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T Cell Tolerance by Clonal Elimination in the Thymus

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Summary

The monoclonal antibody KJ23a reacts with T cell receptors utilizing the Vβ segment Vβ17a. T cells bearing Vβ17a$^+$ receptors react with very high frequency with the MHC class II protein, IE. In this paper we show that T cells expressing Vβ17a are selectively eliminated from the peripheral T cell and mature thymocyte pool of mice expressing IE, but are present in expected numbers in the immature thymocyte population of such animals. These results show that in normal animals tolerance to self-MHC is due to clonal elimination rather than suppression. In addition, they indicate that tolerance induction may occur in the thymus at the time immature thymocytes are selected to move into the mature thymocyte pool.

Introduction

The means by which the immune system discriminates between foreign antigens and potentially immunogenic self-antigens remains poorly understood. Classic experiments established that this tolerance to self-antigens is acquired during the development of the immune system within each individual (Owen, 1945; Billingham et al., 1956). Hence T cell and B cell receptors reactive to self-antigens are encoded in the germ line, but the activity of cells with these receptors is not usually apparent among mature functional lymphocytes. T cell tolerance to products of the major histocompatibility complex (MHC) is a particularly striking example of this phenomenon. T cells within an individual of a species mount a very strong response to products of foreign alleles of this extremely polymorphic complex (Fischer-Lindahl and Wilson, 1977) but fail to respond well to the products of self-MHC alleles.

The mechanism of tolerance induction is unknown. One of the oldest hypotheses is that lymphocyte clones with self-reactivity are eliminated from the popuiation during a critical stage in development. However, based on the demonstration in several in vitro experimental systems of persistent self-reactive clones among mature lymphocytes (Smith and Pasternak, 1978; Battisto and Ponzio, 1981; Glimcher and Shevach, 1982), other hypotheses have been proposed in which tolerance is maintained by preventing the activation of these clones without their actual elimination.

One of the difficulties in designing experiments to test these hypotheses for tolerance induction has been the inability to identify lymphocytes at various stages of ontogeny with receptors of a particular anti-self specificity. In the accompanying paper (Kappler et al., 1987) we described a monoclonal antibody, KJ23a, that reacts with T cell receptors containing Vβ17a. Surprisingly, in SWR mice a very high proportion of T cells that express this Vβ segment were found to be reactive to a number of the allelic forms of the class II MHC molecule, IE. Since SWR mice do not express the IE protein, we have examined the expression of Vβ17a in mice that have a functional IE molecule. Our results show that in these mice cells with Vβ17a$^+$ receptors occur at normal levels among immature thymocytes, but are severely depleted among mature thymocytes and peripheral T cells. These data offer the first direct evidence for clonal elimination as a mechanism for inducing T cell tolerance to self-MHC antigens and indicate the stage of development at which this elimination may normally occur.

Results

Variable Expression of Vβ17a in Various Strains of Mice

In the accompanying report we showed that the polymorphic form of Vβ17, which reacts with the monoclonal antibody KJ23a, is encoded in a Vβ gene segment linked to an unusual Vβ complex haplotype, which is defined by number of restriction fragment length polymorphisms (RFLP) and a large deletion that has eliminated about half of the known Vβ segments including the frequently used Vβ8 family (Behlke et al., 1986). The four independent strains that carry this variant haplotype, SWR, SJL (and its Igh congenic derivative, SJA), C57L, and C57BR, all apparently carried the Vβ17a allele, while other strains had the Vβ17b allele. To confirm this allelic distribution, we examined peripheral T cells from numerous strains of mice stained with KJ23a and the Vβ8-specific antibody, KJ16, to determine the level of expression of Vβ17a and Vβ8. Typical fluorescence histograms for four strains carrying the variant Vβ complex are shown in Figure 1, and a summary of all the results is shown in Table 1.

As we previously reported, no KJ16$^+$ T cells were seen in any of the strains carrying the variant Vβ complex that has deleted the Vβ8 family (Haskins et al., 1984). The level of Vβ8$^+$ T cells in the other strains varied from 11%–20%. As expected, none of the strains with the normal Vβ complex, which contains the Vβ17b allele, expressed any KJ23a$^+$ T cells. Three of the strains with the variant Vβ complex containing Vβ17a expressed detectable levels of KJ23a$^+$ T cells: SWR (~14.0%), SJL/SJA (~9.0%), and C57L (~4.0%). Surprisingly, virtually no KJ23a$^+$ T cells were found in C57BR mice (0.15%) despite the fact that by Southern blot analysis this strain carried a Vβ17a gene

