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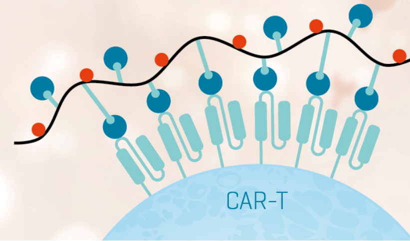

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AN IMMUNE RESPONSE DEFECT DUE TO LOW LEVELS OF CLASS II CELL SURFACE EXPRESSION

Analysis of Antigen Presentation and Positive Selection[1]

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The effects of quantitative differences in class II cell surface expression have been difficult to address in intact animals. This study uses several lines of H-$2^{s/s}$ mice carrying an $A\beta^k$ transgene that differ significantly in terms of class II cell surface expression. Due to inefficient chain pairing, mice carrying 60 to 65 copies of this transgene express only low levels of $A\alpha^s/A\beta^k$ on the cell surface, and cell surface expression of the endogenous $A\alpha^s/A\beta^s$ complex (and total Ia) is severely reduced (to 7–15% control levels). The significant decrease in class II cell surface expression in the thymic cortex of these mice did not affect the frequency of peripheral T cells expressing at least 10 distinct TCR Vβ chains. However, T cell proliferative responses to the $A\alpha^s$/$A\beta^s$-restricted peptide MBP 89-101 were abrogated in high copy number $A\beta^k$ mice. Experiments using bone marrow chimeras demonstrated that both inefficient Ag presentation and failure to positively select appropriate T cells contributed to this lack of response. Inefficient Ag presentation was clearly the dominant defect, and the density of class II cell surface expression required for positive selection appeared to be quite low.

Structural, or qualitative, differences in the MHC class II (Ia) molecules have been shown to affect peptide binding and T cell activation as well as selection of T cells in the thymus. Though less well characterized, quantitative differences in class II expression also affect these processes. T cell activation is dependent on both the density of Ia expressed on APC and the concentration of Ag (1–4). Reduced expression of class II on the surface of APC can abrogate T cell responses to specific Ag (2). Presumably, the density of Ia expressed in the thymus also affects positive and negative selection of T cells. The cell types in the thymus responsible for positive and negative selection have not been unambiguously identified. However, the majority of studies using bone marrow chimeras and transgenic mice have supported the concept that bone marrow derived medullary cells primarily mediate deletion whereas radiation resistant cortical epithelial cells are responsible for positive selection (5–9).

Analysis of transgenic mice expressing a TCR specific for pigeon cytochrome c in association with I-E^k demonstrated that the number of cells expressing the transgene-encoded receptor exported to the periphery correlated with the density of I-E cell surface expression (10). Two- to fourfold differences in cell surface levels of I-E were sufficient to affect positive selection in this experiment. Similarly, the frequency of V$\beta 14^+$ $CD8^+$ cells observed in the periphery of BALB/k mice is approximately twice that observed in (BALB/b × BALB/k)F_1 mice (11). This suggests that the approximate twofold difference in class I cell surface expression observed between homozygote and heterozygote mice is sufficient to affect positive selection of T cells.

If, as the above observations imply, positive selection is in part a stochastic process, a decrease in class II density in the thymus would be expected to affect immune responses restricted by those class II molecules. This possibility has been addressed in (B10.A(4R) × B10.PL)F_1 mice, which express low levels of $E\beta^kE\alpha^u$ on the cell surface due to preferential pairing of $E\alpha^u$ with $E\beta^u$ (4, 12). These animals do not respond to pigeon cytochrome c, though all other strains of mice expressing $E\beta^k$ do. T cell responses elicited in bone marrow chimeras demonstrated that the primary defect was inefficient antigen presentation and indicated that the approximate threefold decrease in cell-surface $E\beta^k$ did not significantly affect positive selection of T cells specific for pigeon cytochrome c in the context of $E\alpha^uE\beta^k$ (12). Thus, in this study, the density of Ia required for positive selection appeared to be lower than that required for Ag presentation.

We have recently described 12 lines of H-$2^{s/s}$ mice carrying from 1 to 65 copies of an $A\beta^k$ transgene that provide a well controlled system in which to assess the effect of drastically reduced Ia density in the thymic cortex on positive selection (13, 14). $A\beta^k$ mRNA expression correlated well with transgene copy number in these animals. Cell surface expression of the transgene was

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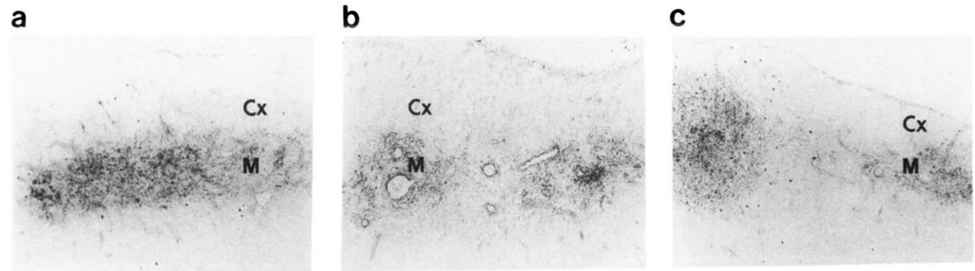

Figure 1. Class I expression, detected by immunoperoxidase staining, in thymus sections from low and high copy number $A\beta^k$ transgenic mice. Thymus sections from a low copy number (*a*) and two high copy number bone marrow chimeras (*b* and *c*) assessed for T cell proliferative responses to MBP 89-101 were stained with 20-8.4.S (anticlass I). Labels identify the cortex (*Cx*) and the medulla (*M*).

