POSTNATAL DISAPPEARANCE OF SELF-REACTIVE ($V_{\beta 6}^+$) CELLS FROM THE THYMUS OF Mls^a 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 (presumably) 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 demonstration 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 ($V_{\beta 6}$) correlates with reactivity to a minor antigen encoded within the Mls^a locus. Our results indicate that self-reactive ($V_{\beta 6}^+$) T cells with a mature ($CD4^+$) surface phenotype are present during the early postnatal period in the thymus of Mls^a mice but rapidly disappear thereafter. Intriguingly, this disappearance is correlated with the transient appearance of $V_{\beta 6}^+$ $CD4^+$ 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 ($H\text{-}2^d$, Mls^b) and (BALB/c × BALB.D2.Mls^a) F_1 ($H\text{-}2^{d/d}$, $Mls^{b/a}$; hereafter referred to as BALB.Mls^a) mice were bred in the animal facility of the Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland. BALB.D2.Mls^a (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).

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-$V_{\beta 6}$; reference 15) and KJ16-133 (anti-$V_{\beta 8.1/8.2}$; 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^a Ontogeny. The stimulatory capacity of spleen cells from newborn or adult BALB.Mls^a mice was assessed by incubating graded doses of cells (10^4–10^6) with 2×10^4 cells of an Mls^a-specific T cell hybrid RG17.16 (17). After 48 h, the IL-2 content of supernatants was measured as described (18).

Immunohistochemical Localization of $V_{\beta 6}^+$ Cells in Mls^a Thymus and Lymph Nodes. Frozen cryosections were prepared and stained with mAbs 44-22-1 (anti-$V_{\beta 6}$) or KJ16-133 (anti-$V_{\beta 8}$) as described in detail elsewhere (19).

Neonatal Tolerance Induction. BALB/c (Mls^b) mice were injected intraperitoneally with 10^8 DBA/2 (Mls^a) spleen cells within 24 h of birth.

Results

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

Localization of $V_{\beta 6}^+$ Cells on Thymus Cryosections. $V_{\beta 6}^+$ cells could be localized on

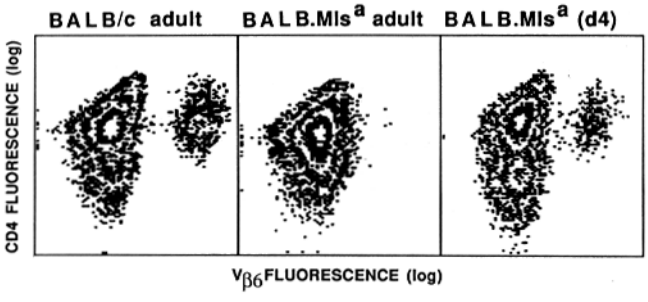

FIGURE 1. Phenotype of brightly staining $V_{\beta 6}^+$ thymocytes in neonatal Mls^a mice. CD8-depleted thymocytes from 4-d-old BALB.Mls^a mice were double stained with mAbs against $V_{\beta 6}$ and CD4. Comparable populations from adult BALB/c or BALB.Mls^a thymus are included for comparison. Cytograms represent 5×10^4 viable cells accumulated using logarithmic fluorescence amplification.