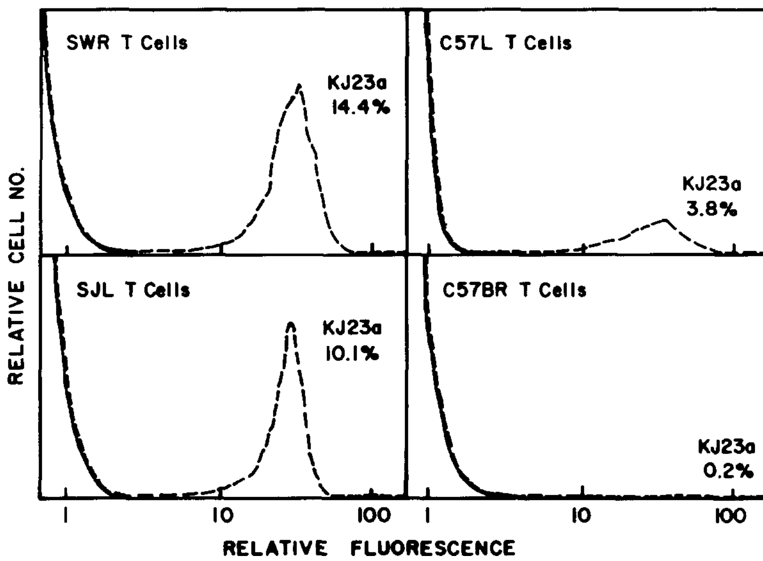

Figure 1. KJ23a Binding to T Cells from Mice Carrying Vβ17a

Fluorescence histograms are shown for peripheral T cells isolated from the strains indicated. The primary antibody was either biotinylated KJ23a (- - -) or, as a negative control, biotinylated KJ16 (———). The secondary reagent was phycoerythrin-streptavidin. The percentage of T cells positive for KJ23a was calculated by curve subtraction within the second decade of fluorescence.

apparently identical to that of the other three strains (Kappler et al., 1987).

Vβ17a Expression Is Suppressed in Mice Expressing an IE Class II MHC Molecule

C57BR is the only strain of the four with the Vβ17a structural gene that carries an MHC haplotype (H-2^k) which can express an IE class II molecule. SJL/SJA and C57L (H-2^s and H-2^b, respectively) fail to express IE because of a deletion of the IE_α chain, and SWR (H-2^q) appears to have a defect in RNA processing for both the IE α and β chains (Mathis et al., 1983; Dr. P. Jones, personal communication). Therefore, one possibility was that, because of the high frequency with which Vβ17a$^+$ T cells react to the various allelic forms of IE, T cells expressing this Vβ segment are eliminated in C57BR during induction of tolerance to MHC molecules. However, there were other possibilities. For example, molecules of the H-2^k haplotype may not select T cells bearing Vβ17a during development in the thymus (Bevan, 1977; Zinkernagel et al., 1978), or some defect in the Vβ17a structural gene in C57BR may preclude efficient expression without affecting our Southern blot analysis. Therefore, we performed a number of experiments to determine whether low levels of T cells bearing Vβ17a were linked to IE expression.

We determined the percentage of T cells expressing KJ23a in a number of heterozygous mice bred to carry the structural gene for Vβ17a and H-2 haplotypes that did or did not allow for IE expression. Two types of mice were bred. First, SWR or SJA mice were bred with other homozygous mice with different H-2 haplotypes. Second, we had previously performed a breeding experiment with B10.Q and (AKR × SWR)F1 mice to map the structural gene for Vβ17a to the Vβ complex (Kappler et al., 1987). The 18 mice from this cross that inherited the Vβ17a structural gene were examined for both the level of KJ23a-expressing T cells and for their expression of the IE^k molecule inherited from AKR. Typical histograms are shown in Figure 2 and Figure 3, and the data are summarized in Figure 4.

Table 1. Expression of Vβ17a and Vβ8 on Peripheral T Cells of Various Strains

			H-2 Genes Expressed						IE Molecules Expressed	% of T Cells Expressing	
Strain	Vβ8	Vβ17	K	Aα	Aβ	Eβ	Eα	D	(α:β)	KJ23a (Vβ17a)	KJ16 (Vβ8.1, Vβ8.2)
SWR	–	a	q	q	q	–	–	q	None	14.2 +/– 0.4 (10)[a]	0.0 +/– 0.1 (3)
SJL	–	a	s	s	s	s	–	s	None	9.4 +/– 0.3 (3)	0.0 +/– 0.0 (3)
SJA	–	a	s	s	s	s	–	s	None	8.5 +/– 0.3 (8)	0.0 +/– 0.0 (3)
C57L	–	a	b	b	b	b	–	b	None	3.7 +/– 0.2 (6)	0.0 +/– 0.0 (3)
C57BR	–	a	k	k	k	k	k	k	k:k	0.1 +/– 0.0 (4)	0.0 +/– 0.0 (3)
BALB/c	+	b	d	d	d	d	d	d	d:d	–0.1 +/– 0.1 (6)	20.0 +/– 0.3 (3)
AKR	+	b	k	k	k	k	k	k	k:k	–0.1 +/– 0.1 (8)	11.6 +/– 0.2 (3)
B10.TL	+	b	s	s	k	k	k	k	k:k	0.0 +/– 0.0 (3)	18.3 +/– 1.3 (3)
B10.HTT	+	b	s	s	s	s	k	d	k:s	0.1 +/– 0.1 (3)	18.3 +/– 0.3 (3)
B10.S(7R)	+	b	s	s	s	s	–	d	None	0.0 +/– 0.0 (3)	17.0 +/– 0.9 (3)
C57BL10	+	b	b	b	b	b	–	b	None	0.1 +/– 0.0 (5)	15.1 +/– 0.4 (3)
B10.Q	+	b	q	q	q	–	–	q	None	0.0 +/– 0.0 (3)	14.2 +/– 0.5 (4)

