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Bone marrow-derived thymic antigen-presenting cells determine self-recognition of Ia-restricted T lymphocytes

(thymus transplant/adult thymus/T-cell development/major histocompatibility complex antigens)

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ABSTRACT **We previously have demonstrated that in radiation-induced bone marrow chimeras, T-cell self-Ia restriction specificity appeared to correlate with the phenotype of the bone marrow-derived antigen-presenting (or dendritic) cell in the thymus during T-cell development. However, these correlations were necessarily indirect because of the difficulty in assaying thymic function directly by adult thymus transplant, which has in the past been uniformly unsuccessful. We now report success in obtaining functional T cells from nude mice grafted with adult thymuses reduced in size by treatment of the thymus donor with anti-thymocyte globulin and cortisone. When (B10 Scn × B10.D2)F_1 nude mice ($I\text{-}A^{b,d}$) are given parental B10.D2 ($I\text{-}A^{d}$) thymus grafts subcutaneously, their T cells are restricted to antigen recognition in association with $I\text{-}A^{d}$ gene products but not $I\text{-}A^{b}$ gene products. Furthermore, thymuses from (B10 × B10.D2)F_1 ($I\text{-}A^{b,d}$) → B10 ($I\text{-}A^{b}$) chimeras transplanted 6 months or longer after radiation (a time at which antigen-presenting cell function is of donor bone marrow phenotype) into (B10 × B10.D2)F_1 nude mice generate T cells restricted to antigen recognition in association with both $I\text{-}A^{d}$ and $I\text{-}A^{b}$ gene products. Thymuses from totally allogeneic bone marrow chimeras appear to generate T cells of bone marrow donor and thymic host restriction specificity. Thus, when thymus donors are radiation-induced bone marrow chimeras, the T-cell *I*-region restriction of the nude mice recipients is determined at least in part by the phenotype of the bone marrow-derived thymic antigen presenting cells or dendritic cells in the chimeric thymus.**

Although the nature of the membrane structure that individual T cells use to recognize foreign antigen in association with self-major histocompatibility complex (MHC) gene products is being defined by the dramatic advances in molecular biology techniques (1, 2) (i.e., message subtraction to yield organ-specific sequences), the process by which the organism generates the family of such T cells that recognizes the universe of foreign antigens (the T-cell repertoire) is still enigmatic. T cells restricted to antigen recognition in association with class I (K/D) antigens undergo thymic (3, 4) and extrathymic (5) influences during development that affect self-MHC recognition. *I*-region restricted T cells appear to require interaction with some cell or cells in a thymus in order to mature (6–10). The MHC genes of precursors of such mature *I*-region-restricted T cells have little effect on the ultimate self-recognition specificity expressed by the cells (9–12). Experiments with radiation-induced bone marrow chimeras in which the marrow donor differs in the MHC from the radiated host [allogeneic bone marrow chimeras (9, 11)] and thymus reconstitution of nude or thymectomized mice (10, 12) have shown that *I*-region self-recognition and the repertoire for foreign antigen recognition in association with *I*-region gene products are determined entirely by the MHC genes of the thymus in which the cells mature. Thus, T cells from type A → type B radiation-induced bone marrow chimeras can interact with B-type *I*-region gene products and make immune response (Ir) gene-controlled responses for which the *B* haplotype is a responder but fail to recognize A-type *I*-region gene products as self and fail to make Ir gene-controlled responses for which the *A* haplotype is a responder and *B* haplotype is a nonresponder.