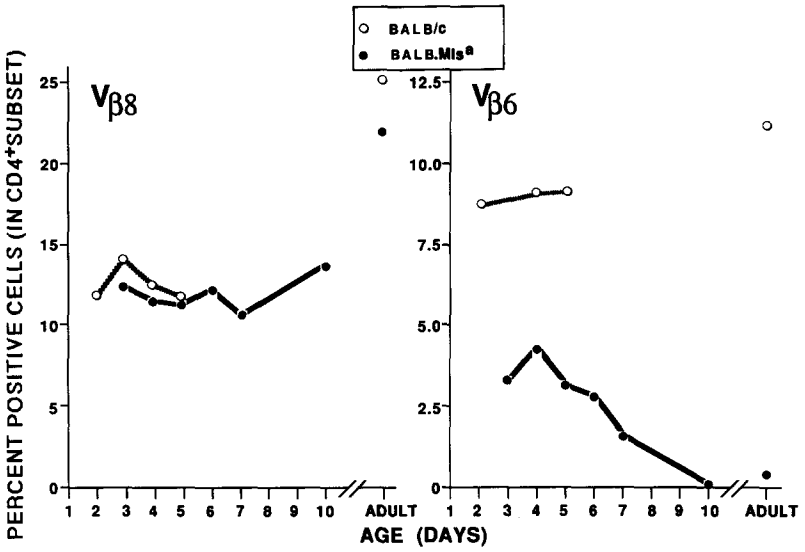

FIGURE 2. Kinetics of disappearance of $CD4^+V_{\beta6}^+$ cells in neonatal Mls^a thymus. CD8-depleted BALB.Mls^a (or control BALB/c) thymocytes were stained with mAbs against $V_{\beta6}$ or $V_{\beta8}$ on the indicated days. Data are expressed as a percentage of $CD4^+$ cells (65–85% of total $CD8^-$ thymocytes) as indicated in Fig. 1.

frozen thymus sections from neonatal DBA/2 (Mls^a) mice (Fig. 3). In contrast to the adult (19), $V_{\beta6}^+$ cells were prominent in the medulla of the Mls^a thymus until day 6. At later times (7–8 d), there was a precipitous decrease in medullary $V_{\beta6}^+$ cells, although disperse staining of $V_{\beta6}^+$ cells throughout the thymus cortex per-

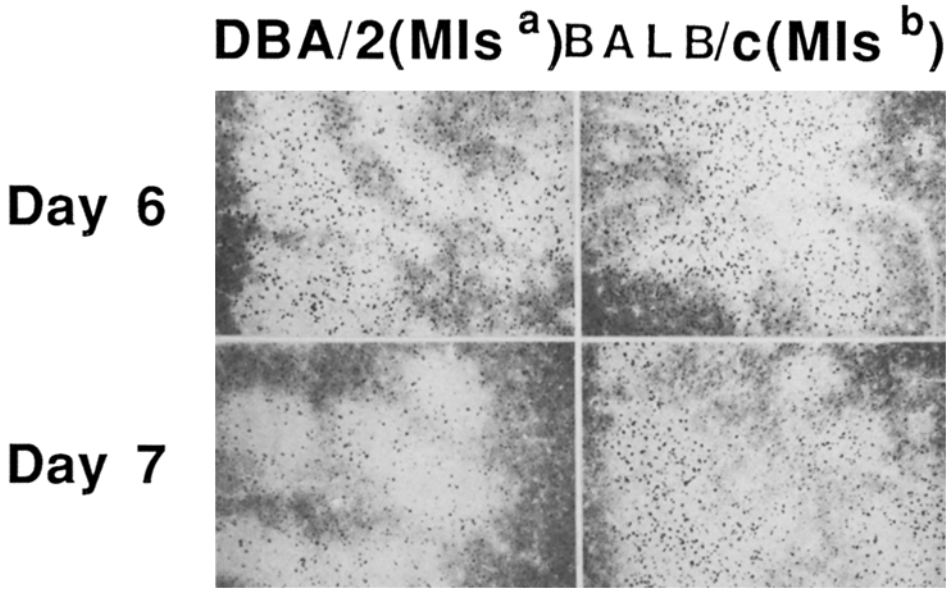

FIGURE 3. Anatomical localization of $V_{\beta6}^+$ cells in early postnatal thymus. Cryosections of BALB/c (Mls^b) or DBA/2 (Mls^a) thymus were stained with anti-$V_{\beta6}$ mAb at the indicated ages and revealed by peroxidase-conjugated anti-rat Ig.