[a] Results shown are the average ± standard error of the mean of the bracketed number of independent determinations.

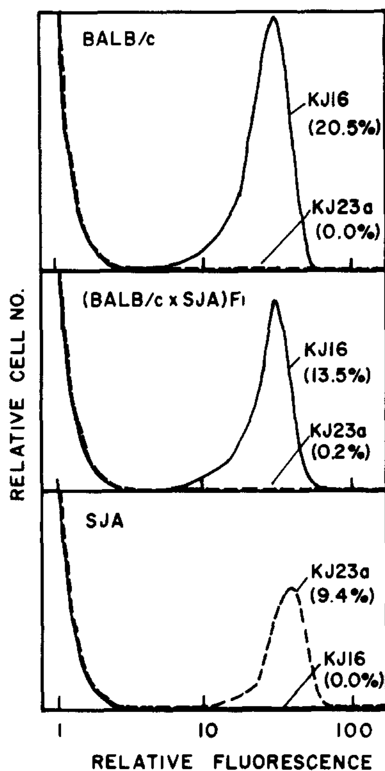

Figure 2. Elimination of Vβ17a Expression in (BALB/c × SJA)F1 Mice

Fluorescence histograms are shown for peripheral T cells from BALB/c, (BALB/c × SJA)F1, and SJA mice. Staining and calculations were as in Figure 1.

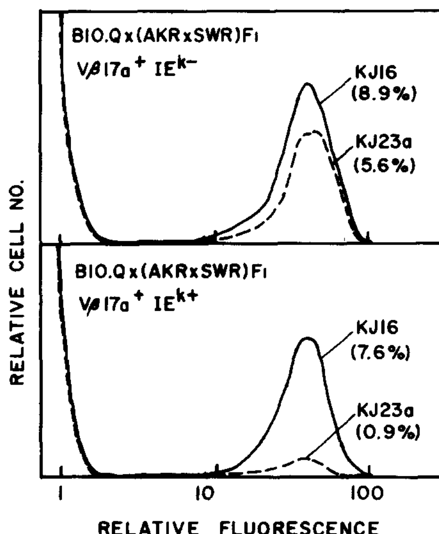

Figure 3. Reduction of Vβ17a Expression in the IE^+, Vβ17a$^+$ Progeny of B10.Q × (AKR × SWR)F1 Mice

B10.Q mice were bred with (AKR × SWR)F1 mice. T cells from 42 progeny were analyzed for staining with KJ23a and KJ16 as in Figure 1. Unseparated lymph node cells from the 18 mice with KJ23a$^+$ T cells were analyzed for expression of the IE^k molecule inherited from AKR using biotinylated Y17 and phycoerythrin–streptavidin. Representative fluorescence histograms of KJ23a and KJ16 staining are shown for one of the mice that was IE^{k-} (<0.2% Y17$^+$ lymph node cells) and one that was IE^{k+} (46.8% Y17$^+$ lymph node cells). The summarized data for all of the KJ23a$^+$ mice are shown in Figure 4.

In each case these mice were heterozygous at the Vβ complex; therefore, we had to take into account the phenomenon of allelic exclusion (which seems to apply in most T cells) in interpreting our results. Since an individual T cell expresses a single β chain, in these heterozygous mice each T cell expressed a Vβ segment from only one of the two Vβ complexes. Thus, if expression were random, the level of expression of a given Vβ segment from one haplotype in the heterozygote should have been about 50% of that seen in the homozygous parent (Roehm et al., 1985). In the case of Vβ17a this expected level may be even less because this variant Vβ complex carries only 12 known Vβ segments compared with about 21 in the normal Vβ complex (Behlke et al., 1986; Barth et al., 1985).