TABLE I
Cytotoxic T cell precursor (CTLp) frequency

Responder	CTLp Frequency	r^{2a}	y-Intercept[b]
High copy number	1/875	0.984	89%
Nontransgenic	1/879	0.981	89%
High copy number	1/587	0.994	120%
Nontransgenic	1/392	0.994	81%

Two pairs of high copy number transgenic mice and nontransgenic littermates were analyzed. [a] r^2 is the coefficient of determination; [b] y-intercept is theoretically equal to 100%.

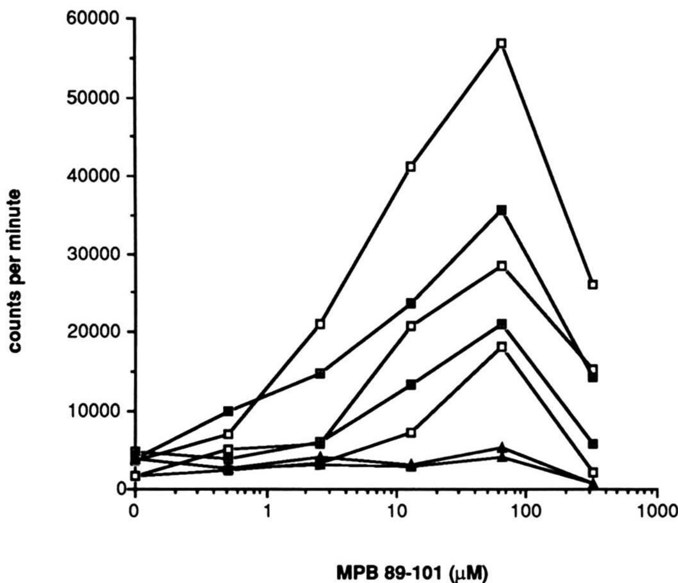

Figure 2. T cell proliferative responses of B10.A(4R) (I-$A^{k/k}$), B10.S × SJL (I-$A^{s/s}$), and 4R × NT (I-$A^{s/k}$) mice to MBP 89-101. Lymph node T cells from immunized mice were cultured in the presence of MBP 89-101 at the concentrations noted. Proliferation was measured by [^{3}H]Tdr incorporation 3 days later. Responses are expressed as the mean incorporation (in cpm) of triplicate cultures; SD were within 20% of the means. 4R × NT, B10.A(4R) × Nontransgenic. □, B10.S × SJL; ⊞, 4R × NT; ▲, B10.A(4R).

dependent on mRNA expression and the type of α-chain available for pairing. Pairing of $A\beta^k$ with $A\alpha^s$ was inefficient, and maximum levels of $A\beta^k/A\alpha^s$ cell surface expression were accompanied by a significant reduction in $A\alpha^s/A\beta^s$ expression. Unpaired or improperly paired chains were not detected intracellularly and appeared to be degraded quite rapidly. Thus, only a fraction of the chains competing for pairing reached the cell surface under conditions of asymmetric chain synthesis in these mice. This resulted in a marked reduction in total Ia cell surface expression in mice carrying 60 to 65 copies of the $A\beta^k$ transgene. Of particular interest was the dramatic difference in endogenous $A\alpha^s/A\beta^s$ surface expression observed between low and high copy number mice, particularly in the thymic cortex. Mice carrying 1 to 6 copies of $A\beta^k$ expressed low levels of $A\alpha^s/A\beta^k$ and control levels of the endogenous $A\alpha^s/A\beta^s$ in the thymic cortex and medulla. In mice carrying 60 to 65 copies of $A\beta^k$, relatively low levels of $A\alpha^s/A\beta^k$ were also observed in the thymus, but cell surface expression of the endogenous $A\alpha^s/A\beta^s$ complex was severely reduced in the thymic cortex and slightly reduced in the medulla. Because these mice expressed very little total Ia in the thymic cortex, they provided a unique system in which to study the requirements for positive selection.

Several additional defects were observed in the high copy number animals, including significantly reduced numbers of mature B cells, increased numbers of granulocytes, and extensive extramedullary hematopoiesis. Despite these abnormalities, the numbers of $CD4^+$ and $CD8^+$ T cells were similar in low copy and high copy number mice (13). To characterize more thoroughly the peripheral T cell repertoires in these animals, we assessed the frequency of lymph node T cells expressing ten distinct TCR Vβ chains. Despite the decrease in overall Ia cell surface expression in the thymuses of high copy number mice, no significant differences were observed in the frequency of peripheral T cells expressing these ten Vβ chains. We then addressed a more specific question: is positive selection of T cells specific for a peptide in the context of I-A^s abrogated in high copy number mice (carrying 60–65 copies of $A\beta^k$), which have a significant decrease in I-A^s (and total Ia) expression, particularly in the thymic cortex? Functional studies using bone marrow chimeras demonstrated that both Ag presentation and positive selection of T cells specific for the I-A^s-restricted peptide MBP[5] 89–101 were inefficient in the high copy number animals. Ag presentation was the dominant defect, and subtle differences in Ia expression in the thymic cortex appeared to affect positive selection. These results support the concept that the threshold of Ia required for Ag presentation is higher than that required for positive selection and suggest that the density of class II expressed on radiation resistant cells in the thymus necessary for positive selection may be quite low.