We previously have suggested that the critical cell in the thymus responsible for determining the *I*-region restriction of the developing T cells is a bone marrow-derived antigen presenting cell (APC) or dendritic cell (13, 14). The data upon which this suggestion was made were indirect and relied on the alteration in T-cell restriction correlating with the rate of turnover of thymic APC after radiation and allogeneic bone marrow reconstitution. First, it was determined that thymic APC are of bone marrow origin (13). When the thymuses of (A × B)F_1 → A radiation-induced bone marrow chimeras were examined several months after radiation, the thymic APC of such chimeras presented antigens to $H\text{-}2^{b}$ lymphocytes. This demonstrated that the thymic APC were derived from donor F_1 bone marrow. If the T cells from (A × B)F_1 → A chimeras were eliminated by *in vivo* treatment with anti-thymocyte globulin (ATS) and cortisone, new T cells generted in the thymus containing F_1 APC behaved like F_1 T cells, recognizing antigen in the context of $H\text{-}2^{a}$, $H\text{-}2^{b}$, or $H\text{-}2^{a\times b}$ gene products (13). The correlation between thymic APC genotype and T-cell *I*-restriction specificity was made stronger by the finding that the rate of thymic APC turnover was affected by the dose of radiation administered to the chimeric hosts (14). Two weeks after 1200 R, essentially all thymic APC are of donor phenotype. On the other hand, 2 weeks after 900 R, the thymic APC are of host phenotype. T cells from (A × B)F_1 → A chimeras made at 900 R are restricted to antigen recognition in the context of host $H\text{-}2^{a}$ gene products. T cells from such chimeras made at 1200 R recognize $H\text{-}2^{a}$, $H\text{-}2^{b}$, or $H\text{-}2^{a\times b}$ gene products as self (14). Thus, it appeared that the bone marrow-derived thymic APC was responsible for the development of *I*-region-restricted T cells.

However, the conclusions of both sets of experiments could be inaccurate because of artifacts in the heavily manipulated chimeric host. The best test of the hypothesis that the thymic APC determines *I*-region self-restriction of T cells would be to directly assess the function of a thymus whose APC genotype differed from that of the other thymic nonlymphoid stromal elements. Ideally one would like to transplant a thymus from a radiation-induced bone marrow

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Abbreviations: MHC, major histocompatibility complex; Ir, immune response; APC, antigen-presenting cell(s); ATS, anti-thymocyte globulin; GLϕ, poly($Glu^{56}Lys^{35}Phe^{9}$); PPD, purified protein derivative of *Mycobacterium tuberculosis*.

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chimera into a naive nude or thymectomized mouse and examine the restriction specificity of the newly generated T cells. Such an experiment has been difficult to perform in the past because adult thymuses have not been reported to be used successfully to reconstitute T-cell immunity in a nude mouse. We have developed a method to use adult thymuses to reconstitute nude mice and have demonstrated directly that thymic APC determine, at least in part, the *I*-region self-recognition of developing T lymphocytes.

MATERIALS AND METHODS

Animals. B10.Scn (*H-2*^{b}), B10.D2(*H-2*^{d}), and (B10 × B10.D2)F_1 nude mice and their normal heterozygous littermates were developed by Carl Hansen and bred for us by Damara Bolte in the Small Animal Section, Veterinary Resources Branch, Division of Research Services, National Institutes of Health. C57BL/10, BALB/c, and B10.D2 mice were purchased from The Jackson Laboratory.

Thymus Transplantation. Neonatal thymus transplant was performed by removing intact neonatal thymuses and implanting one thymus subcutaneously in a nude mouse under light ether anesthesia. Adult thymus donors were treated intraperitoneally with 0.5 ml of a 1:10 dilution of ATS (Microbiological Associates) on days −3 and −1 and with 5 mg of cortisone (Cortone, Merck Sharp & Dohme) on day −2.

Thymuses were removed, and one was implanted subcutaneously into each nude mouse as done for neonatal thymuses.