As seen in Figures 2, 3, and 4, good expression of KJ23a was seen in the mice that did not express an IE molecule; however, in no case was the expression as high as 50% of the KJ23a$^+$ parent, perhaps, as discussed above, reflecting the fewer Vβ segments carried in the variant Vβ complex. However, the level of expression of KJ23a was severely suppressed in all mice expressing an IE molecule. This suppression was virtually complete in (BALB/c × SJA)F1 mice and only partial in other cases. This linkage of the level of KJ23a expression to IE is particularly well-demonstrated with the second group of mice shown in Figure 4. In this case SWR mice were bred with a group of mice congenic at the I region of H-2. In particular, B10.S(7R) and B10.HTT differ only in the expression of the $IE^{k/s}$ molecule, yet they show a dramatic difference in the level of KJ23a$^+$ T cells. The formal linkage to H-2 is also demonstrated by the KJ23a$^+$ progeny of B10.Q mice bred with (AKR × SWR)F1 mice (Figure 3 and the last group in Figure 4). In these animals, there was an exact correlation between the level of KJ23a$^+$ T cells and the inheritance of IE^k. All mice that inherited IE^k from AKR had low KJ23a expression, while those that inherited H-2^q from SWR had high KJ23a expression. These results indicate that mice with an expressed IE molecule eliminate most T cell clones expressing Vβ17a. This represents the first formal demonstration that tolerance to an MHC molecule can occur by the mechanism of clonal elimination.

As a control all mice were also tested for KJ16 expression. As we have previously observed, there was about a 2-fold variation in KJ16 expression among the homozygous strains (Roehm et al., 1985). Among strains congenic at H-2, H-2^b and H-2^q were associated with somewhat lower expression than the other haplotypes. In spite of these minor changes in KJ16 expression, however, we could still see that the level of KJ16$^+$ T cells in the heterozygous mice varied from 50%–70% of the KJ16$^+$ parent, again perhaps reflecting the tendency to express more efficiently from the Vβ complex with a larger complement of Vβ segments.

Suppression of Vβ17a Occurs during Thymocyte Development

We had previously shown, using KJ16, that the level of surface α/β receptor on thymocytes was a reliable marker for their stage of differentiation (Roehm et al., 1984). A por-

Mouse	H-2	IE α:β	KJ23a % T cells Staining	KJ23a % of KJ23a⁺ Parent (20, 40)	KJ16 % T cells Staining	KJ16 % of KJ16⁺ Parent (20, 40, 60)
B10xSJA	b/s	None	2.5±0.3(5)		9.2±0.6(3)	
AKRxSWR	k/q	k:k	0.8±0.1(9)		8.6±0.1(5)	
BALB/cxSJA	d/s	d:d,d:s	0.2±0.1(4)		14.0±0.6(3)	
B10.S(7R)xSWR	t2/q	None	4.5±0.1(3)		7.7±0.2(3)	
B10.HTTxSWR	t3/q	k:s	1.2±0.1(3)		8.0±0.1(3)	
B10.TLxSWR	t1/q	k:k	1.1±0.2(3)		8.3±0.1(3)	
B10.Q x (AKRxSWR)F1	q/q	None	5.5±0.1(4)		8.6±0.2(4)	
	k/q	k:k	1.0±0.1(14)		7.9±0.1(14)	

Figure 4. Reciprocal Relationship between IE and Vβ17a Expression in Vβ Heterozygous Mice

The percentage of T cells binding either KJ23a or KJ16 was determined as in Figure 1 in the indicated F1 mice and in the KJ23a⁺ progeny of B10.Q × (AKR × SWR)F1 mice. The latter mice were also typed for IE^k expression as in Figure 3. The number of mice analyzed in each case is shown in parentheses. Also shown is the frequency of KJ23a⁺ or KJ16⁺ T cells expressed as the percentage of that seen in the KJ23a⁺ or KJ16⁺ parent of the cross (Table 1). In the case of B10.Q × (ARK × SWR)F1 mice, this latter calculation was based on B10.Q mice for KJ16 and on SWR mice for KJ23a.

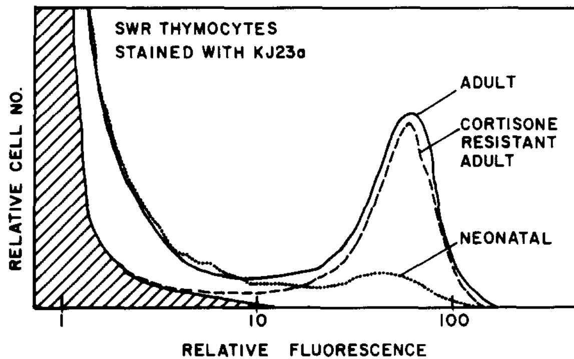

Figure 5. Correlation between the Density of KJ23a Surface Expression and Thymocyte Maturation

Fluorescence histograms are shown for KJ23a staining of thymocytes from adult (4 months old) SWR mice (———), from adult SWR mice treated two days previously intraperitoneally with 2.5 mg of a suspension of hydrocortisone acetate (- - -), from neonatal SWR mice (·····), and as the negative control, from adult BALB/c mice (hatched area). Staining was performed as in Figure 1. All histograms except that for the cortisone-resistant thymocytes are shown at the same scale. The histogram for the cortisone-resistant thymocytes was normalized to the brightly staining population of the normal adult SWR thymocytes (~5× reduction in scale).

tion of immature cortical thymocytes, whether from adult or fetal mice, express the receptor at very low levels (about 20%–10% the level expressed by peripheral T cells). Mature T cells within the thymus medulla express high levels of surface receptor indistinguishable from peripheral T cells. We performed experiments with KJ23a to determine whether this expression pattern was seen with KJ23a as well. Figure 5 shows the staining pattern using KJ23a seen with adult SWR T cells compared with that seen with neonatal SWR T cells (which are enriched in immature thymocytes) or with adult thymocytes from SWR mice treated with cortisone (which are enriched in mature thymocytes). As we have previously seen with KJ16, immature thymocytes express low levels of surface KJ23a, whereas mature thymocytes have high levels of KJ23a.

