POSTNATAL DISAPPEARANCE OF SELF-REACTIVE
(Vβ6+) CELLS FROM THE THYMUS OF Mlsα MICE
Implications for T Cell Development and Autoimmunity

By RETO SCHNEIDER, ROSEMARY K. LEES,* THIERRY PEDRAZZINI,†
ROLF M. ZINKERNAGEL, HANS HENGARTNER,
AND H. ROBSON MACDONALD*

From the Institute of Pathology, University Hospital, 8091 Zurich, Switzerland; the *Ludwig
Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland; and the
†World Health Organization Immunology Research and Training Center,
Institute of Biochemistry, 1066 Epalinges, Switzerland

The mature TCR repertoire is now known to be shaped by both negative and
positive selection events occurring within the thymus. Thus, TCR with high affinity
for constitutively expressed self antigens (in association with appropriate MHC gene
products) are deleted during T cell development (1-7), whereas TCR with (presum-
ably) low affinity for MHC class I and class II molecules are positively selected to
become mature CD8+ and CD4+ T cells, respectively (7-9). The formal demon-
stration that these selective processes occur in both normal and TCR transgenic mice
confirms earlier studies of radiation bone marrow chimeras (10-12) and provides
a framework in which TCR repertoire development can be analyzed.

In the present study, we have undertaken a detailed analysis of the ontogeny of
disappearance of self-reactive cells using a recently described model system (3) in
which a particular TCR β chain variable domain (Vβ6) correlates with reactivity
to a minor antigen encoded within the Mlsα locus. Our results indicate that self-
reactive (Vβ6+) T cells with a mature (CD4+) surface phenotype are present during
the early postnatal period in the thymus of Mlsα mice but rapidly disappear there-
after. Intriguingly, this disappearance is correlated with the transient appearance
of Vβ6+ CD4+ thymocytes bearing reduced levels of surface TCR. These results are
discussed in the context of current models of T cell development and the induction
of autoimmunity.

Materials and Methods

Mice. Congenic BALB/c (H-2k, Mlsα) and (BALB/c × BALB.D2.Mlsα) F1 (H-2k/d, Mlsβα;
hereafter referred to as BALB.Mlsα) mice were bred in the animal facility of the Swiss Insti-
tute for Experimental Cancer Research, Epalinges, Switzerland. BALB.D2.Mlsα (13) breeders
were kindly provided by Dr. Hilliard Festenstein, London Hospital Medical College, U.K.

Cell Suspensions. Neonatal mice were anesthetized by cooling. Thymus, lymph nodes, and
spleen were removed and homogenized to yield single cell suspensions.

mAbs. Cytotoxic rat IgM mAbs against CD4 (RL172.4) and CD8 (3.168.1) were used
in the presence of rabbit complement to deplete thymocyte suspensions of CD4+ or CD8+

This work was supported in part by a grant from the Swiss National Science Foundation (to H. Hengartner).

J. EXP. MED. © The Rockefeller University Press · 0022-1007/89/06/2149/10 $2.00
Volume 169 · June 1989 · 2149–2158
ontogeny of autoreactive T cells, respectively (14). For immunofluorescent staining, the rat IgG mAbs GK-1.5 (anti-CD4) or 53-6.7 (anti-CD8) were used. Rat mAbs to the TCR β chain were 44-22-1 (anti-Vβ; reference 15) and KJ16-133 (anti-Vβ8.1/8.2; reference 16).

Flow Microfluometry. Single and two-color immunofluorescence were performed on a modified FACS II flow cytometer (Becton Dickinson & Co., Sunnyvale, CA) as described previously (3).

Assessment of Mls° Ontogeny. The stimulatory capacity of spleen cells from newborn or adult BALB.Mls° mice was assessed by incubating graded doses of cells (10⁴–10⁶) with 2 x 10⁴ cells of an Mls°-specific T cell hybrid RG17.16 (17). After 48 h, the IL-2 content of supernatants was measured as described (18).

Immunohistochemical Localization of Vβ6° Cells in Mls° Thymus and Lymph Nodes. Frozen cryosections were prepared and stained with mAbs 44-22-1 (anti-Vβ6) or KJ16-133 (anti-Vβ8) as described in detail elsewhere (19).