Radiation-Induced Bone Marrow Chimeras. Chimeras were made at least 6 months before use as thymus donors. B10 or B10.D2 mice (8–12 weeks old) were given 925 R from a ^{137}cesium source at a rate of 128 R/min. Bone marrow donors were either nude mice (15) or normal mice treated *in vivo* with ATS and cortisone, and the marrow was treated twice with anti-T-cell reagents and rabbit complement (Lo-tox, Cedarlane Laboratories, Westbury, NY) as described (8). Bone marrow cells (2×10^7) were administered to irradiated recipients intravenously 18 hr after radiation. All chimeras were typed individually with anti-H-2 antibodies and complement to assure that their lymphohematopoietic cells were entirely of donor origin.

T-Cell Enrichment and Assay. Mice were immunized in the hind footpads with 50 μg of poly(Glu^{56}Lys^{35}Phe^9) (GLϕ) (Miles–Yeda, Rehovot, Israel) and 50 μg of collagen (Sigma) emulsified 1:1 in complete Freund's adjuvant (Difco, *Mycobacterium tuberculosis* strain H37Ra). Eight days later, T cells were enriched from the draining lymph nodes by passage over nylon wool columns and treatment with monoclonal antibodies to *I-A*^{b} and *I-A*^{d} plus complement. T-cell proliferation was measured by plating 4×10^5 T cells in 96-well flat-bottom microtiter plates. Antigen either was added in soluble form along with 2×10^5 (B10 × B10.D2)F_1 irradiated (2000 R) spleen cells as a source of APC or was bound to the surface of APC by preincubating 10^7 irradiated spleen cells with purified protein derivative of *Mycobacterium tuberculosis* (PPD) at 60 μg/ml in 1 ml at 37°C for 60 min, followed by five washes in cold medium not containing PPD. Cells were cultured in ER medium [43.5 ml of RPMI 1640/43.5 ml of Eagle–Hanks' amino acids medium/10 ml of heat-inactivated fetal calf serum (HyClone, Logan, UT) containing 2 mM L-glutamine, 100 units of penicillin per ml, 100 μg of gentamicin per ml, and 20 μM 2-mercaptoethanol (final volume, 100 ml)]. Triplicate cultures in 0.2-ml volumes were incubated in 5% CO_2/95% air at 37°C. On day 3 of culture, 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine (6.7 Ci/mmol, New England Nuclear) was added to each well, and 18 hr later the cultures were harvested onto glass-fiber filters with an automated sample harvester, and radioactivity was measured in a liquid scintillation counter. All values are arithmetic means of triplicate values ± SEM. All responding T cells were documented to be F_1 in origin by cytotoxicity assay using appropriate antibodies to MHC products.

RESULTS AND DISCUSSION

The major barrier to directly assaying thymus function in radiation-induced bone marrow chimeras has been the inability to transplant adult thymuses. In general, histologic examinations of adult thymus grafts have shown failure of the organ to be vascularized. We wondered if shrinking the adult thymus to the approximate size of a neonatal thymus by treating the thymus donors with ATS and cortisone might facilitate the engraftment of adult thymuses. The data in Table 1 demonstrate that such treatment of thymus donors did result in successful engraftment of adult thymuses in nude mice and the genetic restriction of the nude mouse T-cell repertoire to thymus type *I*-region interactions.

(B10 × B10.D2)F_1 nude mice were given thymus grafts from untreated neonatal B10 or B10.D2 mice or from adult B10 or B10.D2 mice that had been treated with 0.5 ml of 1:10 ATS intraperitoneally on days −3 and −1 and 5 mg of cortisone intraperitoneally on day −2. Thymuses (one per mouse) were implanted subcutaneously on day 0 under light ether anesthesia. Three months later, nude mouse recipients of thymus grafts were immunized and T cells were isolated. T cells from (B10 × B10.D2)F_1 nude mice reconstituted with either neonatal or adult B10 thymuses behaved similarly, responding to PPD and to collagen (to which B10 is a responder and B10.D2 is a nonresponder) and failing to respond to GLϕ (to which B10 is a nonresponder but B10.D2 is a responder). Recipients of neonatal or adult B10.D2 thymuses responded to PPD and GLϕ, but not to collagen. Thus, adult thymuses reduced in size (and perhaps activated) by treatment with ATS and cortisone function similarly to neonatal thymus grafts in nude mice and restrict the developing nude mouse T-cell repertoire to thymus-type *I*-region interactions. The use of untreated adult normal thymuses failed to reconstitute T-cell immunity in nude mice (data not shown).