Another marker that distinguishes mature from immature thymocytes is the distribution of the differentiation antigens, L3T4 and Lyt2. Whereas the bulk of cortical immature thymocytes express both of these antigens, mature thymocytes and peripheral T cells express one or the other (Scollay et al., 1984; Ceredig et al., 1983). We examined adult SJL thymocytes for coexpression of L3T4, Lyt2, and KJ23a. The results are shown in the left half of Table 2. The bulk of SJL thymocytes with low levels of KJ23a carried both the L3T4 and the Lyt2 markers, whereas the majority of cells with high levels of KJ23a expressed either L3T4 or Lyt2, again confirming the level of receptor expression as an indicator of thymocyte maturation.

Therefore, we examined thymocytes from mice that carried the Vβ17a gene and expressed an IE molecule to determine whether the IE-mediated elimination of Vβ17a clones occurred within the thymus. The results with C57BR mice are shown in Figure 6, and those with (AKR × SWR)F1 mice are shown in Figure 7. In both cases thymocytes with low levels of surface KJ23a expression were detected with a frequency not significantly different from that calculated from SWR or SJL; however, virtually no thymocytes were seen in either case with high levels of surface KJ23a expression. In the case of C57BR we also examined the low level KJ23a-expressing population for coexpression of L3T4 and Lyt2. These results are

Table 2. Distribution of KJ23a Binding among Thymocyte Subpopulations

Thymocyte Subpopulation	% of SJL Thymocytes	% Dull KJ23a	% Bright KJ23a	% of C57BR Thymocytes	% Dull KJ23a	% Bright KJ23a
L3T4 only	20	0.34	1.97	10	0.08	0.06
Lyt2 only	6	0.04	0.36	4	−0.06	0.01
L3T4/Lyt2	74	2.88	0.15	86	2.59	0.06

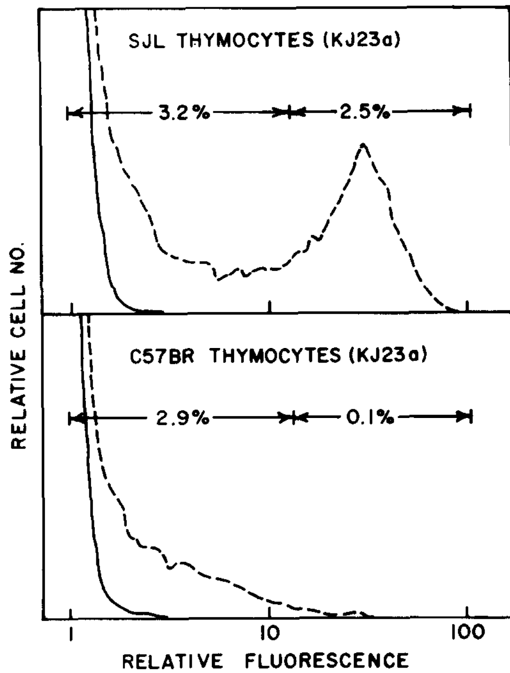

Figure 6. Elimination of KJ23a Expression in IE+ Mice among Mature, but Not Immature, Thymocytes (SJL vs. C57BR)

Fluorescence histograms are shown for adult thymocytes from either SJL or C57BR stained with biotinylated KJ23a and phycoerythrin–streptavidin (- - -) or, as the negative control, with phycoerythrin–streptavidin alone (———). Percentages of stained cells in the indicated regions were determined by curve subtraction. The demarcation between the dully and brightly staining populations was set to include in the brightly staining region ~95% of KJ23a+ SJL lymph node T cells analyzed in parallel (data not shown).

