at the level of mature T cells. Here it is necessary to postulate that MHC class specificity is not absolute and that T cells do have some capacity to recognize the "wrong" class of MHC molecules. In considering this possibility it should be stressed that the evidence that $CD8^+$ cells are unresponsive to class II molecules refers to helper-independent responses, that is, responses that do not depend on the presence of exogenous lymphokines. These responses might be restricted to T cells with strong reactivity for antigen. Revealing weak reactivity might require the presence of exogenous lymphokines such as IL-2.

Testing whether $CD8^+$ cells can mount helper (IL-2)–dependent responses to class II antigens in vitro is difficult because addition of IL-2 leads to high background responses with syngeneic APC. For in vivo responses, however, priming mice with class II–different spleen cells followed by secondary stimulation in vitro yields $CD8^+$ cells exhibiting class II–restricted cytotoxic activity (*8*). The simplest explanation for this finding on intact mice is that $CD8^+$ cells do express class II reactivity, but only in the presence of help from $CD4^+$ cells.

The experiments discussed below were designed to test whether short-term exposure of a mixture of $CD4^+$ and $CD8^+$ cells to I-E antigens in vivo is capable of stimulating both T cell subsets. This question was addressed with the aid of an antibody specific for $V_\beta11$; as for $V_\beta17a$, the expression of $V_\beta11$ TCR is as high on $CD8^+$ cells as on $CD4^+$ cells (7).

The response of T cells to antigen in vivo occurs in two stages (*2, 9*). Within the first 1 to 2 days, antigen-specific T cells cease their normal pattern of blood-to-lymph recirculation and become selectively trapped in the lymphoid tissues, presumably as a manifestation of binding to APCs. After this period of specific sequestration, the progeny of the antigen-reactive cells reenter the lymph as blast cells. The experiment in Table 1 indicates that this sequence of T cell sequestration, followed by generation of circulating blast cells, applies to $V_\beta11^+$ cells (7). The data show $V_\beta11$ expression on T cells obtained from thoracic duct lymph (TDL) of heavily irradiated $I\text{-}E^+$ hosts given a large intravenous dose of unseparated T cells from $I\text{-}E^-$ mice. During the stage of sequestration (20 to 36 hours), the donor $V_\beta11^+$ cells disappear from TDL; this depletion is V_β specific and is near complete for $CD4^+$ cells (>90%) and partial for $CD8^+$ cells (30%). During the stage of blast cell generation (>60 hr), $V_\beta11^+$ cells reenter the lymph as activated T cells and account for up to 25% of the total blasts. Significantly, this enrichment applies to $CD8^+$ cells as well as to $CD4^+$ cells.

These findings are unexpected because, in vitro, even $CD4^+$ $V_\beta11^+$ T hybridomas fail to respond to I-E antigens (*10*). Although it is unclear why $V_\beta11^+$ cells show I-E reactivity only in vivo, we favor the idea that in vivo systems are simply more sensitive. The key finding in the experiment, however, was that in vivo responses to I-E antigens involve both $CD4^+$ and $CD8^+$ cells. Before discussing the implications of this finding, it is useful to consider T cell reactivity to Mls antigens.

The minor lymphocyte stimulating (Mls) molecules are a class of poorly characterized cell surface molecules expressed predominantly on B cells (*11*). Although the precise receptor-ligand interactions involved in response to Mls antigens are still controversial, these responses are known to involve TCR contact with self class II molecules, especially I-E molecules (*12*). There are two main forms of Mls molecules, Mls^a and Mls^c. Responses to Mls^a antigens are controlled principally by T cells expressing $V_\beta6$ and $V_\beta8.1$ TCR (*13*). These T cells, including both $CD4^+$ and $CD8^+$ cells, are selectively deleted in Mls^a mice. There is thus a strong parallel to the deletion of $V_\beta17a^+$ and $V_\beta11^+$ cells in $I\text{-}E^+$ hosts. The question therefore arises whether immune responses to Mls^a antigens are restricted to $CD4^+$ cells. Many workers appear to be of this opinion (*11*), and some workers assert that CD8 expression precludes Mls^a reactivity (*14*).

Using the in vivo approach discussed above, we investigated whether transfer of unseparated Mls^a-negative T cells to irradiated Mls^a-positive hosts was capable of stimulating both $CD4^+$ and $CD8^+$ cells (*15*). The results shown in Table 2 illustrate that this is indeed the case. Blast cells entering the TDL of the host after 3 days comprise a 4:1 mixture of $CD4^+$ and $CD8^+$ blasts. Both populations show a marked enrichment for $V_\beta6^+$ and $V_\beta8.1^+$ cells.