MATERIALS AND METHODS

Mice. SJL mice were obtained from The Jackson Laboratories (Bar Harbor, ME), and B10.S mice from Olac (Bicester, U.K.). B10.A(4R), and F_1 offspring from these strains were bred and maintained in our colony. The $A\beta^k$ transgenic mice have been described (13, 14). The bone marrow chimeras were constructed using "high copy" number mice from the $A\beta^k$-40 and -105 lines (which carry 60–65 copies of the $A\beta^k$ transgene) and "low copy" number mice from the $A\beta^k$-124 and -115 lines (which carry 1–6 copies of $A\beta^k$).

Preparation of bone marrow chimeras. Mice 7 to 8 wk of age were irradiated with 750 to 900 rad using an x-ray source, allowed to rest 6 to 8 h, and then were reconstituted with 1.5 to 7 × 10^6 bone marrow cells. Bone marrow cells were extruded from the femurs of 6- to 8-wk-old mice with cold PBS, washed three times, and passed through nylon mesh. Cells were resuspended at 4 to 10 × 10^7 cells/

[5] Abbreviations used in this paper: MBP, myelin basic protein; 4R, B10.A(4R); BM, bone marrow.

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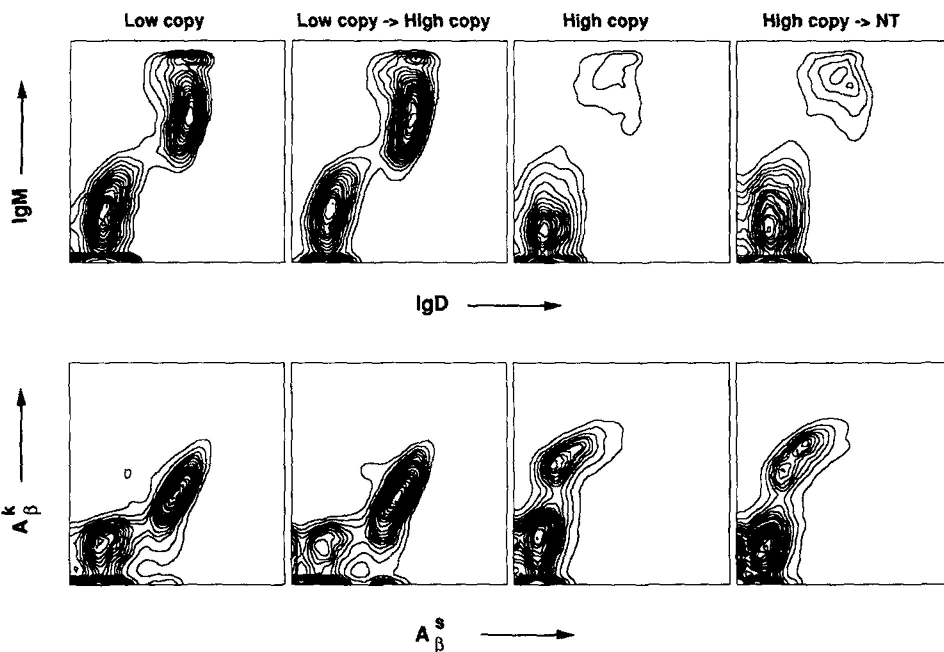

Figure 3. FACS analysis of spleen cells from control mice and radiation-induced bone marrow chimeras. Cells were stained with anti-IgM, anti-IgD, anti-$A\beta^k$ (40.N), and anti-$A\beta^s$ (MKS4) as noted. Data are presented in 5% probability contour plots with log fluorescence intensity on each axis. Low copy ($A\beta^k$-124, 1 copy of $A\beta^k$); high copy ($A\beta^k$-40, 60–65 copies $A\beta^k$); low copy → high copy (low copy bone marrow → irradiated high copy recipient); high copy → NT (high copy number bone marrow → irradiated nontransgenic recipient). Mice were analyzed 2 mo postreconstitution.

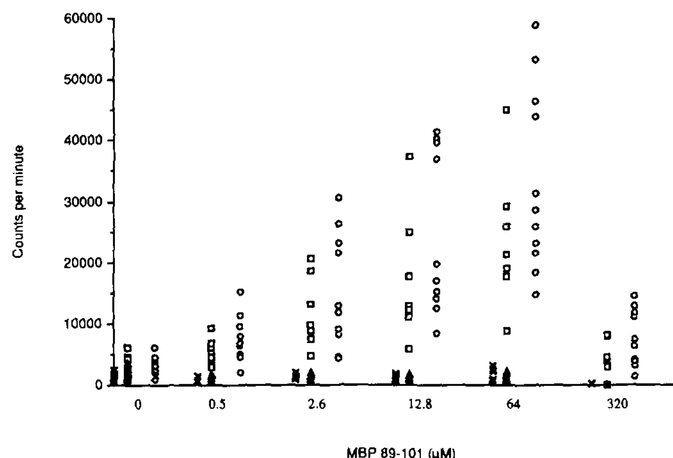

Figure 4. T cell proliferative responses of control mice to MBP 89-101. Lymph node T cells from immunized mice were cultured in the presence of MBP 89-101 at the concentrations noted. Proliferation was measured by [^{3}H]Tdr incorporation 3 days later. Responses are expressed as the mean incorporation of triplicate cultures; SD were within 20% of the means. Compiled data are presented in Table II. ○, Nontransgenic; ▲, B10.A(4R); □, low copy; x, high copy.

ml and injected into the tail veins of irradiated mice (0.2 ml/mouse). Mice were placed on antibiotic water 1 wk before or immediately after irradiation. Long term survival was >90% for the majority of groups. Chimeras were allowed to rest 2 mo before immunization. When mice were killed for the T cell proliferation assays, spleens were removed for FACS analysis and thymuses for immunohistochemistry.