Neonatal Tolerance Induction. BALB/c (Mls°) mice were injected intraperitoneally with 10⁸ DBA/2 (Mls°) spleen cells within 24 h of birth.

Results

Ontogeny of Vβ6° Expression in the Mls° Thymus. We have previously shown that Vβ6° T cells are absent in peripheral lymphoid tissues and mature thymus subsets of adult (4–6 wk old) Mls° mice (3), but are present as a dull staining subpopulation among CD4°Vβ6° thymocytes in the thymus cortex (19). In preliminary experiments (not shown), we observed that early postnatal thymi from BALB.Mls° mice contained bright (as well as dull) Vβ6° cells. Depletion of CD8° cells followed by double staining (Fig. 1) demonstrated that most of the bright Vβ6° cells in day 4 BALB.Mls° thymus were of the CD4° phenotype. A detailed analysis of the kinetics of disappearance of bright Vβ6° cells (in the CD4° compartment) is illustrated in Fig. 2. It can be seen that CD4°Vβ6° cells reached a maximum (3–4%) on day 4 in BALB.Mls° mice and declined rapidly thereafter, reaching adult levels (<0.5%) by day 10. Control congenic BALB/c (Mls°) thymus contained a higher proportion (~8%) of CD4°Vβ6° cells early after birth and this proportion remained constant thereafter. The disappearance of CD4°Vβ6° cells in BALB.Mls° thymus was selective since CD4°Vβ6° cells were present at levels comparable with control BALB/c mice (Fig. 2). A parallel kinetic analysis of CD8° thymocytes in neonatal BALB.Mls° mice revealed that Vβ6° cells accounted for only 0.6% on day 3–4 and declined thereafter. In contrast, 7.2% of CD8° thymocytes expressed Vβ6 on day 4 in control BALB/c mice.

Localization of Vβ6° Cells on Thymus Cryosections. Vβ6° cells could be localized on

![Figure 1](image-url)
Kinetics of disappearance of CD4+ Vβ6+ cells in neonatal Mlsa thymus. CD8-depleted BALB.Mlsa (or control BALB/c) thymocytes were stained with mAbs against Vβ6 or Vβ8 on the indicated days. Data are expressed as a percentage of CD4+ cells (65-85% of total CD8+ thymocytes) as indicated in Fig. 1.

Frozen thymus sections from neonatal DBA/2 (Mlsa) mice (Fig. 3). In contrast to the adult (19), Vβ6+ cells were prominent in the medulla of the Mlsa thymus until day 6. At later times (7-8 d), there was a precipitous decrease in medullary Vβ6+ cells, although disperse staining of Vβ6+ cells throughout the thymus cortex per-

**Figure 2.** Kinetics of disappearance of CD4+ Vβ6+ cells in neonatal Mlsa thymus. CD8-depleted BALB.Mlsa (or control BALB/c) thymocytes were stained with mAbs against Vβ6 or Vβ8 on the indicated days. Data are expressed as a percentage of CD4+ cells (65-85% of total CD8+ thymocytes) as indicated in Fig. 1.

**Figure 3.** Anatomical localization of Vβ6+ cells in early postnatal thymus. Cryosections of BALB/c (Mlsb) or DBA/2 (Mlsa) thymus were stained with anti-Vβ6 mAb at the indicated ages and revealed by peroxidase-conjugated anti-rat Ig.
sisted until adulthood (compare with reference 19). In control BALB/c (Mlsb) thymus, Vβ6+ cells were present in both cortex and medulla at all ages tested.

Selectively Decreased Intensity of Vβ6 Staining in Neonatal Mlsa Thymus. Careful analysis of the staining profiles of CD4+ thymocytes from neonatal BALB.Mlsa mice revealed a progressive shift in the mean Vβ6 fluorescence intensity as compared with age-matched BALB/c controls. At early times (day 2–3), Vβ6+CD4+ cells stained equally brightly in Mlsa and Mlsb thymus (data not shown). However, beginning around day 4–5, CD4+ thymocytes from BALB.Mlsa mice stained less brightly for Vβ6 than the corresponding BALB/c (Mlsb) thymocytes (Fig. 4). This difference, which persisted as long as Vβ6+ cells could be detected in Mlsa mice (i.e., until day 7–10), was highly reproducible (18.6 ± 1.6 log fluorescence channels, mean ± SEM, n = 8) and corresponded in absolute terms to a 40–50% decrease in TCR density. The intensity of Vβ8 staining was, however, comparable for CD4+ thymocytes from
Mls\textsuperscript{a} and Mls\textsuperscript{b} mice throughout neonatal ontogeny (e.g., see Fig. 4), emphasizing that the decrease was selective for V\beta\textsuperscript{a}.