These experiments indicate that $CD8^+$ cells show overt reactivity not only to class II alloantigens (I-E) but also to class II–restricted antigens (Mls^a). These responses fail to occur in the absence of $CD4^+$ cells, which implies that the response of $CD8^+$ cells to Mls^a antigens and I-E alloantigens is heavily helper dependent (*16*). This requirement for exogenous help does not apply for typical responses of $CD8^+$ cells to class I alloantigens (*5*).

Why the reactivity of $CD8^+$ cells for I-E antigens and Mls^a antigens is helper dependent is unclear. Helper dependency might be a reflection of low binding affinity of T cells to APCs: class II–reactive $CD8^+$ cells are able to bind to class II^+ APCs via their TCR,

Table 1. $V_\beta11^+$ cells in TDL of irradiated $I\text{-}E^+$ B10.A(2R) mice injected with T cells from $I\text{-}E^-$ B10.A(4R) mice. The 2R and 4R hosts were exposed to 900-rad γ irradiation and injected intravenously with 1.2×10^8 LN T cells from normal 4R mice. After 16 hours, thoracic duct cannulas were inserted in the recipients, and lymph was collected continuously for the next 3 days. Thoracic duct lymph cells were pooled from each group (two to three mice per group) and analyzed simultaneously for V_β expression ($V_\beta11$, $V_\beta8.1 + 8.2$) and CD8 expression by dual fluorescence and flow cytometry. After 4R T cell transfer to 2R hosts, the first collection of TDL cells (20 to 36 hours) was selectively depleted of $V_\beta11^+$ cells: a reflection of sequestration in the lymphoid tissues; this collection of cells consisted almost entirely of small lymphocytes. The blast cells appearing in later TDL collections comprised a 1 to 1 ratio of $CD4^+CD8^-$ to $CD8^+$ T ($Thy\text{-}1^+$) cells; both subsets showed a selective enrichment for $V_\beta11^+$ cells. With syngeneic transfer of 4R T cells to 4R hosts, the proportion of $V_\beta11^+$ cells remained constant throughout the experiment and no blasts were generated. Another experiment gave similar results. Data adapted from (7).

Donor T cells transferred to irradiated hosts	Time of TDL collection (hours)	Percent of $CD4^+$ cells expressing		Percent of $CD8^+$ cells expressing		$CD8^-/CD8^+$ ratio
		$V_\beta11$	$V_\beta8$	$V_\beta11$	$V_\beta8$	
4R T → 4R	20–36	4.2	18.1	5.9	19.2	0.7
	48–60	3.6	17.9	5.7	19.1	0.5
	72–88	3.7	18.4	6.1	19.7	0.6
4R T → 2R	20–36	0.2	21.0	4.1	19.5	1.0
	60–65	20.4	5.5	21.3	7.0	1.1
	72–88	10.8	7.5	12.3	7.5	0.7

Table 2. $V_\beta6^+$ cells in TDL of irradiated *Mls*^{a} AKR/J (*H-2*^{k}) mice injected with T cells from *Mls*^{b} B10.BR (*H-2*^{k}) mice. The experiment was set up essentially as described for Table 1, except that the T cells were transferred in much lower doses (3×10^6 per mouse) and the hosts were cannulated on day 3. The TDL cells collected after this time were nearly all blast cells. Control B10.P (*H-2*^{p}, *Mls*^{b}) hosts were used to generate H-2–activated blast cells. Another experiment gave similar results. Data adapted from (*15*).

Donor T cells transferred to irradiated hosts	Time of TDL collection (hours)	Percent of $CD4^+$ cells expressing			Percent of $CD8^+$ cells expressing			$CD4^+/CD8^+$ ratio
		$V_\beta6$	$V_\beta8.1$	$V_\beta8.2$	$V_\beta6$	$V_\beta8.1$	$V_\beta8.2$	
B10.BR T → AKR/J (response to Mls^a)	72–87	75.6	21.8	<0.1	64.3	25.5	<0.1	5.0
	87–92	76.7	18.7	2.4	65.9	23.5	<0.1	4.5
	96–116	77.3	15.3	1.9	65.2	24.7	<0.1	4.4
B10.BR T → B10.P (response to H-2^p)	87–92	11.8	6.1	16.3	13.3	16.3	6.7	5.2
	96–116	12.8	5.3	16.6	16.1	12.9	10.0	2.5
Normal unprimed LN T cells	B10.BR	9.5	3.1	15.4	13.2	11.3	12.4	1.2
	AKR/J	<0.1	<0.1	12.9	<0.1	0.4	14.5	2.6