The capacity to use adult thymuses to reconstitute nude mice allowed us to examine directly the function of chimeric thymus glands in which the MHC genes of the APC (bone

Table 1. Neonatal vs. ATS- and cortisone-treated adult thymus reconstitution of (B10 × B10.D2)F_1 nude mice

Prototype responses				Thymus donor			
				B10		B10.D2	
B10 (*I-A*^{b})	B10.D2 (*I-A*^{d})	(B10 × B10.D2)F_1 (*I-A*^{b,d})	Antigen	Neonatal	Adult	Neonatal	Adult
—	—	—	Medium (no antigen)	3,423 ± 388	1,658 ± 401	2,009 ± 554	2,764 ± 212
+	+	+	PPD (20 μg/ml)	75,922 ± 5,241	63,266 ± 7,123	110,269 ± 8,812	80,074 ± 5,589
−	+	+	GLϕ (100 μg/ml)	3,702 ± 616	1,927 ± 314	63,343 ± 3,986	48,675 ± 3,421
+	−	+	Collagen (200 μg/ml)	32,311 ± 3,585	24,661 ± 3,012	2,398 ± 367	2,488 ± 568

T-cell proliferative responses of (B10 × B10.D2)F_1 nude mice reconstituted with neonatal or adult thymuses are shown in cpm ± SEM. T cells were isolated and cultured as described.

marrow-derived) differed from those of the other nonlymphoid stromal cells (host-derived). We reconstituted (B10 × B10.D2)F_1 nude mice with thymuses from B10 → B10 or (B10 × B10.D2)F_1 → B10 radiation-induced bone marrow chimeras. Thymus donors were used at least 6 months after radiation, a time at which APC function is of donor bone marrow phenotype (data not shown). Three months after thymus transplant, the nude mice were immunized and their T-cell function was examined. The results in Table 2 indicate that B10 → B10 chimera thymuses restrict the F_1 nude T cells to recognizing collagen, an antigen to which B10 is a responder, but not GLϕ, to which B10 is a nonresponder. Furthermore, the T cells from F_1 nude mice with B10 → B10 chimeric thymuses respond to PPD predominantly when presented by $I\text{-}A^b$ APC. Thus, thymuses from mice that have been radiated behave similarly to those from nonirradiated adults and neonates in reconstituting nude mouse T-cell function and restricting T-cell antigen recognition to thymus-type MHC gene products.

When (B10 × B10.D2)F_1 → B10 thymuses are used to reconstitute (B10 × B10.D2)F_1 nude mice, the T cells from the recipient behave like (B10 × B10.D2)F_1 T cells. They respond to both collagen and GLϕ and proliferate in response to PPD on either B10 or B10.D2 APC. Since the nonlymphoid stromal elements of the thymus from (B10 × B10.D2)F_1 → B10 chimeras are the same as those from B10 → B10 chimeras (see Table 3), the difference in the T-cell restriction specificity between the two groups of nude mice must relate to the difference in the bone marrow donor between the two types of chimeras. When the thymus contains B10 APC, the T cells are restricted to $I\text{-}A^b$. When the thymus contains (B10 × B10.D2)F_1 APC, the T cells recognize antigen in association with $I\text{-}A^b$ or $I\text{-}A^d$. An identical pattern of T-cell restriction was obtained when thymuses from (B10 × B10.D2)F_1 → B10.D2 chimeras were implanted in (B10 × B10.D2)F_1 nude mice (Table 2). One difficulty with this experiment is that it is not possible to determine conclusively by *H-2* typing whether the responding T cells came from the nude recipient or the thymus donor since both were (B10 × B10.D2)F_1. There are several reasons to believe they are host-derived. First, in other experiments with neonatal, adult, and chimera thymus donors, the responding T cells were always of nude host genotype. Furthermore, the T cells of the (B10 × B10.D2)F_1 → B10 chimeras from which the thymuses came were restricted to $I\text{-}A^b$ recognition (data not shown; ref. 15), and those of the recipient of the thymus interact with both $I\text{-}A^b$- and $I\text{-}A^d$-bearing APC. Finally, the thymus donors are treated with ATS and cortisone, which results in virtually complete elimination of T-cell function. Thus, it seems unlikely that the results are an artifact of T-cell carryover from the thymus donor to the recipient.