Peripheralization of V\beta\textsuperscript{b} Cells in Mls\textsuperscript{a} Mice. The disappearance of CD4\textsuperscript{+}V\beta\textsuperscript{b} cells from the thymus of neonatal Mls\textsuperscript{a} mice raised the question of the subsequent fate of these cells. As shown in Fig. 5, a small (but significant) proportion of V\beta\textsuperscript{b} cells could be detected in tissue sections of mesenteric lymph nodes from neonatal DBA/2 (Mls\textsuperscript{a}) mice. By flow microfluorometry, V\beta\textsuperscript{b} cells accounted for \sim1.5\% of BALB.Mls\textsuperscript{a} lymph node cells on days 4 and 6, as compared with 0.2\% in the adult.

Ontogeny of Mls\textsuperscript{a} Stimulatory Capacity. The ontogeny of cells capable of stimulating an Mls\textsuperscript{a}-specific T cell response was investigated using a T cell hybrid (RG 17.16) that secretes IL-2 in response to Mls\textsuperscript{a} antigens presented in association with appropriate MHC class II gene products (particularly E\textsuperscript{k} and E\textsuperscript{d}) (17). Adult BALB.Mls\textsuperscript{a} spleen was a potent stimulator of RG17.16, whereas neonatal (1 d) spleen did not induce a detectable response (Fig. 6). Further analysis indicated that Mls\textsuperscript{a} stimulatory capacity was first detected at 4 d (Fig. 6).

Disappearance of V\beta\textsuperscript{b} Cells in Neonatal Mls\textsuperscript{b} Thymus after Transfer of Mls\textsuperscript{a} Spleen Cells. In view of the uncertainty regarding the ontogeny of expression of the Mls\textsuperscript{a} antigen, the kinetics of disappearance of CD4\textsuperscript{+}V\beta\textsuperscript{b} cells was also investigated in BALB/c (Mls\textsuperscript{b}) mice that had received a neonatal injection of DBA/2 (Mls\textsuperscript{a}) spleen cells. As shown in Table I, CD8\textsuperscript{+} thymocytes from 4-d-old “Mls\textsuperscript{a}-tolerant” BALB/c mice already had slightly reduced numbers of V\beta\textsuperscript{b} cells, as compared with age-matched controls. Furthermore, the mean intensity of V\beta\textsuperscript{b} staining was significantly diminished in the tolerant animals (data not shown), as previously observed for newborn BALB.Mls\textsuperscript{a} mice (Fig. 4). By day 8, CD4\textsuperscript{+}V\beta\textsuperscript{b} cells were present at very low levels in the DBA/2-injected mice, as compared with controls (Table I). Staining with the control V\beta\textsuperscript{a}-specific mAb KJ16 did not reveal a significant decrease in the proportion of positive cells in neonatally Mls\textsuperscript{a}-tolerant animals (Table I), nor was there a shift in V\beta\textsuperscript{b} fluorescence intensity (data not shown).

![Figure 6](image-url)  
**FIGURE 6.** Ontogeny of Mls\textsuperscript{a} stimulatory capacity. Graded numbers of spleen cells from neonatal BALB.Mls\textsuperscript{a} mice of various ages were used to stimulate IL-2 production by the Mls\textsuperscript{a}-specific T cell hybrid RG17.16 (O). Adult BALB.Mls\textsuperscript{a} spleen cells served as a positive control in each experiment (●).