but the overall avidity of binding is too low to lead to effective cell triggering; addition of help (lymphokines) somehow overcomes this deficit. This line of reasoning ignores the potential of CD8 molecules to interact with the class I molecules on APCs. This interaction, combined with TCR–class II interaction, could result in high-affinity binding. On this point other, workers have suggested that CD4 and CD8 molecules function not only as adhesion molecules but are also important in cell triggering (*17*). To augment triggering, however, the accessory molecules and the TCR have to associate and make contact with the same MHC molecule. This would explain why the MHC-class specificity of $CD4^+$ and $CD8^+$ cells is generally very precise. The capacity of exogenous lymphokines to cause a partial breakdown of MHC-class specificity might reflect that the action of lymphokines can at least partially substitute for the signaling role of accessory molecules.

T Cell Tolerance in the Thymus

The generation of TCR $\alpha\beta^+$ self-MHC–restricted T cells in the thymus occurs by a process of positive selection whereby early thymocytes expressing both CD4 and CD8 molecules and a low density of surface TCR molecules interact with MHC molecules expressed on thymic epithelial cells (TECs) (*2, 18, 19*). T cell binding to MHC molecules on TECs probably involves both the TCR and either CD8 or CD4 molecules (for class I or class II recognition, respectively). Downregulation of the redundant accessory molecule leads to maturation of the thymocytes into $CD4^+CD8^-$ and $CD8^+CD4^-$ cells that express a high TCR density. These cells move from the cortex to the medulla and are then exported.

The mechanism of positive selection is still unclear. Proliferation is apparently not involved because cell division ceases when T cells first begin to show surface TCR expression (*20*). We (*21*) and others (*22*) favor the idea that positive selection simply prevents programmed cell death: T cells binding to MHC molecules on cortical TECs receive some form of protective signal that allows these T cells to survive and make their way to the medulla; T cells not receiving this signal undergo rapid autodestruction. Because cell death in the thymus is massive (*23*), positive selection probably rescues <5% of thymocytes.

Some thymocytes would be expected to express TCR molecules with high MHC affinity. Although it has long been argued that these potentially autoaggressive cells have to be destroyed during ontogeny, the first direct evidence that T cells are subject to self tolerance induction (negative selection) came from the discovery that I-E^+ mice show a selective deletion of thymocytes expressing a high density of $V_\beta17a$ TCR molecules [(*3*) and above]. The critical finding is that I-E^+ mice contain normal numbers of immature $CD4^+CD8^+$ cells expressing $V_\beta17a$ TCR at a low density. This observation indicates that $V_\beta17a^+$ T cells are destroyed at a comparatively late stage of differentiation.

Although the deletion of $V_\beta17a^+$ cells probably occurs largely in the medulla or the cortico-medullary junction, it is possible that deletion can also occur in the cortex. This possibility is supported by the finding that class I tolerance in TCR transgenic mice is induced early in ontogeny, at or before positive selection (*19, 24*). The physiological significance of this finding is difficult to evaluate, however, because TCR transgenic mice show abnormally early expression of T cells with high TCR density.

Which cell types in the thymus control tolerance induction is controversial. There are three main candidates: thymocytes, APCs, and TECs.

Direct evidence that MHC expression on thymocytes can be tolerogenic has come from studies involving intrathymic transfer of $CD4^-CD8^-$ thymic stem cells into MHC-different mice (*25*). The tolerogenicity of thymocytes is limited to class I molecules, probably because class II expression on thymocytes is low.

Bone marrow (BM)–derived cells with APC function are known to be important in tolerance induction (*21*). These cells are rare in the cortex and are concentrated in the medulla and the cortico-medullary junction. Several groups have reported that T cells differentiating in MHC-different thymuses selectively depleted of BM-derived cells show only limited tolerance to the MHC antigens of the thymus graft (*26, 27*, and below). However, addition of purified populations of APCs (dendritic cells) to BM cell–depleted thymuses leads to full tolerance induction (*28*). These and other data have led to the view that induction of complete tolerance to class I and class II antigens requires the presence of BM-derived APCs.