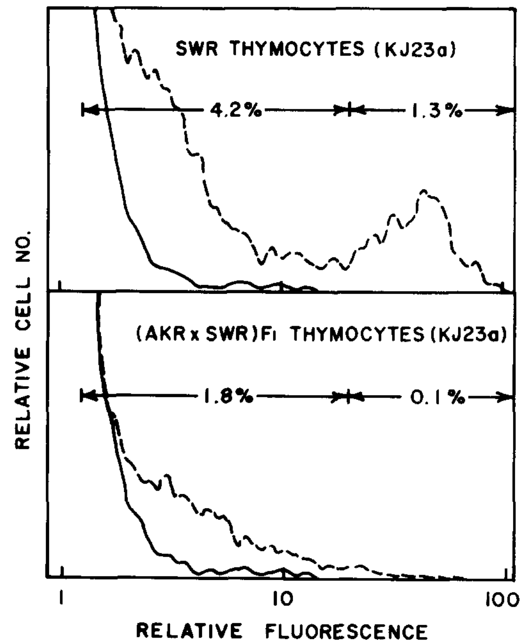

Figure 7. Elimination of KJ23a Expression in IE+ Mice among Mature, but Not Immature, Thymocytes (SWR vs. (AKR × SWR)F1

Fluorescence histograms are shown for adult thymocytes from SWR and (AKR × SWR)F1 mice (- - -) or, as a negative control, from AKR mice (———) stained first with unconjugated KJ23a, then with fluoresceinated 187.1 (rat anti-mouse κ), and finally with fluoresceinated RG7-9.1 (mouse anti-rat κ). The percentages of cells in the dully and brightly staining regions were calculated as in Figure 6.

shown in the right half of Table 2. As predicted, these cells expressed both L3T4 and Lyt2. These results establish that in IE-expressing strains, Vβ17a expression among the immature thymocytes is normal, with elimination of Vβ17a+ clones occurring at some time during thymocyte maturation.

Discussion

A number of processes are known to affect the repertoire for antigen of mature, peripheral T cells. One of these is the phenomenon of selection for self-MHC restriction, a process that occurs in the thymus whereby maturing thymocytes are selected for recognition of antigen in association with MHC proteins expressed in the thymus. T cells released from the thymus will therefore be more likely to respond to antigen in association with self-MHC proteins than antigen bound to foreign MHC products (Bevan, 1977; Zinkernagel et al., 1978).

Another phenomenon that affects the repertoire of peripheral T cells is tolerance, simply defined as unresponsiveness to an antigen even though the animal generates T cells that could respond to that antigen. The problem of how and when tolerance to self-antigens is induced has long been posed and remains one of the major problems confronting immunologists today. At least two important questions are unresolved: In unmanipulated animals, to what extent is tolerance only induced during T cell development rather than after emigration of mature T cells from the thymus? Is tolerance caused by the elimination of self-reactive T cells, or is it maintained by the suppression of their activation?

Tolerance must be established in T cells both for self-MHC proteins and for the many other potential self-antigens that might be recognized in association with self-MHC. Since many of these non-MHC antigens are never expressed within the thymus, it is reasonable to assume that tolerance to these antigens must be established after emigration of fully differentiated T cells from the thymus. The results in several experimental systems support this idea. For example, globular foreign protein antigens, when injected in an aggregate-free form, induce a profound state of T cell tolerance to subsequent injection with normally immunogenic forms of the same antigen (Dresser et al., 1962; Chiller et al., 1971). Although there is no consensus on the mechanism of this experimentally induced tolerance, there is little doubt that it involves the induction of unresponsiveness in mature peripheral T cells.

The site and timing of tolerance to MHC–antigens has been a thornier issue, since T cells are exposed to these proteins throughout their development and in fact several models for selection of self-MHC restriction in the thymus intimately involve simultaneous induction of tolerance to self-MHC–antigens. The data on the subject have not

been conclusive. Some time ago experiments were performed in which thymuses bearing foreign MHC–antigens were grafted into T cell–depleted mice. The T cells that subsequently developed in these mice were tolerant to the MHC–antigens of the graft and the host (Kindred, 1978). In vitro introduction of allogeneic fetal liver cells into fetal thymus yields thymocytes tolerant to MHC of both the fetal liver and the thymus (Good et al., 1983). In more recent experiments using thymus grafts depleted of hemopoetically derived cells (macrophages and dendritic cells) tolerance to the MHC–antigens of the graft was not observed (Zinkernagel et al., 1980; Jenkinson et al., 1985; von Boehmer and Schubiger, 1986). This has led to the idea that tolerance is induced in the thymus during thymocyte interaction with cells of extra-thymic origin.

The mechanism of the maintenance of tolerance has been a most controversial area. The longest standing hypothesis proposes that self-reactive clones are eliminated via interaction with antigen. For example, at a particular stage in development antigen binding to the T cell receptor may send a lethal rather than stimulatory signal to the cell. In mature T cells antigen binding to the receptor in the absence of sufficient secondary signals (interleukin-1?) from the antigen-presenting cells may cause the T cell to self-destruct (Lafferty and Woolnough, 1977). However, an alternate view is that self-reactive T cells are not eliminated, but rather are prevented from responding to antigen by other regulatory elements in the immune system. For example, suppressor T cells have been credited with the inactivation of other T cells in an antigen-specific manner. The best evidence for suppressor T cells comes from experimental models involving induction of nonresponsiveness to foreign antigens (Green et al., 1983). However, their role has been postulated in tolerance to self-MHC antigens as well (Brondz et al., 1984), because of the persistent demonstration in vitro of T cells reactive to self-MHC (Smith and Pasternak, 1978; Battisto and Ponzio, 1981; Glimcher and Shevech, 1982). The possibility has been raised that these T cells are not apparent in vivo because of some suppressive mechanism that is lost in vitro. More recently, several experimental transplantation models have involved the induction of tolerance to transplanted foreign tissue, without the loss of T cells reactive to the foreign MHC–antigens of the graft (Talmage et al., 1986). These results raise the possibility that tolerance to self-MHC–antigens may be maintained by mechanisms other than clonal elimination.