Discussion

The recent demonstration that TCR utilizing V\beta\textsuperscript{b} (3) or V\beta\textsuperscript{b,1} (2) react preferentially with Mls\textsuperscript{a}-encoded antigens and that mature V\beta\textsuperscript{b} or V\beta\textsuperscript{b,1} T cells are deleted intrathymically in mice expressing the Mls\textsuperscript{a} gene product provides a unique
model system in which to analyze the mechanisms underlying tolerance induction in vivo. In this report, we have analyzed the thymic ontogeny of \( V_{\beta 6} \) expression in BALB.Mls\(^a\) mice. Surprisingly, we find that some brightly staining \( V_{\beta 6}^- \) T cells are not deleted until 7-10 d after birth. These \( V_{\beta 6}^- \) thymocytes are predominantly of the CD4\(^+\) phenotype and are initially present in the medulla of the developing thymus. Furthermore, the intensity of TCR expression by these CD4\(^+\) cells is significantly reduced as compared with age-matched congenic BALB/c controls. On the basis of these findings, we would like to suggest that CD4\(^+\) \( V_{\beta 6}^- \) thymocytes in neonatal BALB.Mls\(^a\) mice are undergoing a physiological response to tolerogenic signals in vivo.

Current models of T cell development within the thymus (20-24) argue in favor of a differentiation pathway in which CD4\(^-\)8\(^-\) (TCR\(^-\)) precursor cells give rise to CD4\(^+\)8\(^+\) (TCR\(^+\)) "cortical" thymocytes, a proportion of which are further selected to become mature CD4\(^+\) or CD8\(^+\) T cells. The status of CD4\(^+\)8\(^+\) thymocytes as developmental intermediates (rather than "dead end" cells) has been greatly strengthened by recent studies in which developing T cells bearing a self-reactive TCR have been followed by anti-TCR mAbs. Thus, in both normal (3) and transgenic (4, 7) animals, clonal deletion of autoreactive TCR specific for MHC class I- or class II-restricted antigens has been found to occur in both CD4\(^+\) (class II-restricted) and CD8\(^+\) (class I-restricted) mature subsets, arguing that elimination may occur at a stage when both CD4 and CD8 are expressed. Furthermore, inhibition of the clonal deletion process by in vivo administration of anti-CD4 mAbs (in the case of class II MHC-restricted TCR) was found to restore autoreactive cells in the complementary (CD8\(^+\)) subset (22, 23). Collectively, these results make a strong case that the CD4\(^+\)8\(^+\) thymocyte is a precursor of both mature T cell lineages as well as a target for the negative selection process.

It is of interest to try to relate the findings described in this report to such a developmental model. In this regard, the presence of some CD4\(^+\) \( V_{\beta 6}^- \) cells in the early postnatal BALB.Mls\(^a\) thymus could be interpreted as being due simply to the absence of tolerogen (Mls\(^a\) antigen) at this stage of development, a possibility that is difficult to evaluate experimentally (see below). On the other hand, the fact that CD4\(^+\) \( V_{\beta 6}^- \) cells are considerably less frequent (even shortly after birth) in BALB.Mls\(^a\) thymus as compared with age-matched BALB/c controls suggests that
some manifestation of self-tolerance is occurring. Moreover, the selectively reduced levels of TCR expression on these CD4+ thymocytes that coincides temporally with their disappearance from the thymus (day 4–7) provides suggestive evidence that they may be responding to a tolerogenic stimulus in vivo. If this interpretation is correct, our results raise the possibility that thymocytes bearing autoreactive MHC class II-restricted TCR are either deleted at a CD4+ stage of development or alternatively progress to the CD4+ stage after receiving a tolerogenic signal in an earlier (presumably CD4+8+) compartment. It is noteworthy that CD8+ Vβ6+ thymocytes are selectively absent in neonatal Mlsa mice, in contrast to the situation in neonatal or adult Mlsb thymus. Such asymmetry would suggest that commitment of Vβ6+ cells to the CD4+ lineage has already occurred at the CD4+8+ developmental stage in Mlsa thymus, a possibility that can be reconciled with either positive or negative selection mechanisms (24).

Nothing is currently known about the mechanism of deletion of autoreactive cells within the thymus, although various models including specific veto cells (25, 26) or programmed cell death (27) have been proposed. In this regard, the selectively decreased TCR density on CD4+Vβ6+ cells from either the neonatal BALB.Mlsa thymus or the thymus of Mlsb-tolerant BALB/c mice could be interpreted as a manifestation of TCR downregulation (or modulation) resulting from recent contact with a tolerogenic signal. Similar decreases in TCR density have been observed on mature T cells after stimulation with anti-TCR mAbs (28) or phorbol esters (29). Alternatively it is possible that CD4+Vβ6+ thymocytes with constitutively low TCR density selectively accumulate in Mlsa mice because of a corresponding reduced avidity for Mlsa/class II MHC tolerogens.