Whether TECs contribute to tolerance induction is controversial (*2*). A popular approach to this question is to examine tolerance induction in T cells differentiating in MHC-different thymuses treated with deoxyguanosine (dGuo) in vitro (*29*); this treatment destroys BM-derived cells but leaves TECs intact. In the case of class I tolerance, stem cell differentiation in dGuo thymuses causes little or no tolerance of $CD8^+$ cells (*27, 28, 30*). Thus, when strain A stem cells differentiate in strain B dGuo-treated thymuses, the $CD8^+$ cells formed in these thymuses generate strong CTL activity against strain B (but not strain A) target cells after stimulation with strain B APCs in vivo. Although these findings suggest that TECs are essentially nontolerogenic for $CD8^+$ cells, the situation for $CD4^+$ cells is more complex. We have found that strain A $CD4^+$ cells differentiating in strain B dGuo-treated thymuses show considerable though not complete tolerance to strain B APCs in terms of primary

mixed lymphocyte reactions (MLRs) (*30*). Comparable findings apply to T cells differentiating in parent → F_1 BM chimeras (*31*) and in a line of transgenic mice in which MHC antigens are expressed selectively in TECs (*32*).

Several studies suggest that the tolerogenicity of TECs for $CD4^+$ cells is especially pronounced within the thymus itself (*30, 31, 33*). In the case of our own studies (*30, 31*), experiments with dGuo-treated thymus–grafted mice and BM chimeras have shown that tolerance induced by TECs is nearly complete for the mature component of $CD4^+CD8^-$ thymocytes, but only partial for lymph node (LN) $CD4^+$ cells (Table 3). The strong tolerance seen at the level of $CD4^+CD8^-$ thymocytes is associated with only partial elimination of $V_\beta11^+$ cells in $I\text{-}E^-$ → $I\text{-}E^+$ combinations. Thus, the tolerance of $CD4^+$ thymocytes is not solely a reflection of clonal deletion. In addition, some other form of unresponsiveness is apparently involved.

The different degrees of tolerance seen in the thymus and LN in the above experiments suggest that contact with TECs can induce two forms of tolerance in $CD4^+$ cells: clonal deletion and anergy (*31, 33*). In the thymus we hypothesize that virtually all $CD4^+$ cells recognizing class II MHC molecules on TECs receive tolerogenic signals. Some cells succumb to these signals and undergo clonal deletion. Others are not deleted but are rendered temporarily anergic. To explain the lower level of tolerance in LN, we suggest that anergy is reversible and disappears when $CD4^+$ cells move from the thymus into LN (*34*).

When exported from the thymus, the subset of $CD4^+$ cells that evades tolerance induction by TECs has interesting properties: although these cells show only partial tolerance in terms of proliferative responses (MLRs), full tolerance applies to skin graft rejection and induction of lethal GVHD (*30*). The proliferative response of the $CD4^+$ cells in vitro can thus be viewed as a "sterile" response that is not associated with the generation of effector cells. Since this response is unusually susceptible to inhibition with MAb to class II (anti–class II) (*31*), the response appears to be mediated by low-affinity cells.

These findings lead us to speculate that TECs, like APCs, are fully capable of inducing strong tolerance of high-affinity $CD4^+$ cells, which we envisage as cells able to mediate graft rejection and GVHD. Tolerance of these cells is probably due to clonal deletion. For $CD4^+$ cells of lower affinity (cells producing a sterile MLR), contact with TECs results in an inefficient form of tolerance: the cells are rendered temporarily anergic but recover from this signal upon export to the periphery. Full tolerance of these low-affinity $CD4^+$ cells requires intrathymic contact with APCs.

Because class II^+ APCs are rare in the cortex of the thymus, tolerance induction by APCs presumably occurs largely in the medulla. The site of tolerance induction mediated by TECs, however, is difficult to define because these cells are prominent throughout the thymus. Since epithelial cells in the cortex and medulla have different embryonic origins (*35*), it does not necessarily follow that all TECs have tolerogenic properties. A priori, it is possible that only medullary epithelium can induce tolerance. T cell selection in the thymus would then be compartmentalized, with epithelial cells in the cortex controlling positive selection (*36*) and the combined actions of medullary epithelium and APCs in the medulla inducing negative selection. Without direct evidence on the tolerogenicity of cortical versus medullary epithelium, this scenario is difficult to assess.