Peptide synthesis. MBP 89-101 (VHFFKNIVTPRTP) was synthesized by solid phase methods using an automated Applied Biosystems (Foster City, CA) 431A peptide synthesizer. Tert-butyloxycarbonyl protected amino acids and tert-butyloxycarbonyl protected Proline coupled to resin were purchased from Applied Biosystems. Amino acid composition of the peptide was correct and purity was >95% as determined by HPLC.

mAb. The mAb 20-8.4.S (anticlass I) (15) 40.N (anti-$A\beta^{k,f,r,u}$) (16), MKS4 (anti-$A\beta^{s,f,u}$) (17), were grown in ascites and purified by ammonium sulfate precipitation followed by protein A chromatography. RA3-6b2 (anti-B220) (18) was generously provided by Drs. Alan Stall and Nabuko Uchida, Stanford University.

FACS analysis. FACS analysis of spleen cells was done as described by Hayakawa et al. (19). Approximately 10^6 cells were incubated for 30 min at 4°C in 50 to 100 μl of PBS containing 0.1% NaN_3, 0.5% BSA, and an appropriate dilution of biotinylated or fluoresceinated mAb. Cells were washed four times and resuspended in PBS/BSA/NaN_3 with Texas Red-Avidin (Becton Dickinson, Mountain View, CA) and 2 μg/ml propidium iodide (Sigma). After incubating for 30 min on ice, cells were washed and analyzed on a dual laser FACS IV (Becton Dickinson Immunocytometry) equipped with a log amplifier. Dead cells were excluded by propidium iodide staining.

Immunohistochemistry. Tissue samples were cut into small pieces, embedded in OCT-compound (Tissue-Tek, Miles Scientific, Napewich, IL), and frozen in dry ice/isopentane without fixation. Cryosections 4-μm thick were cut and air dried for 1 h, fixed with cold acetone for 10 min, and washed three times in PBS. For staining, the sections were first blocked with 1% BSA (fraction V, Sigma) then incubated for 30 min in an appropriate dilution of a biotinylated primary antibody. Slides were washed with PBS, then incubated with avidin-peroxidase for 30 min, washed, and developed with 3,3'-diaminobenzidine-4 HCl (Sigma). Slides were then rinsed in H_2O and mounted under coverslips.

T cell proliferation assays. Mice were immunized subcutaneously at the base of the tail with 100 μg of MBP 89-101 in 100 μl of CFA containing 400 μg of heat-killed *Mycobacterium tuberculosis* H37Ra (Difco, Detroit, MI). Ten days later mice were killed and inguinal and paraaortic lymph nodes removed. Cells were washed twice in PBS and resuspended in RPMI 1640 (GIBCO, Grand Island, NY), 1% normal mouse serum, Pen-Strep, L-glutamine, and 5×10^{-5} M 2-ME. Lymphocytes were cultured at 37°C, 5% CO_2 in microtiter plates (5×10^5 cells/well in a total volume of 100 μl) in the presence of MBP 89-101 at concentrations noted, 20 μg/ml of purified protein derivative of *M. tuberculosis* (Statens Seruminstitut, Copenhagen, Denmark), or 4 μg/ml of Con A. After 72 h, wells were pulsed with 1 μCi of [^{3}H]Tdr for 16–18 h. DNA was harvested onto glass fiber filters and [^{3}H]Tdr incorporation determined in a scintillation counter. Mean thymidine incorporation was calculated for triplicate cultures and standard deviations were within 20% of the means.

Controls for each experiment included nontransgenic littermates and age-matched, unmanipulated mice from each line. Due to the small numbers of cells obtained from the high copy mice and (high copy BM → low copy) chimeras, lymph node cells from two of these animals were occasionally pooled for proliferation assays. All other mice were assayed individually.

Cytotoxic T cell precursor frequency determination. CTLp frequency for allogeneic BALB/c stimulator cells was determined by a modification of the method of Isakov and Bach (20). Briefly, 100 to 6400 responder spleen cells were cultured with 10^6 irradiated (3000 rad) stimulator spleen cells per well in flat or round-bottom microtiter plates. Culture medium was RPMI 1640 supplemented with 10% FCS (GIBCO), 5×10^{-5} M 2-ME, Pen-Strep, L-glutamine, and mouse rIL-2 (a kind gift of Dr. Anne O'Garra, DNAx, Palo Alto, CA). After 10 days of incubation, cultures were washed once and assessed for ability to lyse ^{51}Cr-labelled BALB/c Con A blasts. Wells were scored as positive if ^{51}Cr released was greater than 2 SD above background. CTLp frequencies were calculated according to the method of Taswell et al. (21). For each determination, the coefficient of determination

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TABLE II

T cell proliferative responses of control mice and radiation-induced bone marrow chimeras to MBP 89-101 and purified protein derivation (see Figs. 4-6)[a]

Mice	MBP 89-101 (64 nM)		PPD (20 ng/ml)
	Dcpm	%PPD	Dcpm
Controls			
Nontransgenic (NT) (11)	29,702 ± 15,093	39 ± 13	72,831 ± 16,273
Low copy no. (7)	20,722 ± 12,418	29 ± 14	69,610 ± 9,884
B10.A(4R) (7)	0	0	77,892 ± 8,516
High copy no. (8)	**652 ± 814**	**2 ± 3**	38,552 ± 27,515
Chimeras (BM → host)			
Low copy → low copy (8)	12,766 ± 4,819	21 ± 7	60,401 ± 11,453
NT → NT (3)	20,264 ± 16,272	32 ± 22	59,758 ± 6,985
High copy → low copy/NT (6)	**195 ± 416**	**0.3 ± 0.8**	29,535 ± 24,378
Low copy → high copy			
R (9)	8,767 ± 2,760	14 ± 4	61,098 ± 8,959
NR (6)	**1,863 ± 1,351**	**4 ± 2**	42,732 ± 19,840
Total (15)	6,006 ± 4,155	10 ± 6	53,752 ± 16,529

[a] Numbers of mice immunized in each group are in parentheses. Responses significantly different from controls ($p < 0.01$, two tailed Student's t test) are in bold type. D cpm = response (cpm) in the presence of Ag or PPD at the concentrations noted − response (cpm) in absence of Ag. NT, nontransgenic; R, responder, NR nonresponder.