Table 3. Summary of results

Thymus donor	Thymic APC	Thymic epithelium	T-cell restriction* B10 + X	T-cell restriction* B10.D2 + X
B10	B10	B10	+	−
B10 → B10	B10	B10	+	−
(B10 × B10.D2)F_1				
B10	(B10 × B10.D2)F_1	B10	+	+
B10.D2	(B10 × B10.D2)F_1	B10.D2	+	+
B10.D2 → B10	B10.D2	B10	+/−	+
B10 → B10.D2	B10	B10.D2	+	+/−

*X, Any environmental antigen.

The results presented thus far cannot exclude the possibility that both the bone marrow-derived thymic APC and the host-derived thymic nonlymphoid stromal elements act in concert to determine the T-cell I-region restriction specificity because the T cells recognize at least $I\text{-}A^b$ as self in both experiments, and the nonlymphoid stromal elements express $H\text{-}2^b$ products (including $I\text{-}A^b$). To discern whether the nonlymphoid stromal cells play a genetically specific role in determining the T-cell receptor repertoire, we used thymuses from allogeneic bone marrow chimeras to reconstitute (B10 × B10.D2)F_1 nude mice (Table 2). T cells from F_1 nude mice reconstituted with BALB/c → B10 or B10.D2 → B10 chimeras proliferate in response to GLϕ to which $H\text{-}2^d$ strains respond, but no response is made to collagen, an antigen to which $H\text{-}2^b$ strains respond but $H\text{-}2^d$ strains do not. The response of the F_1 T cells to PPD is predominately, but not exclusively, in the context of $I\text{-}A^d$. Similarly, T cells from F_1 nude mice reconstituted with thymuses from B10 → B10.D2 chimeras respond to collagen ($I\text{-}A^b$ strains are responders) and do not respond to GLϕ ($I\text{-}A^d$ strains are responders). The PPD

Table 2. Chimeric thymus reconstitution of (B10 × B10.D2)F_1 [(*B* × *D*)F_1] nude mice

Antigen	Thymus donor: B → B	(B × D)F_1 → B	(B × D)F_1 → D	D → B* Exp. 1	D → B* Exp. 2	B → D Exp. 1	B → D Exp. 2
Medium (no antigen)	3,001 ± 456	1,589 ± 502	2,166 ± 442	1,015 ± 201	4,212 ± 641	3,701 ± 559	2,668 ± 357
PPD	76,289 ± 8,023	32,884 ± 2,606	45,415 ± 3,233	29,311 ± 2,585	88,955 ± 9,525	40,306 ± 4,217	51,197 ± 6,336
GLϕ	2,769 ± 1,005	19,851 ± 1,181	24,754 ± 3,034	11,238 ± 1,277	31,931 ± 2,782	4,925 ± 661	3,319 ± 217
Collagen	15,667 ± 1,249	9,697 ± 559	10,910 ± 993	1,422 ± 322	4,678 ± 358	8,925 ± 1,102	11,055 ± 954
B10 spleen†							
PPD on surface	42,238 ± 3,148	14,029 ± 957	17,586 ± 2,139	1,919 ± 275	16,386 ± 1,223	21,772 ± 2,834	30,632 ± 5,821
Alone	3,243 ± 721	2,132 ± 458	3,008 ± 712	1,492 ± 408	4,511 ± 664	4,566 ± 614	2,974 ± 412
Net cpm	38,995	11,897	14,578	427	11,875	17,206	27,658
B10.D2 spleen†							
PPD on surface	3,714 ± 344	10,479 ± 815	18,211 ± 1,998	12,366 ± 1,459	34,113 ± 4,111	8,191 ± 933	12,253 ± 969
Alone	3,655 ± 514	1,956 ± 388	2,456 ± 479	1,371 ± 190	4,397 ± 336	5,023 ± 464	3,149 ± 501
Net cpm	59	8,523	15,755	10,995	29,716	3,168	9,104