Extrathymic Tolerance of T Cells

Whereas immature T cells in the thymus are highly sensitive to tolerance induction, exposure of mature T cells in the periphery to antigen generally leads to immunity rather than tolerance. Nevertheless, under certain conditions, contact with antigen in the post-thymic environment does lead to unresponsiveness. As discussed below, most evidence suggests that tolerance induction of mature T cells is a consequence of defective presentation of antigen (*37, 38*).

In the case of normal immune reactions, responses of resting T cells to antigen are controlled by specialized APCs, especially dendritic cells (*2, 39*). For effective triggering of unprimed T cells, APCs not only have to present antigen-MHC complexes, but also need to deliver appropriate "second signals" (*2, 40*). These signals are ill-defined: soluble lymphokines may participate, but some second signals appear to be cell bound.

Although the precise function of APC second signals is still obscure, a consensus of opinion is emerging that T cell recognition of antigen in the absence of these signals tends to cause tolerance rather than immunity (*40*). Thus, exposure of unprimed T cells to antigen expressed on cells other than "professional" APCs leads to an unresponsive (anergic) state in which the T cells are refractory to subsequent stimulation by normal APCs. This is supported by

Table 3. Mixed lymphocyte reactions by mature $CD4^+$ cells prepared from thymus and LN of long-term parent → F_1 BM chimeras. (B6 × CBA)F_1 mice were exposed to heavy irradiation (1300 rad) and reconstituted with T cell–depleted B6 ($H\text{-}2^b$, Thy-1.2) BM cells. To ensure elimination of host APCs, we further irradiated the chimeras after 6 months and reconstituted with Thy-1–marked B6.PL ($H\text{-}2^b$, Thy-1.1) BM cells. Purified $Thy\text{-}1.2^-$ $CD4^+CD8^-$ cells prepared from LN and thymus of the chimeras were stained for $V_\beta11$ expression (the CBA strain is $I\text{-}E^+$) and tested in MLR for reactivity to irradiated spleen stimulators expressing host-type $H\text{-}2^k$ antigens (CBA/Ca). The LN $CD4^+$ cells from the chimeras show partial tolerance to the host, whereas thymocyte $CD4^+$ cells show near complete tolerance; $V_\beta11$ deletion in the chimeras is partial and no more marked for thymus than LN. Data adapted from (*31*). TdR, thymidine deoxyribose.

Cells donors	$CD4^+CD8^-$ cells tested	Percent of $CD4^+$ cells expressing		Day of assay	[^{3}H]TdR incorporation (cpm × 10^{-3}) with stimulators*		
		$V_\beta11$	$V_\beta8$		CBA/Ca ($H\text{-}2^k$)	B6/PL ($H\text{-}2^b$)	bm12 ($H\text{-}2^{bm12}$)
B6.PL	Thymus	4.5	16.3	5	78.0	0.3	39.1
B6.PL → F_1 chimera	Thymus	1.9	16.6	5	1.8	0.4	30.8
				6	3.2	0.2	62.7
	LN	1.4	17.8	5	13.6	1.6	89.6
				6	63.6	1.5	10.4
Normal F_1	Thymus	0.4	12.5				
			Not tested				
	LN	0.1	14.9				

*MLR (mean of triplicate cultures) with 1×10^5 responders and 5×10^5 stimulators; SDs were generally within 10 to 20% of the mean values.

studies with MHC transgenic mice in which foreign class I or class II genes are expressed selectively in the β cells of the pancreas or the hepatocytes of the liver (*38*). Some of these transgenic lines show systemic unresponsiveness to the transgenic MHC antigens, despite the paucity of lymphatic infiltration in the organ expressing the transgene. Certain other MHC transgenic lines, however, show little or no evidence of tolerance induction (*41*).