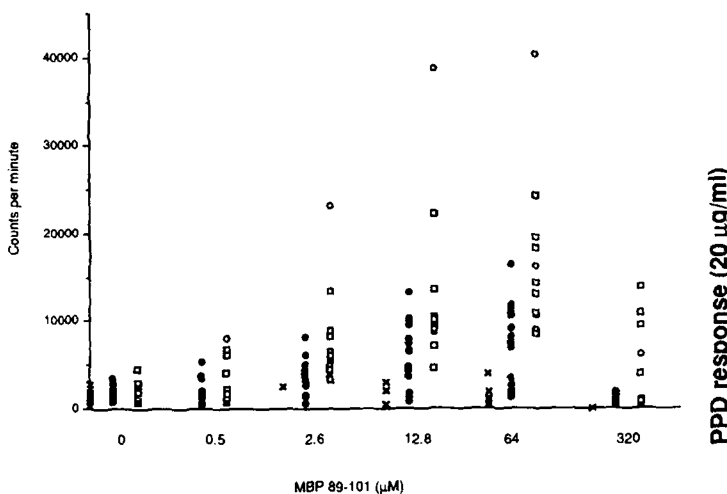

Figure 5. T cell proliferative responses of radiation-induced bone marrow chimeras to MBP 89-101. Lymph node T cells from immunized mice were cultured in the presence of MBP 89-101 at the concentrations noted. Proliferation was measured by [^{3}H]Tdr incorporation 3 days later. Responses are expressed as the mean incorporation of triplicate cultures; SD were within 20% of the means. Compiled data are presented in Table II. O, nontransgenic (NT) BM → NT; □, low copy BM → low copy; ●, low copy BM → high copy; x, high copy BM → low copy.

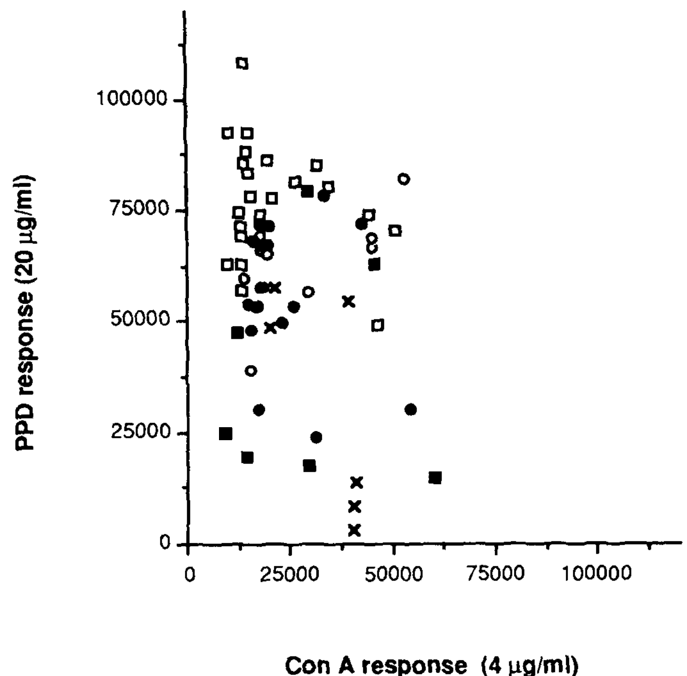

Figure 6. T cell proliferative responses of control mice and bone marrow chimeras to purified protein derivation and Con A. Cells from mice immunized with MBP 89-101 (see Figs. 4 and 5; Table II) were cultured for 3 days in the presence of 20 μg/ml of PPD or 4 μg/ml of Con A. Proliferative responses were assessed by [^{3}H]Tdr incorporation. Responses are expressed as the mean incorporation (in cpm) of triplicate cultures; SD were within 20% of the means. □, Controls (4R/nontransgenic (NT)/low copy); ■, high copy; O, NT/low copy BM → NT/low copy; ●, low copy → high copy; x, high copy BM → NT/low copy.

r^2 and the y-intercept (theoretically equal to 100) were calculated as a test for the goodness of fit of the data.

RESULTS

TCR expression and allogeneic immune responses in transgenice mice. Several approaches were used to assess T cell selection and function in the high copy number transgenic mice. As stated above, high and low copy number mice had normal numbers of CD4$^+$ and CD8$^+$ T cells, which implied that selection of T cells was not grossly skewed by the decrease in cell surface Ia in the high copy number mice. To more thoroughly characterize T cells in these animals, the proportion of peripheral T cells expressing Vβ 2, 3, 5.1, 5.2, 6, 7, 8.1, 8.2, 8.3, or 11 was examined. No significant differences in the frequency of CD4$^+$ or CD8$^+$ lymph node T cells expressing these ten Vβ chains were observed in any of the Aβ^k transgenic mice, again indicating that T cell selection was not dramatically affected by the decrease in Ia cell surface expression in the high copy number mice (data not shown).