T-cell proliferative responses are shown in cpm ± SEM.

*Data shown are from B10.D2 → B10 chimeras. Similar results were obtained with BALB/c → B10 chimeras.

†Antigen was added to the cultures only in cell-associated form. Spleen cells were incubated with soluble antigen and washed thoroughly, and 2×10^5 antigen-pulsed or unpulsed cells were added to the cultures. Proliferation induced by unpulsed cells is subtracted from proliferation induced by antigen-pulsed cells to obtain the net antigen-specific proliferation.

response in these animals is also predominately, but not exclusively, restricted to the $I\text{-}A^b$ haplotype—that of the bone marrow donor. Thus, the T-cell *I*-region restriction of the nude mice recipients is determined, at least in part, by the phenotype of the bone marrow-derived thymic APC or dendritic cell in the chimeric thymus. There are cells restricted to the chimeric thymus host haplotype; however, it is not clear whether this represents the effects of the host nonlymphoid stromal cells on the developing T cells or reflects the fact that the bone marrow-derived APC or dendritic cells have not completely replaced the host cells at the time of the transplant into F_1 nude animals.

The question of the role of the nonlymphoid stromal elements in T-cell development has recently been addressed by Ready *et al.* (16) and von Boehmer and Schubiger (17). Ready *et al.* (16) demonstrated that thymic stromal cells depleted of bone marrow-derived cells by *in vitro* culture with deoxyguanosine were unable to induce tolerance in adoptive allogeneic hosts despite the fact they continued to express class I and class II donor MHC molecules. Furthermore, such treated thymus grafts were not rejected, implying that immune recognition of MHC antigens is not solely a function of expression of MHC molecules. von Boehmer and Schubiger (17) have also found that deoxyguanosine-treated thymic nonlymphoid stromal cells were incapable of inducing tolerance to class I MHC molecules. Thus, in systems in which the nonlymphoid stromal cells are isolated or in which they are of a distinct genotype from the bone marrow-derived elements of the thymus, it appears that the nonlymphoid stromal cells are not capable of exerting an influence on the developing T-cell repertoire. It may be that the bone marrow-derived thymic APC and the nonlymphoid stromal cells act in concert to shape the T-cell repertoire.

Although the mechanism by which T cells are generated is poorly understood, several facts are known about the process (18). The thymus is divided histologically into a cortex (85–90% of all cells) and a medulla (10–15% of all cells), and the cells in each region have discrete cell-surface phenotypes. The cells of the cortex are functionally immature, turnover about once every 3 days, and appear to die within the thymus rather than emigrate to the periphery. On the other hand, medullary cells are functionally mature, similar in phenotype to the cells that leave the thymus to populate peripheral lymphoid tissues, and, despite being a small fraction of all thymocytes, could account for all of the cells emigrating from the thymus. Cortical thymocytes are susceptible to cortisone, and medullary thymocytes are resistant to cortisone. The findings that the medullary Ia-bearing cells are derived from bone marrow (19) and are capable of presenting soluble antigens to T cells *in vitro* when exposed to antigen *in vivo* (20) support our notion that these cells play an important role in the generation of the class II-restricted T-cell repertoire for antigen recognition.