We have used two approaches to assess the notion that presentation of MHC antigens on cells other than typical APCs leads to tolerance induction. The first system involves a thymus-grafting model in which thymectomized (A × B)F_1 mice are given supralethal irradiation (1300 rad) and reconstituted with T-depleted strain A BM cells (*42*). These mice are rapidly repopulated with donor-derived cells and eventually show complete disappearance of host-derived APCs. After several months the mice receive strain A thymus grafts. It is then possible to determine whether the strain A T cells differentiating in these syngeneic thymus grafts become tolerant to host strain B antigens after export to the secondary lymphoid tissues, where the MHC antigens of the host are encountered on various cell types, but not on BM-derived APCs. To date we have only examined tolerance at the level of $CD4^+$ cells, using primary MLR and $V_\beta 11$ expression to define tolerance. By these parameters, the thymus-grafted mice show no evidence of tolerance induction. Thus the $CD4^+$ cells prepared from these mice give strong primary MLR in response to normal host-type strain B APCs in vitro and, in $I\text{-}E^- \rightarrow I\text{-}E^+$ combinations, show no deletion of $V_\beta 11^+$ cells. One could argue that the lack of tolerance in the thymus-grafted mice merely reflects a quantitative deficiency of host class II antigens. We think this possibility is unlikely because injecting the mice with normal strain A $CD4^+$ cells causes most of the host-reactive component of these cells to leave the circulation and become selectively trapped in the spleen. By this parameter the thymus-grafted mice do show significant expression of host class II molecules. Under optimal staining conditions, low-level expression of host class II molecules is evident on vascular endothelial cells and on a subset of germinal center cells.

The second model we are using to search for peripheral tolerance induction involves transferring large doses of T cells into heavily irradiated MHC-different mice and then testing whether the donor T cells recovered from these mice show tolerance to the host (*43*). This approach might sound impractical because the recipients would be expected to die rapidly from GVHD. To avoid this problem we make use of the finding that $CD4^+$ cells given in large doses provide strong protection against lethal forms of GVHD (*44*), probably by enabling the host to mount effective immunity against pathogens (chronic infection being the main cause of death from GVHD). The approach is to transfer large doses of unseparated spleen cells (a mixture of $CD4^+$ cells, $CD8^+$ cells, and B cells) into heavily irradiated (1000 rad) mice differing either at class I (B6 → bm1) or class II (B6 → bm12) loci. Through the protective function of the donor $CD4^+$ cells, the recipients show low mortality rates and survive for a year or more. In interpreting the results considered below, it should be borne in mind that the recipients show very rapid disappearance of host BM–derived APCs; after the first month, host antigens are encountered almost exclusively on non-BM–derived cells.

For the class I–different B6 → bm1 combination, irradiated (B6 × bm1)F_1 mice killed at 6 to 12 months after injection of 10^8 B6 spleen cells show definite evidence of subclinical chronic GVHD: the mice look reasonably healthy but the lymphoid tissues show severe atrophy. For examining tolerance induction, donor B6 $CD8^+$ cells prepared from these spleen chimeras are tested in vitro for reactivity to normal host-type (bm1) APCs. When $CD8^+$ cells from the chimeras are stimulated in vitro with normal bm1 APCs in the presence of exogenous lymphokines, the cells mount strong antigen-specific cytotoxic T lymphocyte (CTL) activity to bm1 target cells in typical ^{51}Cr-release assays (Table 4). By this parameter, the $CD8^+$ cells from the chimeras show no obvious tolerance to the class I antigens of the host, despite having been exposed to these antigens for a year or more in vivo. For proliferative responses, purified $CD8^+$ cells from the chimeras give good MLR to host-type APCs in the presence of IL-2, but low MLR (25% of normal) in the absence of IL-2. Thus, the long-term residence of donor $CD8^+$ cells in the chimeras causes the cells to shift from a helper-independent to a helper-dependent state.

Table 4. Anti–class I CTL activity by T cells from (B6 × bm1)F_1 mice given 1000 rad plus 10^8 unseparated B6 spleen cells 1 year before. Purified T cells prepared from spleen of the chimeras were cultured with normal (B6 × bm1)F_1 stimulators (irradiated spleen) plus lymphokines for 4 days and then tested for CTL activity (^{51}Cr release) with standard techniques and concanavalin A (Con A)–stimulated blasts as targets. T cells from normal B6 mice were used as a control.

Donors of CTL precursors	Killer: target ratio	CTL activity (^{51}Cr release) to Con A blasts*		
		B6	bm1	bm9
Normal B6	10:1	<1	47	1
	1:1	<1	16	<1
B6 spleen → 1000 rad (B6 × bm1)F_1	10:1	6	57	6
	1:1	3	25	2

*Mean of triplicate cultures; SDs were generally within 10 to 20% of the mean values.

For class II–different recipients, transfer of 10^8 B6 spleen cells to irradiated (1000 rad) (B6 × bm12)F_1 hosts leads to only limited mortality. In this situation, however, the long-term recipients do not exhibit pathology; the spleen and LN are of normal size and contain normal numbers of T and B cells. For MLR, the $CD4^+$ cells from the chimeras show little evidence of tolerance induction. Even 1 year after transfer, the donor $CD4^+$ cells give strong MLR to normal host-type bm12 APCs in vitro (Table 5).