Early postnatal thymocytes have been shown to react to self antigens (30, 31), including those apparently encoded by the MHC class II locus (32). In this context, it is also well established that effective functional tolerance to foreign antigens (including Mlsa) can be induced in mice by injection of the antigen (or antigen-bearing cells) within a short period after birth (33–35). Furthermore, as shown here and elsewhere (36), such neonatally induced tolerance (at least in the Mlsa system) is accompanied by rapid intrathymic deletion of mature Vβ6+ cells. Taken together, these observations raise the possibility that post-natal elimination of autoreactive cells is not unique to the Mlsa antigen but, rather, represents a normal physiological pathway of self tolerance during development. Although seemingly potentially harmful to the organism, delayed induction of tolerance may be necessitated by limitations in the rate of entry of tissue-specific antigens and/or hematopoietic-derived (tolerance-inducing?) cells into the thymus after birth. Alternatively, it is possible that the Mlsa antigen is unusual in that its expression is delayed during ontogeny. As shown here and elsewhere (37), the ability of splenic cells to stimulate an Mlsa-specific T cell response in vitro cannot be detected at birth; however, it is not clear whether this represents a delayed transcriptional activation of the Mlsa gene or simply a delayed development of those cells (most likely a subset of B lymphocytes [38, 39]) that are best able to stimulate an Mlsa response in vitro. The fact that the postnatal kinetics of elimination of CD4+Vβ6+ cells is similar in the thymus of Mlsa mice or Mlsb mice injected at birth with Mlsa spleen cells would be consistent with the latter interpretation. However, molecular (or serological) definition of the Mlsa gene product will be required to completely resolve this issue.
If the Mls system described herein does prove to be a representative model of self tolerance, then it follows that a previously underestimated population of potentially autoreactive T cells are generated (and peripheralized) during early life. Although these cells would be dramatically diluted during the further course of normal T cell development, it remains to be investigated whether they persist in the adult as autoreactive cells, or alternatively, are functionally inactivated by some peripheral tolerance mechanism (40). The potential of a neonatal cohort of self-reactive T cells to provoke autoimmunity is strongly supported by the finding that thymectomy of mice or rats within a short time period after birth leads to a greatly increased incidence of a wide range of autoimmune disorders (41-45). Such a result would be consistent with the delayed kinetics of elimination of autoreactive cells in our model system.

Summary

The postnatal ontogeny of potentially autoreactive T cells has been studied in a model system where a particular TCR β chain variable domain (Vβ6) is correlated with reactivity to a minor antigen encoded by the Mls locus. Although absent among mature (CD4+ or CD8+) T cells in adult mice expressing Mls, brightly staining Vβ6+ cells were readily detectable in the thymus of neonatal animals, reaching a maximum after 4 d and decreasing rapidly thereafter. These Vβ6+ thymocytes were predominantly of the CD4+ phenotype and were localized in the medulla of the developing thymus. Furthermore, the intensity of TCR expression by these CD4+ cells was significantly (twofold) reduced as compared with age-matched Mlsb controls. A rapid disappearance of CD4+Vβ6+ cells (and corresponding decrease in TCR density) could also be observed in the thymus of Mlsb mice that had been injected neonatally with Mlsa spleen cells. Taken together, these results raise the possibility that some autoreactive T cells may persist after birth and that TCR downregulation may occur as a physiological response to tolerogenic signals in vivo.

We thank P. Zaech and C. Knabenhans for performing the flow cytometry, B. Odermatt for assistance with the immunohistochemistry, and A.-F. Brunet for preparing the manuscript.

Received for publication 28 December 1988 and in revised form 15 February 1989.