To further assess T cell function in the high copy number mice, allogeneic immune responses were examined. Mixed lymphocyte responses were equivalent in high copy number mice and nontransgenic littermates (data not shown). Cell surface expression of class I was not affected in the thymuses of high copy number mice (Fig. 1), and cytotoxic T lymphocyte precursor frequency was similar in high copy number mice and nontransgenic littermates (Table I). These data indicated that the T cells in the high copy number transgenic mice were functional and suggested that positive selection of class I-restricted T cells was normal in the high copy number animals.

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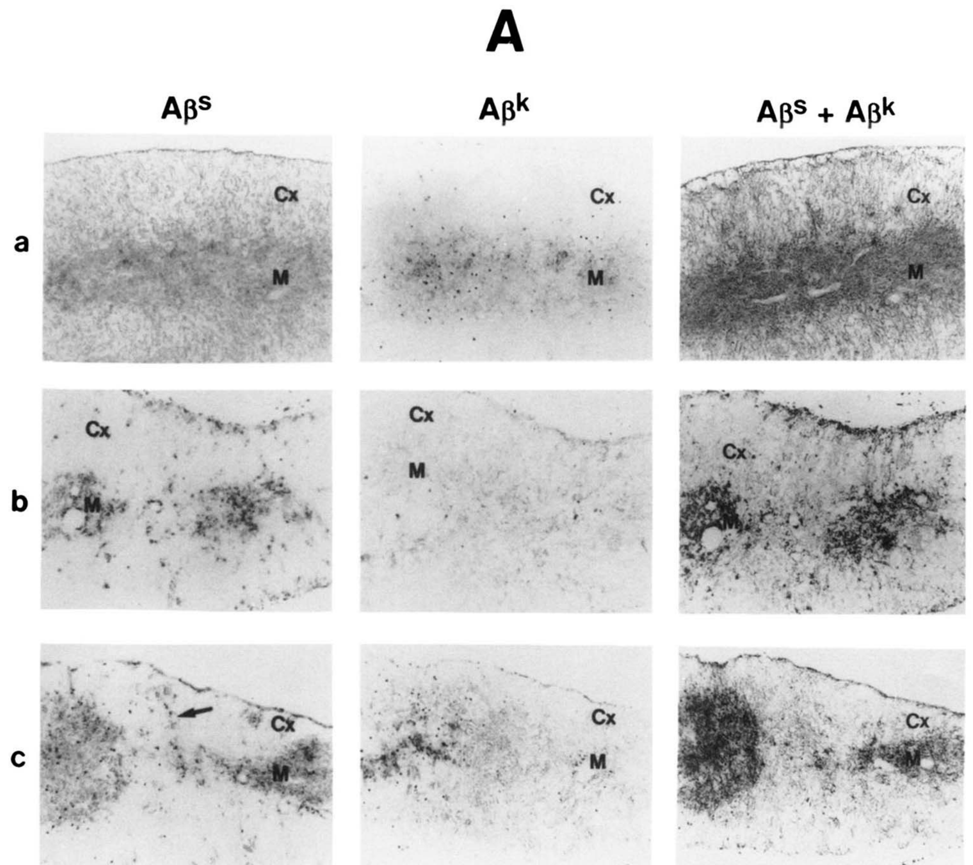