The difficulty in interpreting these experimental results in terms of the mechanism and site of tolerance induction has been that they rely on the detection of self-reactive T cell clones solely on the basis of their reactivity. The discovery of the anti-Vβ antibody, KJ23a, has allowed us to examine these questions in a new way by tracking the fate of a set of potentially self-reactive clones from the moment they develop a surface receptor. The results reported in our accompanying paper (Kappler et al., 1987) showed that in SWR mice T cell receptors that use the Vβ region to which this antibody binds, Vβ17a, react with IE molecules with very high frequency, apparently regardless of the rest of the components of the receptor. Thus $V\beta17a^+$ T cell receptors selected by $H\text{-}2^q$ thymuses (the MHC haplotype expressed in SWR animals) often react with IE. This also seems to be true of $V\beta17a^+$ receptors selected by other MHC haplotypes, as suggested by the data in this paper and by our own preliminary surveys. Therefore, we could use this antibody to locate T cells with these receptors regardless of their state of differentiation or potential inducibility with antigen and observe the fate of these cells in animals that expressed an IE protein.

The data in this paper show that $V\beta17a^+$ T cells are severely depleted peripherally in mice expressing IE. Examination of F1 animals demonstrated that this was not due to the fact that $V\beta17a^+$ T cells were not selected in the thymuses of these mice. For example, such T cells are selected very effectively in SJA ($H\text{-}2^s$) animals and constitute about 10% of their peripheral T cell pool. When SJA animals are crossed with BALB/c, however, the F1 progeny, which now express both IE^d and $IE^{d/s}$, contain virtually no $V\beta17a^+$ T cells. The inescapable conclusion of this, and other similar experiments, is that expression of IE eliminates these cells from the peripheral T cell pool, thus clearly demonstrating that in this case, tolerance to self-MHC in an unmanipulated animal is due to clonal deletion.

The profundity of elimination did not vary within a particular strain or strain combination, but did vary among the different IE^+ mice. In (BALB/c × SJA)F1 or C57BR animals, for example, $V\beta17a^+$ T cells were almost completely absent. In (AKR × SWR)F1 mice, on the other hand, residual $V\beta17a^+$ T cells were found, although their numbers were still far less than those predicted had selective elimination not occurred. There are several possible explanations for this variation. Variation in the degree of elimination of Vβ17a-expressing cells could be due to differences in the repertoire of these cells selected in the thymus by different MHC types. $H\text{-}2^k$, for example, may select very strongly for T cells bearing $V\beta17a^+$ receptors that will react with IE^k. On the other hand, $V\beta17a^+$ receptors selected in the thymus by $H\text{-}2^q$ may not all react with IE^k. Indeed our own data show that this latter selection leads to only about 70% reactivity with IE^k. A second possibility is that the efficiency of elimination of $V\beta17a^+$ T cells may be related to differences in dose of IE expressed in the different animals. Homozygous C57BR mice express IE^k from both MHC-bearing chromosomes. (BALB/c × SJA)F1 mice express both IE^d and $IE^{d/s}$, although the exact amount of protein that reaches the cell surface in such animals is controlled by the level of IE_α chain synthesis from the $H\text{-}2^d$ complex. By contrast (AKR × SWR)F1 animals express only one haploid dose of IE, because both the IE α and β chains encoded by $H\text{-}2^q$ are nonfunctional (Mathis et al., 1983; Dr. P. Jones, personal communication). Sorting out these possibilities will require the breeding of mice carrying the Vβ17a gene and homozygous at H-2.

During the last 10 years much has been learned about pathways of thymocyte maturation. Monoclonal antibodies have been used to identify thymocytes at different stages of their life histories, and it is now agreed that early thymocytes, which constitute about 3% of the cells in the