References

1. Kappler, J. W., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. Cell. 49:273.
2. Kappler, J. W., U. Staerz, J. White, and P. Marrack. 1988. Self tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature (Lond.). 332:35.
3. MacDonald, H. R., R. Schneider, R. K. Lees, R. C. Howe, H. Acha-Orbea, H. Festeinstein, R. M. Zinkernagel, and H. Hengartner. 1988. T cell receptor Vβ use predicts reactivity and tolerance to Mls-encoded antigens. Nature (Lond.). 332:40.
4. Kisielow, P., H. Bluthmann, U. D. Staerz, M. Steinmetz, and H. von Boehmer. 1988. Tolerance in T cell receptor transgenic mice involves deletion of nonmature CD4+8+ thymocytes. Nature (Lond.). 333:742.
5. Pullen, A. M., P. Marrack, and J. W. Kappler. 1988. The T-cell repertoire is heavily
influenced by tolerance to polymorphic self antigens. Nature (Lond). 335:796.
6. Fry, A. M., and L. A. Matis. 1988. Self-tolerance alters T-cell receptor expression in an antigen-specific MHC restricted immune response. Nature (Lond). 335:830.
7. Sha, W. C., C. A. Nelson, R. D. Newberry, D. M. Kranz, J. H. Russell, and D. Y. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. Nature (Lond). 336:73.
8. Kisielow, P., H. S. Teh, H. Blüthmann, and H. von Boehmer. 1988. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. Nature (Lond). 335:730.
9. MacDonald, H. R., R. K. Lees, R. Schneider, R. M. Zinkernagel, and H. Hengartner. 1988. Positive selection of CD4+ thymocytes controlled by MHC class II gene products. Nature (Lond). 336:471.
10. Bevan, M. J., and P. J. Fink. 1978. The influence of thymus H-2 antigens on the specificity of maturing killer and helper cells. Immunol. Rev. 42:3.
11. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T cell restriction specificity, function and responsiveness. Adv. Immunol. 27:51.
12. Sprent, J., D. Lo, E.-K. Gao, and Y. Ron. 1988. T cell selection in the thymus. Immunol. Rev. 101:173.
13. Festenstein, H., and L. Berumen. 1974. Balb.D2.Mls+: a new congenic mouse strain. Transplantation (Baltimore). 37:322.
14. Budd, R. C., M. Schreyer, G. C. Miescher, and H. R. MacDonald. 1987. T cell lineages in the thymus of lpr/lpr mice. Evidence for parallel pathways of normal and abnormal T cell development. J. Immunol. 139:2200.
15. Payne, J., B. T. Huber, N. A. Cannon, R. Schneider, M. W. Schilham, H. Acha-Orbea, H. R. MacDonald, and H. Hengartner. 1988. Two monoclonal rat antibodies with specificity for the 3-chain variable region Vb of the murine T-cell receptor. Proc. Natl. Acad. Sci. USA. 85:7695.
16. Haskins, K., C. Hannum, J. White, N. Roehm, R. Kubo, J. Kappler, and P. Marrack. 1984. The major histocompatibility complex-restricted antigen receptor on T cells. VI. An antibody to a receptor allotype. J. Exp. Med. 160:452.
17. MacDonald, H. R., A. L. Glasebrook, R. Schneider, R. K. Lees, H. Pircher, T. Pedrazzini, O. Kanagawa, J.-F. Nicolas, R. C. Howe, R. M. Zinkernagel, and H. Hengartner. 1989. T cell reactivity and tolerance to Mls4-encoded antigens. Immunol. Rev. 107:89.
18. Landegren, U. 1984. Measurement of cell number using an endogenous enzyme, hexosaminidase. Applications for the detection of lymphokines and cell surface antigens. J. Immunol. Methods. 67:379.
19. Hengartner, H., B. Odermatt, R. Schneider, M. Schreyer, G. Walle, H. R. MacDonald, and R. M. Zinkernagel. 1988. Deletion of self-reactive T cells before entry into the thymus medulla. Nature (Lond). 336:388.
20. Möller, G. 1988. T cell precursors. Immunol. Rev. Vol. 104.
21. Smith, L. 1987. CD4+ murine T cells develop from CD8+ precursors in vivo. Nature (Lond). 326:798.
22. Fowlkes, B. J., R. H. Schwartz, and D. M. Pardoll. 1988. Deletion of self reactive thymocytes occurs at a CD4+8+ precursor stage. Nature (Lond). 334:620.
23. MacDonald, H. R., H. Hengartner, and T. Pedrazzini. 1988. Intrathymic deletion of self reactive cells prevented by neonatal anti-CD4 antibody treatment. Nature (Lond). 335:174.
24. Janeway, C. A. 1988. Accessories or coreceptors? Nature (Lond). 335:208.
25. Miller, R. G. 1986. The veto phenomenon and T-cell regulation. Immunol. Today. 7:112.
26. Fink, P. J., R. P. Shimonkevitz, and M. J. Bevan. 1988. Veto cells. Annu. Rev. Immunol. 6:115.
27. Duvall, E., and A. H. Wyllie. 1986. Death and the cell. Immunol. Today. 7:115.
28. Meuer, S. C., K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983. Clonotypic structures involved in antigen-specific human T cell function: relationship to the T3 molecular complex. J. Exp. Med. 157:705.