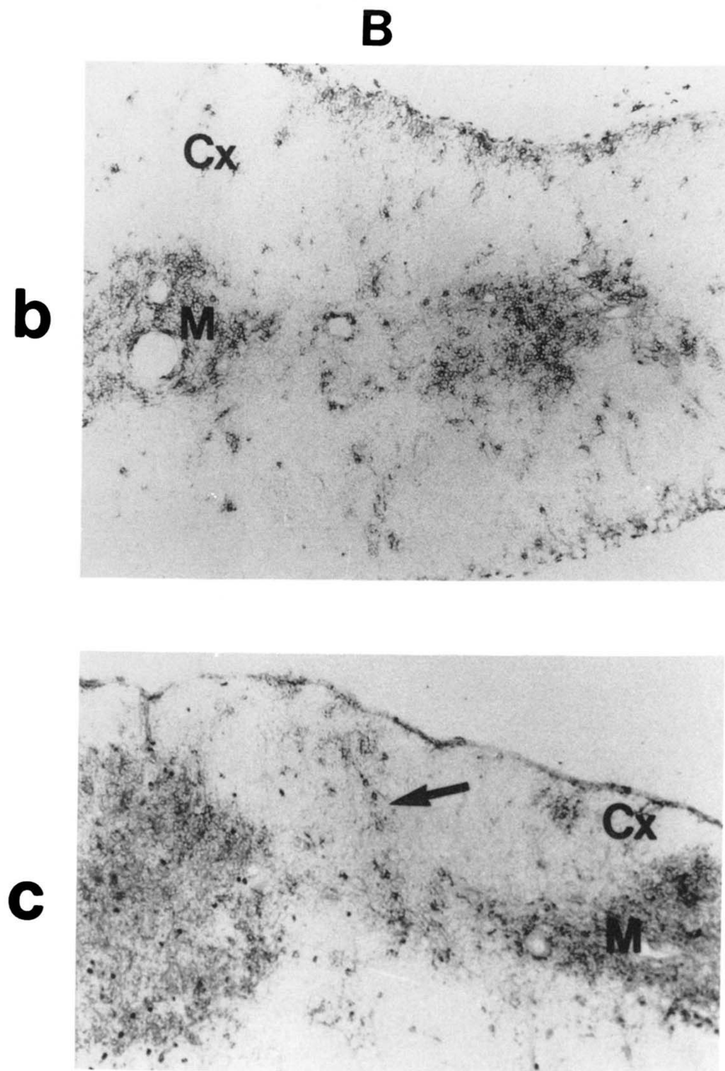

Figure 7. Ia expression, detected by immunoperoxidase staining, in thymus sections from low and high copy number $A\beta^k$ transgenic mice. *A*, Thymus sections from a low copy number (*a*) and two high copy number bone marrow chimeras (*b* and *c*) assessed for T cell proliferative responses to MBP 89-101 were stained with 40.N (anti-$A\beta^k$), MKS4 (anti-$A\beta^s$), and 10-3.6 (anti-$A\beta^k$/$A\beta^s$, total Ia). These are serial sections from the same thymi shown in Figure 1. Labels identify the cortex (*Cx*) and the medulla (*M*). The high copy number chimera shown in *b* did not respond to MBP 89-101, whereas that in *c* did. The *arrow* in *c* points to an area of relatively higher class II expression observed from the chimera that responded to MBP 89-101. *B*, Enlargement of *b* and *c* stained with MKS4 (anti-$A\beta^s$).

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Assessment of positive selection and Ag presentation. To assess functional consequences of decreased expression of I-A^s in the high copy number animals, mice carrying 1 to 6 or 60 to 65 copies of the transgene were immunized with the $A\alpha^s/A\beta^s$-restricted peptide MBP 89-101. I-$A^{s/s}$ and I-$A^{s/k}$ mice respond to this peptide whereas I-A^k mice do not (Fig. 2). Low copy mice and nontransgenic littermates responded equally well to this peptide. In contrast, none of the high copy number mice responded significantly better than did B10.A(4R) controls (see Fig. 4). These data indicated that the levels of I-A^s expressed on the cell surface in the high copy number mice were not sufficient to generate a response to MBP 89-101.

To distinguish between a probable defect in Ag presentation and a possible defect in positive selection, we constructed a set of bone marrow chimeras. Bone marrow cells from low copy number mice were transferred into irradiated high copy number mice. These (low copy BM → high copy) chimeras had functional antigen presenting cells and T cells selected in thymuses expressing low levels of I-A^s on radiation-resistant epithelial cells. Conversely, bone marrow cells from high copy number mice were transferred into low copy number recipients. These (high copy BM → low copy) chimeras had APC expressing low levels of I-A^s and T cells selected by thymic epithelial cells expressing control levels of I-A^s. Control chimeras included irradiated low copy number animals reconstituted with low copy number BM and nontransgenic animals reconstituted with nontransgenic BM. As shown in Fig. 3, more than 95% of lymphocytes in the chimeras were of donor origin as assessed by I-A cell surface expression and B cell phenotype.

All unmanipulated nontransgenic and low copy number mice responded significantly better to MBP 89-101 than did B10.A(4R) controls, and a fairly wide range of responses was observed (Fig. 4; Table II). None of the high copy number animals responded significantly better than did B10.A(4R) controls. The chimeras demonstrated clearly that a bone marrow cell derived (antigen presenting) defect contributed to their inability to respond. Responses elicited in the (high copy BM → low copy) mice were similar to those of unmanipulated high copy number controls (Fig. 5; Table II). We were not able to overcome this defect by immunizing mice with twice as much Ag (200 μg) and adding H-$2^{s/s}$ (B10.S × SJL) APC *in vitro* (data not shown). In addition, responses to PPD were fairly low in several of the high copy number animals and (high copy BM → low copy) chimeras (Fig. 6), suggesting that *in vivo* priming was inefficient in these animals. Consistent with these observations, barely detectable levels of $A\beta^s/A\alpha^s$ cell surface expression were induced by IFN-γ on peritoneal macrophages from the high copy number mice (data not shown).

Of the 15 (low copy BM → high copy) chimeras immunized, six did not respond significantly better to MBP 89-101 than did unmanipulated high copy number mice (Fig. 5; Table II). This result implies that positive selection of T cells specific for MBP 89-101 was inefficient in approximately 40% of the high copy number animals. The responses elicited in the remaining nine chimeras were relatively low, but not significantly different from those observed in control chimeras. Because the magnitude of the responses to MBP 89-101 did not correlate at all with the number of bone marrow cells transferred, it is highly unlikely that residual mature T cells in the donor bone marrow were responsible for these responses. Individual thymi from the (low copy BM → high copy) chimeras were examined to determine whether heterogeneity in thymic Ia expression might account for the variable responses observed. Indeed, subtle differences in the amount of I-A^s expressed in the thymic cortex correlated well with the responses observed (Fig. 7). When unidentified sections were analyzed by a "blind" observer, the low and high responders were consistently and unambiguously identified. In those chimeras that responded, I-A^s was expressed at higher levels and in larger patches on the cortical epithelial cells. In the low responders, almost no expression was observed on these cells and class II expression in the cortex seemed to be limited primarily to single, fairly brightly staining cells.

DISCUSSION

These $A\beta^k$ transgenic mice have provided a well controlled system in which to assess the effect of decreased I-A cell surface expression in the thymus on selection of T cells. We focused on positive selection because the decrease in Ia cell surface expression is more marked in the thymic cortex of the high copy number mice. Previous characterization of the $A\beta^k$ transgenic mice indicated that the defect in class II cell surface expression did not affect other cell surface proteins. Class I cell surface expression was similar in thymuses from low and high copy number mice. In addition, CTL precursor frequency was identical in high copy number animals and nontransgenic littermates, indicating that the thymuses are functional in high copy number mice. Thus, T cells in the high copy number animals appeared to be functional by several criteria independent of Ag presentation.