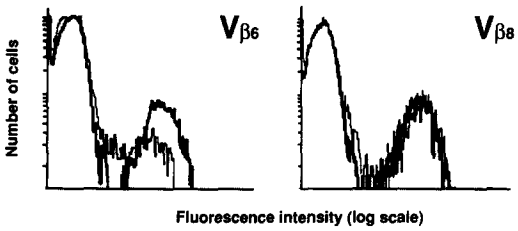

FIGURE 4. Selectively reduced TCR density on $CD4^+V\beta6^+$ cells in neonatal Mls^a thymus. CD8-depleted thymocytes from 5-d-old BALB.Mls^a (*thin lines*) or BALB/c (*heavy lines*) mice were stained with mAbs against $V\beta6$ or $V\beta8$ and analyzed by flow microfluorometry. A logarithmic scale of cell number has been used in order to visualize the small proportion of $V\beta6^+$ cells.

sisted until adulthood (compare with reference 19). In control BALB/c (Mls^b) thymus, $V\beta6^+$ cells were present in both cortex and medulla at all ages tested.

Selectively Decreased Intensity of $V\beta6$ Staining in Neonatal Mls^a Thymus. Careful analysis of the staining profiles of $CD4^+$ thymocytes from neonatal BALB.Mls^a mice revealed a progressive shift in the mean $V\beta6$ fluorescence intensity as compared with age-matched BALB/c controls. At early times (day 2–3), $V\beta6^+CD4^+$ cells stained equally brightly in Mls^a and Mls^b thymus (data not shown). However, beginning around day 4–5, $CD4^+$ thymocytes from BALB.Mls^a mice stained less brightly for $V\beta6$ than the corresponding BALB/c (Mls^b) thymocytes (Fig. 4). This difference, which persisted as long as $V\beta6^+$ cells could be detected in Mls^a 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 $V\beta8$ staining was, however, comparable for $CD4^+$ thymocytes from

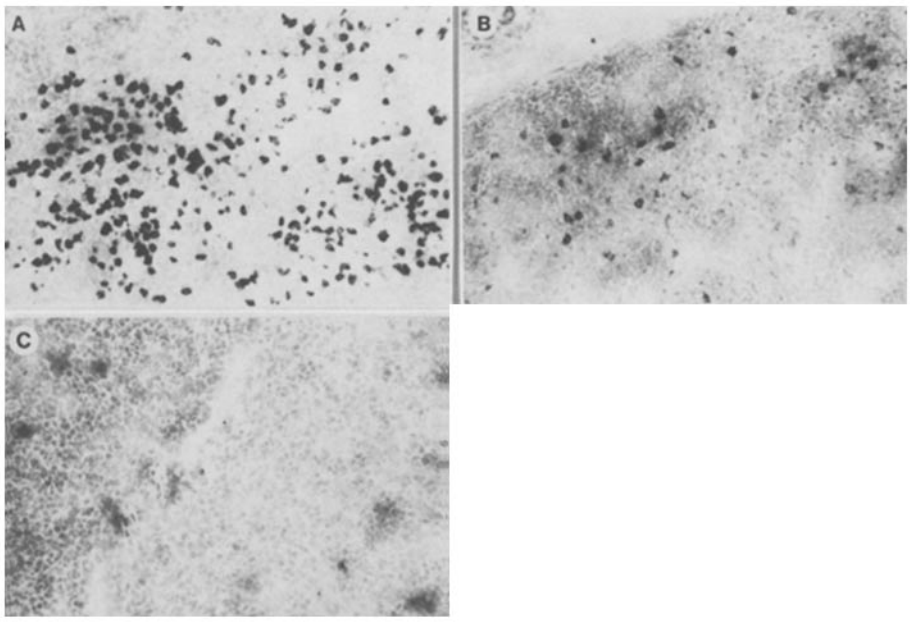

FIGURE 5. Immunohistochemical detection of $V\beta6^+$ cells in lymph nodes of neonatal Mls^a mice. Mesenteric lymph nodes from 2–3-d-old DBA/2 (Mls^a) mice were stained with mAbs against $V\beta8$ (*A*), $V\beta6$ (*B*), or nothing (*C*), followed by peroxidase-conjugated anti-rat Ig.