There are some findings that are not consistent with this notion. For example, Kyewski *et al.* (20) appear to find that medullary APC turnover is so rapid in radiation-induced bone marrow chimeras that one would predict that the chimeras would never demonstrate host-restricted T-cell recognition if these cells were truly critical to the development of the repertoire. Our own initial studies on the rate of thymic APC turnover relied on presentation to antigen-primed T-cell populations (13, 14) rather than to T-cell clones (20). It is conceivable that our approach was less sensitive than that used by Kyewski *et al.* (20) and led us to conclude that the turnover was slower. On the other hand, the direct assay of the effects of the chimeric thymus on T-cell development reported here strongly supports a role for these cells in the selection of self. It is possible that more complex mechanisms are at work in the radiation-induced bone marrow chimera that account for the apparent host restriction (21). Smith and Miller (21) have suggested that priming of nonhost parent-restricted T cells is specifically suppressed in $F_1 \rightarrow P$ chimeras.

Another set of experiments that argue against a role for the medullary APC or dendritic cell in the development of the T-cell repertoire was reported by Zinkernagel (22). Using a somewhat different method of depleting $F_1 \rightarrow P$ chimeras of their mature T cells than that reported initially by us (13), he was unable to demonstrate that newly developing T cells were restricted to donor type class I MHC recognition in the cytotoxic T-cell response to vaccinia. Yet there are now a number of different lines of evidence suggesting that class I-restricted T cells have both intrathymic and extrathymic influences on their development (5, 15), and, in the light of this evidence, it seems possible that his inability to detect F_1-restricted cytotoxic T cells was due to the fact that the cells were primed to vaccinia primarily by cells of parental host haplotype.

The data presented here suggest that medullary APC or dendritic cells play a crucial role in the development of the class II-restricted T-cell repertoire. This notion is strongly supported by another series of experiments in a different experimental model, the anti-Ia-suppressed mouse. Mice treated from birth with anti-Ia antibody develop class I-restricted but not class II-restricted T cells (23). We have shown that such Ia-suppressed mice have no detectable thymic APC function and lack the $Lyt\text{-}2^-$ $L3T4^+$ population both in the thymus and the periphery. When Ia-suppressed mice are allowed to recover from the effects of the antibody, thymic APC function recovers quickly and $Lyt\text{-}2^-$ $L3T4^+$ T cells appear in the thymus before they reappear in the periphery (24). These data suggest that the development of the class II-restricted T-cell repertoire is dependent on the thymic APC.

Another issue addressed in part by our data is the nature of the thymic defect in nude mice. Jenkinson *et al.* (25) have noted that the embryonic nude thymus contains cells bearing H-2K antigens but not Ia antigens, whereas the normal thymus at the same stage of development contains cells expressing both class I and class II antigens. A possible explanation for this defect is that nude mice lack Ia-bearing cells capable of homing to the thymus. In our experiments, nude mice were the source of bone marrow for constructing most of the chimeras. Thus, the thymuses from these chimeras possess Ia-bearing cells from nude mice. Since these thymuses appeared to function normally to reconstitute T-cell function in the nude recipients, the defect in the developing nude mouse thymus is not related to defective bone marrow-derived Ia-bearing cells.

Our results are most consistent with the notion that class II-restricted T cells result from the interaction of a medullary thymocyte with a medullary bone marrow-derived Ia-bearing APC or dendritic cell. Other interpretations are possible. How such an interaction results in the development of clonally distributed receptors for environmental antigens and alloantigens is unclear. The results do have implications for human allogeneic bone marrow transplantation. If the clinician could induce the rapid replacement of host thymic APC by donor bone marrow-derived APC (for example by thymic radiation of the recipient), our results predict that the developing T cells should then recognize donor MHC gene products as self, cooperate with the bone marrow-derived APC that repopulate the lymphatic system, and be tolerant to the host MHC antigens encountered during their ontogeny.

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