These experiments suggest that, both for class I– and class II–different combinations, prolonged exposure of T cells to MHC antigens in irradiated hosts results in little tolerance induction, at least by the parameters of MLRs and CTL generation in vitro. It does not necessarily follow, however, that the host reactivity of the recovered T cells is qualitatively normal. In this respect, ongoing experiments suggest that the T cells recovered from long-term spleen chimeras exhibit a reduced capacity to elicit GVHD upon further transfer to normal host-type irradiated mice. Thus, the host-reactive T cells surviving in the chimeras might consist predominantly of low-affinity cells, the high-affinity cells (cells mediated GVHD) having been tolerized. This idea is certainly possible, but one is faced with defining tolerance in mechanistic terms. Thus even if one could prove that the chimeras do become depleted of cells with defined high affinity, several different mechanisms could be invoked to explain the failure to find these cells. Classic tolerance through clonal deletion or anergy is merely one possibility. An alternative possibility is that the T cells terminally differentiate into effector cells that home to mucosal surfaces and are eventually excreted from the body (*45*). It is also conceivable that the T cells merely switch their functional phenotype, for example, by changing their patterns of lymphokine release.

The data on thymus-grafted mice and long-term spleen chimeras do not disprove the hypothesis that antigen encountered on cells other than typical APCs leads to tolerance induction rather than immunity. Nevertheless, the data do place constraints on this idea. Although it is incontestable that defective presentation of antigen does lead to tolerance induction under defined conditions, it

remains to be proved whether this is a general phenomenon applicable to a broad range of T cell functions.

The Mechanism of T Cell Anergy

Much of the speculation in this article is based on the assumption that the affinity of T cells for antigen is heterogeneous and that cells of different affinities have different triggering requirements and functions. We now discuss this issue further, with particular reference to anergy.

Although the notion that T cell tolerance can reflect either clonal deletion or anergy is now unquestioned, the essential difference between these two forms of tolerance is unclear. A central issue is why clonal deletion is the major mechanism of tolerance in some situations, whereas anergy predominates in others. We suggest that the answer may lie in the avidity of the interaction between T cells and APCs.

The avidity of T cell–APC interactions presumably reflects the combined effects of multiple factors, including (i) the binding affinity of TCR molecules for antigenic epitopes on APCs, (ii) the density of antigen on APCs, and (iii) the efficiency of the interactions between the complementary accessory molecules on T cells and APCs. In the case of mature T cells, one can envisage that efficient triggering depends on strong (high-avidity) binding of T cells to APCs; under these conditions the cells enter DNA synthesis and then proliferate extensively as a consequence of endogenous lymphokine (IL-2) production. If the avidity of T cell–APC interactions is reduced below a certain threshold, T cells undergo only partial triggering; IL-2 synthesis is limited and the cells proliferate only when supplemented with exogenous IL-2. According to this view, the helper dependency of T cell responses is largely a reflection of the avidity of T cell–APC interactions. High-avidity interactions lead to helper-independent responses, whereas low-avidity interactions elicit helper-dependent responses. Any reduction in the avidity of T cell–APC interaction, for example, lowering the dose of antigen on APCs, favors the production of helper-dependent responses.

With regard to TCR affinity, it is generally assumed (though not formally proven) that the intrinsic affinity of TCR molecules for particular antigen-MHC complexes varies considerably from one T cell to another. The avidity of T cell–APC interaction would be expected to correlate closely with TCR affinity, T cells with low-affinity TCR forming weaker conjugates with APCs than high-affinity T cells. Under physiological conditions with normal (professional) APCs, we suggest that helper-independent T cell responses are mediated by T cells with high TCR affinity, whereas helper-dependent responses involve T cells with low-affinity TCRs (*2, 46*).