Mls^a and Mls^b mice throughout neonatal ontogeny (e.g., see Fig. 4), emphasizing that the decrease was selective for Vβ6.

Peripheralization of Vβ6$^+$ Cells in Mls^a Mice. The disappearance of CD4$^+$Vβ6$^+$ cells from the thymus of neonatal Mls^a mice raised the question of the subsequent fate of these cells. As shown in Fig. 5, a small (but significant) proportion of Vβ6$^+$ cells could be detected in tissue sections of mesenteric lymph nodes from neonatal DBA/2 (Mls^a) mice. By flow microfluorometry, Vβ6$^+$ cells accounted for ~1.5% of BALB.Mls^a lymph node cells on days 4 and 6, as compared with 0.2% in the adult.

Ontogeny of Mls^a Stimulatory Capacity. The ontogeny of cells capable of stimulating an Mls^a-specific T cell response was investigated using a T cell hybrid (RG 17.16) that secretes IL-2 in response to Mls^a antigens presented in association with appropriate MHC class II gene products (particularly E^k and E^d) (17). Adult BALB.Mls^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^a stimulatory capacity was first detected at 4 d (Fig. 6).

Disappearance of Vβ6$^+$ Cells in Neonatal Mls^b Thymus after Transfer of Mls^a Spleen Cells. In view of the uncertainty regarding the ontogeny of expression of the Mls^a antigen, the kinetics of disappearance of CD4$^+$Vβ6$^+$ cells was also investigated in BALB/c (Mls^b) mice that had received a neonatal injection of DBA/2 (Mls^a) spleen cells. As shown in Table I, CD8$^-$ thymocytes from 4-d-old "Mls^a-tolerant" BALB/c mice already had slightly reduced numbers of Vβ6$^+$ cells, as compared with age-matched controls. Furthermore, the mean intensity of Vβ6 staining was significantly diminished in the tolerant animals (data not shown), as previously observed for newborn BALB.Mls^a mice (Fig. 4). By day 8, CD4$^+$Vβ6$^+$ cells were present at very low levels in the DBA/2-injected mice, as compared with controls (Table I). Staining with the control Vβ8-specific mAb KJ16 did not reveal a significant decrease in the proportion of positive cells in neonatally Mls^a-tolerant animals (Table I), nor was there a shift in Vβ8 fluorescence intensity (data not shown).

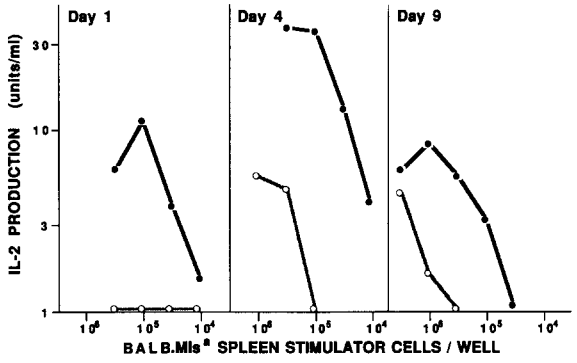

FIGURE 6. Ontogeny of Mls^a stimulatory capacity. Graded numbers of spleen cells from neonatal BALB.Mls^a mice of various ages were used to stimulate IL-2 production by the Mls^a-specific T cell hybrid RG17.16 (○). Adult BALB.Mls^a spleen cells served as a positive control in each experiment (●).