organ of a mature mouse, bear neither Lyt-2 or L3T4 nor α/β T cell receptors, but are positive for receptors for interleukin-2 (Raulet, 1985; Fowlkes et al., 1985). Once in the thymus these cells proliferate, rearrange, and express their T cell receptor genes, giving rise to the predominant cell type in the thymus (Born et al., 1985; Snodgrass et al., 1985). This cell type is found in the thymus cortex, is cortisone-sensitive, and bears both Lyt2 and L3T4, i.e., it is "double positive" (Ceredig et al., 1983; Scollay et al., 1984). About 50% of the cells of this type bear surface T cell receptors, though at much lower concentrations than peripheral T cells (Roehm et al., 1984). Some of these cells continue to divide. Finally mature thymocytes that are almost indistinguishable from peripheral T cells, are cortisone-resistant, and bear either Lyt2 or L3T4 ("single positive") and high levels of T cell receptor (Ceredig et al., 1983; Scollay et al., 1984; Roehm et al., 1984; Fowlkes et al., 1985) appear. Two theories have been suggested to account for the development of mature thymocytes. One suggests that they appear via an almost undetectable immature intermediate and that thymocytes which have not been selected to mature differentiate into the predominant Lyt2$^+$, L3T4$^+$ cortical population, all of which is destined to die. Another theory suggests that all mature thymocytes are selected from the intermediate, double positive pool, but that because thymus selection picks out only a small percentage of all cells, most of the cells in this intermediate pool go on to die. There is little direct evidence at present to distinguish these theories.

The second major conclusion of our experiments is that, at least for this set of T cell clones, the establishment of tolerance by clonal elimination occurs within the thymus at an apparently late stage of differentiation. We found that tolerance induction occurs while or before thymocytes mature into single positive, cortisone-resistant medullary cells. Interestingly, we have previously shown that if thymocytes differentiate in the presence of anti-receptor antibodies their maturation is blocked at a similar stage. Immature, double positive cells are unaffected by such antibodies, but the appearance of mature thymocytes with which the anti-receptor antibodies can react is inhibited (McDuffie et al., 1986; Born et al., 1987). Taken together, these data lead us to suggest that selection for self-MHC restriction and tolerance to self-MHC itself may occur almost at the same time in thymocyte maturation, at the point at which mature cells are selected to arise from cells within the immature, cortical thymocyte pool. These results are not incompatible with either theory on the origins of mature thymocytes, since both hypothesize that the processes of selection and tolerance do not operate on the majority of the cells in this immature pool.

Experimental Procedures

Mice

All mice were either bred in our own facility or obtained from the Jackson Laboratory, Bar Harbor, ME.

Flow Cytometric Analyses

Analyses were performed essentially as previously described (Roehm et al., 1984) using a Coulter Epics C Flow cytometer. The following monoclonal antibodies were used in these experiments: KJ23a, mouse IgG, specific for Vβ17a (Kappler et al., 1987); KJ16, rat IgG, specific for Vβ8.1 and Vβ8.2 (Haskins et al., 1984); 2.43, rat IgG, specific for Lyt2.2 (Sarmiento et al., 1982); GK-1.5, rat IgG specific for L3T4 (Dialynas et al., 1983); Y17, mouse IgG, specific for all IE molecules except IE^d (Lerner et al., 1980); 187.1, rat IgG, specific for mouse κ chain (Yelton et al., 1981); RG7/9.1, mouse IgG, specific for rat κ chain (a gift from Dr. T. Springer).

Single color analyses were performed with biotin conjugated to KJ23a, KJ16, or Y17 as the primary reagent and phycoerythrin–streptavidin (Becton Dickinson, Mountain View, CA) as the secondary reagent. In a few experiments unconjugated KJ23a was used as the primary reagent, fluorescein–187.1 as the secondary reagent, and fluorescein–RG7/9.1 as the tertiary reagent. Various negative controls were used including the secondary (or secondary and tertiary) reagents alone, the full set of reagents used with cells from a different mouse strain not reactive with the primary antibody, or a different primary antibody not reactive with the cells in the experimental group. In each case, the fluorescence seen in these negative controls was not significantly different from the autofluorescence seen with the cells alone. The number of cells in a particular region of a fluorescence histogram was calculated by subtraction of the number of cells in that region of the appropriate negative control from the number of cells in that region of the experimental group. This calculation was most accurate for brightly staining cells whose fluorescence did not significantly overlap the negative control. The calculation was somewhat of an underestimate for dully staining cells whose fluorescence significantly overlapped the negative control.

Two color analyses of thymocytes were performed with biotinylated KJ23a/phycoerythrin–streptavidin for red fluorescence and fluorescein conjugated to 2.43 and/or GK-1.5 for green fluorescence. Various combinations of three reagents were used to calculate the percentage of immature L3T4$^+$/Lyt2$^-$ and mature L3T4$^-$/Lyt2$^+$ or L3T4$^+$/Lyt2$^-$ thymocytes, as well as the percentage of each of these three populations staining brightly or dully with KJ23a. In making these calculations, the small (~2%) portion of very immature thymocytes that were L3T4$^-$/Lyt2$^-$ were assumed to be KJ23a$^-$. Also, no correction was made for this population in calculating the percentages of L3T4$^+$/Lyt2$^+$, L3T4$^+$/Lyt2$^-$, or L3T4$^-$/Lyt2$^+$ cells, so that the portion of L3T4$^+$/Lyt2$^+$ cells was slightly underestimated, and that for the other two populations, slightly overestimated.

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