Differences in the frequency of T cells expressing particular Vβ chains in the periphery of mice have been attributed to both clonal deletion and positive selection (22–28). Ia density has not been implicated as an important parameter in these studies, perhaps because relatively slight differences in Ia cell surface expression have been addressed (for instance the approximate twofold differences between homozygotes and heterozygotes). In one study, however, the approximate twofold difference in class I cell surface expression observed between homozygote and heterozygote mice was sufficient to affect positive selection of T cells expressing Vβ14 (11). Despite the decrease in Ia cell surface expression in the high copy number $A\beta^k$ transgenic mice, no significant differences were observed in the frequency of T cells expressing ten Vβ chains in the periphery of these animals. Although T cells staining with a particular anti-TCR Vβ antibody use the same Vβ region, these TCR are quite heterogeneous, given their expression of different Dβ, Jβ, Vα, and Jα gene segments. A number of T cells may have been exported from the thymus less efficiently in the high copy number mice, but these differences were not detected by the Vβ-specific antibodies used. As yet, the effect of Ia density on positive selection has been quantitively demonstrated most clearly in transgenic mice expressing a TCR of known specificity on a high percentage of T cells (10).

Functionally, failure to positively select appropriate T cells can result in nonresponse to specific Ag. Expression

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of $E\beta^k$ in the thymus appears to be necessary for selection of T cells specific for pigeon cytochrome c in context of $E\beta^k/E\alpha$ (12). The effect of $E\beta^k$ density on positive selection of T cells specific for pigeon cytochrome c in association with $E\alpha^u/E\beta^k$ has been addressed in (B10.A(4R) × B10.PL)F_1 mice (12). These mice express approximately 30% B10.A (I-$E^{k/k}$) levels of $E\beta^k$ on the cell surface, and do not respond to pigeon cytochrome c. Consistent with previous studies (3, 4), Kovak and Schwartz demonstrated that failure to respond was primarily due to inefficient Ag presentation in these mice. In addition, T cell proliferation responses elicited in bone marrow chimeras constructed to assess positive selection were approximately 50% of those elicited in control chimeras (but these differences were not statistically significant). Interpretation of these results is complicated by the fact that no mice express $E\alpha^u/E\beta^k$ on the cell surface at control levels. Structural differences between the $E\alpha^u$ and $E\alpha^k$ chains may also contribute to the lack of response observed in (B10.A(4R) × B10.PL)F_1 mice.

Our $A\beta^k$ transgenic mice have provided an additional system in which to assess functional effects of class II density on positive selection. This system is unique in that very little total Ia is expressed in the thymic cortex of these mice. Moreover, the reduction in I-A^s cell surface expression observed in the high copy $A\beta^k$ mice is more severe than the relatively modest reduction in $E\beta^k$ observed in (B10.A(4R) × B10.PL)F_1 mice (12). In addition, the differences in Ia cell surface expression assessed in the transgenic mice were solely quantitative; cell surface expression of the endogenous $A\beta^s/A\alpha^s$ complex differed significantly whereas expression of $A\beta^k/A\alpha^s$ was similar in the low and high copy number mice analyzed. The $A\beta^k/A\alpha^s$ heterodimer may have contributed to the responses observed in these mice. A proportion of T cells selected by this heterodimer may be able to recognize MBP 89-101 in the context of $A\beta^k/A\alpha^s$. Despite this possibility, the decrease in Ia cell surface expression was sufficient to affect positive selection of T cells specific for MBP 89-101/I-A^s. We have not formally ruled out peripheral effects, which will require thymus transfer experiments. However, because the magnitude of responses observed in the chimeras correlated well with subtle differences in I-A^s and total Ia expression in the thymic cortex, our results strongly suggest that T cells specific for MBP 89-101 were not positively selected in 40% of the (low copy BM → high copy) chimeras. Thus, the low levels of Ia expressed in the thymic cortex of these mice were sufficient for positive selection of a grossly "normal" $CD4^+$ repertoire, yet a smaller (functionally significant) subset was absent in the periphery of some animals. That extremely subtle differences in cortical Ia cell surface expression appeared to affect selection of precursors specific for MBP 89-101 bound to $A\beta^sA\alpha^s$ supports our conclusion that the threshold of class II expression required for positive selection is quite low.

Although positive selection was inefficient in a proportion of the high copy number mice, Ag presentation was clearly the dominant defect. Other alterations in the high copy number mice could possibly have contributed to this defect, but several lines of evidence suggest that severely reduced class II cell surface expression was the predominant factor. If other problems (such as low numbers of B cells) were primarily responsible for the priming and presentation defects, processing of complex Ag as well as peptides should have been affected. T cell responses to complex Ag were not significantly reduced in the high copy number mice. Data presented in this report indicate that PPD was being adequately processed and presented. In addition, an extensive analysis of antibody responses has been carried out in these mice (29). Again, responses to a complex Ag (BSA) were normal to high copy number mice, whereas responses to synthetic peptides were much more variable and correlated with levels of class II expression. Given the extremely low levels of class II cell surface expression in the high copy number animals, the Ag presentation defect is not unexpected, and is consistent with the analysis of (B10.A(4R) × B10.PL)F_1 mice (12). Collectively, these data imply that relatively low levels of Ia expression in the thymic cortex are sufficient for some degree of positive selection. If so, quantitative differences in class II cell-surface expression may be functionally significant primarily in the periphery. Failure to activate appropriately selected T cells in the periphery is probably more likely to generate an immune response defect than failure to select them in the thymus.

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