29. Cantrell, D. A., A. A. Davies, and M. J. Crump. 1985. Activators of protein kinase C down-regulate and phosphorylate the T3/T-cell antigen receptor complex of human T lymphocytes. Proc. Natl. Acad. Sci. USA. 82:8158.

30. Howe, M., A. Goldstein, and J. Battisto. 1970. Isogenic lymphocyte interaction: recognition of self antigens by cells of the neonatal thymus. Proc. Natl. Acad. Sci. USA. 67:613.

31. von Boehmer, H., and W. Byrd. 1972. Responsiveness of thymic cells to syngeneic and allogeneic lymphoid cells. Nature (Lond.). 235:50.

32. Lattime, E., S. Gillis, G. Pecoraro, and O. Stutman. 1982. Ia-dependent interleukin 2 production in syngeneic cellular interactions. J. Immunol. 128:480.

33. Billingham, R. E., L. Brent, and P. B. Medawar. 1953. Actively acquired tolerance of foreign cells. Nature (Lond.). 172:603.

34. Macphail, S., S. T. Ishizaka, M. J. Bykowsky, E. C. Lattime, and O. Stutman. 1985. Specific neonatally induced tolerance to Mls locus determinants. J. Immunol. 135:2967.

35. Hosono, M., T. Kina, T. Hosokawa, and Y. Katsura. 1986. Neonatal tolerance induction in the thymus to MHC class II associated antigens. I. Preferential induction of tolerance to Mls antigens and resistance to allo MHC antigens. Cell. Immunol. 103:1.

36. MacDonald, H. R., T. Pedrazzini, R. Schneider, J. A. Louis, R. M. Zinkernagel, and H. Hengartner. 1988. Intrathymic elimination of Mlsa-reactive (V06') cells during neonatal tolerance induction to Mlsa-encoded antigens. J. Exp. Med. 167:2005.

37. Ahmed, A., I. Scher, and K. W. Sell. 1976. Functional studies of the ontogeny of the M-locus product: a surface antigen of mature B lymphocytes. In Leukocyte Membrane Determinants Regulating Immune Reactivity. V. P. Eijssvoogel, D. Roos, and W. P. Zeijlemaker, editors. Academic Press, New York. 703-709.

38. von Boehmer, H., and J. Sprent. 1974. Expression of M locus determinants by B cells but not T cells. Nature (Lond.). 249:363.

39. Ahmed, A., and I. Scher. 1976. Studies on non-H-2-linked lymphocyte activating determinants. II. Nonexpression of Mls determinants in a mouse strain with an X-linked B lymphocyte immune defect. J. Immunol. 117:1922.

40. Nossal, G. J. V. 1983. Cellular mechanisms of immunologic tolerance. Annu. Rev. Immunol. 1:33.

41. Yunis, E. J., R. Hong, M. A. Grewe, C. Martinez, E. Cornelius, and R. A. Good. 1967. Postthymectomy wasting associated with autoimmune phenomena. I. Antilglobulin-positive anemia in A and C57BL/6 Ks mice. J. Exp. Med. 125:947.

42. Nishizuka, Y., and T. Sakakura. 1969. Thymus and reproduction: sex-linked dysgenesis of the gonad after neonatal thymectomy in mice. Science (Wash. DC). 166:733.

43. Taguchi, O., and Y. Nishizuka. 1981. Experimental autoimmune orchitis after neonatal thymectomy in the mouse. Clin. Exp. Immunol. 46:425.

44. Silverman, D. A., and N. R. Rose. 1974. Neonatal thymectomy increases the incidence of spontaneous and methylcholanthrene-enhanced thyroiditis in rats. Science (Wash. DC). 184:162.

45. Tung, K. S. K., S. Smith, C. Teuscher, C. Cook, and R. E. Anderson. 1987. Murine autoimmune oophoritis, epididymoorchitis, and gastritis induced by day 3 thymectomy. Am. J. Pathol. 126:293.