Discussion

The recent demonstration that TCR utilizing Vβ6 (3) or Vβ8.1 (2) react preferentially with Mls^a-encoded antigens and that mature Vβ6$^+$ or Vβ8.1$^+$ T cells are deleted intrathymically in mice expressing the Mls^a gene product provides a unique

TABLE I

Disappearance of CD4$^+$ Vβ6$^+$ Cells from the Thymus of BALB/c (Mls^b) Mice Injected Neonatally with DBA/2 (Mls^a) Spleen Cells

	Percent positive cells at day 4		Percent positive cells at day 8	
Mice	Vβ6	Vβ8	Vβ6	Vβ8
Control	8.5	9.9	5.8	8.2
Mls^a tolerant	4.8, 6.5	9.4, 8.6	1.3, 1.5	7.0, 7.3

CD8$^-$ thymocytes from control or neonatally injected (Mls^a tolerant) BALB/c mice were stained with mAbs directed against Vβ6 or Vβ8. Data are presented as percent positive cells (after subtraction of background staining with the fluorescent anti-Ig conjugate alone). Data from individual mice are separated by commas.

model system in which to analyze the mechanisms underlying tolerance induction in vivo. In this report, we have analyzed the thymic ontogeny of Vβ6 expression in BALB.Mls^a mice. Surprisingly, we find that some brightly staining Vβ6$^+$ T cells are not deleted until 7–10 d after birth. These Vβ6$^+$ 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$^+$Vβ6$^+$ 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$^+$Vβ6$^+$ 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$^+$Vβ6$^+$ 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 $CD8^+$ $V_{\beta 6}{}^+$ thymocytes are selectively absent in neonatal Mls^a mice, in contrast to the situation in neonatal or adult (3) Mls^b thymus. Such asymmetry would suggest that commitment of $V_{\beta 6}{}^+$ cells to the $CD4^+$ lineage has already occurred at the $CD4^+8^+$ developmental stage in Mls^a 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 $CD4^+V_{\beta 6}{}^+$ cells from either the neonatal BALB.Mls^a thymus or the thymus of Mls^a-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 $CD4^+V_{\beta 6}{}^+$ thymocytes with constitutively low TCR density selectively accumulate in Mls^a mice because of a corresponding reduced avidity for Mls^a/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 Mls^a) 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 Mls^a system) is accompanied by rapid intrathymic deletion of mature $V_{\beta 6}{}^+$ cells. Taken together, these observations raise the possibility that post-natal elimination of autoreactive cells is not unique to the Mls^a 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 Mls^a 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 Mls^a-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 Mls^a 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 Mls^a response in vitro. The fact that the postnatal kinetics of elimination of $CD4^+V_{\beta 6}{}^+$ cells is similar in the thymus of Mls^a mice or Mls^b mice injected at birth with Mls^a spleen cells would be consistent with the latter interpretation. However, molecular (or serological) definition of the Mls^a gene product will be required to completely resolve this issue.

If the Mls^a 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 ($V_{\beta 6}$) is correlated with reactivity to a minor antigen encoded by the Mls^a locus. Although absent among mature ($CD4^+$ or $CD8^+$) T cells in adult mice expressing Mls^a, brightly staining $V_{\beta 6}{}^+$ cells were readily detectable in the thymus of neonatal animals, reaching a maximum after 4 d and decreasing rapidly thereafter. These $V_{\beta 6}{}^+$ 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 Mls^b controls. A rapid disappearance of $CD4^+V_{\beta 6}{}^+$ cells (and corresponding decrease in TCR density) could also be observed in the thymus of Mls^b mice that had been injected neonatally with Mls^a 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. Festenstein, R. M. Zinkernagel, and H. Hengartner. 1988. T cell receptor V_β use predicts reactivity and tolerance to Mls^a-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: 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 β-chain variable region $V_{\beta 6}$ 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 Mls^a-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. Crumpton. 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 Mls^a-reactive ($V_{\beta 6}^+$) cells during neonatal tolerance induction to Mls^a-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. Eijsvoogel, 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. Antiglobulin-positive anemia in A and C_{57}BL/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).* 166:753.
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.