Low avidity T cell–APC interactions could reflect a quantitative or qualitative change in the accessory molecules on APCs. This brings us to the phenomenon of anergy. As discussed earlier, anergy tends to result when normal mature T cells are exposed to antigen expressed on cells other than professional APCs. Two forms of antigen presentation can lead to anergy: (i) presentation of antigen by cells that lack classic APC function (for example, pancreatic β cells) and (ii) presentation of antigen by normal APCs treated with certain chemicals (*37, 38*). Anergy in these two situations is usually taken to reflect the absence of requisite second signals by APCs. An alternative possibility is that the APCs in these situations show a reduced or defective expression of accessory molecules. This results in low-avidity T cell–APC interactions and suboptimal (helper-dependent) triggering. In considering this possibility, it is significant that T cell anergy can be overcome by adding IL-2. Anergic responses thus show close similarities to typical helper-dependent responses. We suggest that both responses reflect a common mechanism and are largely a consequence of low-avidity T cell–APC interactions. The observation that anergic cells are refractory to subsequent stimulation by normal APCs might reflect that T cell triggering is inevitably followed by a period of transient unresponsiveness, even after optimal stimulation (*47*). This notion hinges on the assumption that anergy is reversible: protracted anergy is indicative of constant exposure to antigen.

The above discussion refers to the response of mature extrathymic T cells. Extrapolation to the response of immature T cells in the thymus is difficult because the essential difference between mature and immature T cells is still unclear. The critical issue is why immature T cells show much greater susceptibility to tolerance induction. One possibility is that, unlike mature T cells, immature T cells cannot withstand strong signaling. Overstimulation results and the cells succumb to apoptosis (*48*). The end result is clonal deletion. With lower levels of stimulation the T cells evade self destruction (deletion) and enter an anergic state. According to this rationale, the form of unresponsiveness induced in the thymus is largely a reflection of the avidity of T cell–APC interactions: strong T cell–APC interactions lead to tolerance via clonal deletion, whereas weak interactions result in anergy. This model implies that the capacity of TECs to induce widespread anergy but only partial clonal deletion reflects that, unlike thymic APCs, TECs enter into only weak associations with T cells (perhaps because TECs lack certain accessory molecules). The avidity of interaction between T cells and TECs is sufficient to cause anergy but is generally too low to cause clonal deletion. Clonal deletion does occur but is limited to T cells expressing TCRs of the highest affinity.

The notion that the form of tolerance, clonal deletion or anergy, is largely a reflection of the avidity of T cell–APC interaction is probably an oversimplification, because it could be argued that T cells receive a number of qualitatively different signals (rather than one signal of varying intensity). Until T cell signaling is understood in more detail, however, no model of tolerance induction can be put forward with any degree of confidence. In fact, for anergy, even the definition of tolerance is still inexact. Thus, the only direct method currently available for assessing preexisting anergy of T cells is to test whether exposure of the cells to antibodies to TCRs or CD3 in vitro is capable of triggering helper-independent proliferation (or IL-2 production). In the case of normal $CD4^+$ cells, however, separation of these cells into "virgin" ($CD45R^{hi}$) and "memory" ($CD45R^{lo}$) cells reveals that only the memory cells give helper-independent proliferative response to antibody to CD3 (*49*). The $CD45R^{hi}$ cells are unresponsive. According to the current defini-

Table 5. Anti-class II MLR by $CD4^+$ cells from (B6 × bm12)F_1 mice given 1000 rad plus 10^8 unseparated B6 spleen cells 10 months before. Purified LN $CD4^+$ cells were cultured with irradiated spleen stimulators and pulsed with [^{3}H]TdR (1 μCi per well) on day 4. The strong anti-bm12 response of B6 $CD4^+$ cells from the spleen chimeras contrasts with the weak anti-bm12 response of B6 $CD4^+$ cells prepared from 1000 rad (B6 × bm12)F_1 mice reconstituted with B6 BM cells (a situation where the B6-derived $CD4^+$ cells are tolerized in the host thymus).

Donors of LN $CD4^+$ cells	MLRs (cpm × 10^{-3})* against		
	B6 (H-2^b)	bm12 (H-2^{bm12})	B10.BR (H-2^k)
Normal B6	1.2	44.3	22.5
B6 BM → 1000 rad (B6 × bm12)F_1	0.8	4.2	28.4
10^8 B6 spleen → 1000 rad (B6 × bm12)F_1	5.9	54.6	30.2

*MLR (mean of triplicate cultures) with 2 × 10^5 responders and 5 × 10^5 stimulators; SDs were generally within 10 to 20% of the mean values.

tion, one is therefore led to the unsatisfactory conclusion that typical virgin $CD4^+$ cells are "naturally" anergic. Anergy needs to be defined with more precision.

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50. We thank B. Marchand for typing the manuscript. Supported by grants CA38355 and CA25803 from the National Cancer Institute and AI21487 from the National Institute of Allergy and Infectious Diseases.