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Immunology

Most γδ T cells develop normally in β_2-microglobulin-deficient mice

(major histocompatibility complex class I/positive selection/T-cell development)

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ABSTRACT **The specificity of T cells bearing γδ T-cell receptors (γδ⁺ T cells) is poorly characterized. Earlier studies suggest that like αβ⁺CD8⁺ T cells, some γδ⁺ T cells may recognize antigens associated with class I major histocompatibility complex molecules. αβ⁺CD8⁺ T cells are nearly absent in class I-deficient mice (mutant for β_2-microglobulin), reflecting a requirement for intrathymic "positive selection" of these cells by class I molecules. Here, we examine whether the development of γδ⁺ T cells is altered in the β_2-microglobulin mutant mice. We show that the cellularity, marker expression, repertoire, and functional competence of γδ⁺ T cells are not detectably deficient in β_2-microglobulin mutant mice. We conclude that class I expression is unnecessary for the development of most γδ⁺ T cells.**

T cells bearing the γδ T-cell receptor (TCR) represent a minor subset of T cells in the secondary lymphoid organs but are often the major T-cell type among lymphocytes in epithelial tissues (for review, see refs. 1 and 2). Although the *in vivo* specificity of γδ T cells remains unclear, recent studies have identified candidate physiological antigens for γδ⁺ T cells, including mycobacterial antigens, heat shock proteins or peptides, and an autologous keratinocyte antigen (3–9). However, it is unclear whether recognition of any of these antigens is restricted by molecules related to the class I or class II major histocompatibility complex (MHC) molecules that restrict recognition by αβ⁺ T cells.

Several examples of cloned γδ⁺ T cells that react with foreign or even self class I or class II MHC molecules have been reported, including those reactive with the related class I molecules that associate with β_2-microglobulin (β_2m) and are structurally similar to MHC class I (MHC-I) molecules (10–14). Only a few cases of MHC-restricted antigen recognition by γδ T cells have been reported (15, 16). The success at isolating γδ⁺ T cells reactive to class I molecules has reinforced the speculation that γδ⁺ T cells generally recognize antigens in a class I-restricted fashion.

It is also unclear whether γδ⁺ T cells undergo a positive selection step, akin to that which operates on αβ⁺ T cells during their differentiation within the thymus. Some features of γδ T-cell ontogeny and the properties of γδ T cells localized to different peripheral sites might be rationalized by invoking a selection process. Distinct waves of γδ T cells differing in Vγ and Vδ variable gene usage and extent of TCR diversity appear in the thymus in an ordered sequence in ontogeny (17, 18) and apparently migrate to distinct peripheral sites. For example, γδ T cells in the first thymic wave, which use Vγ3 and Vδ1 variable (V) region genes to encode their TCR and exhibit no V–joining (J) junctional sequence diversity, migrate to the epidermis where they are known as dendritic epidermal cells or skin-associated intraepithelial lymphocytes (s-IELs) (19, 20). Later waves express different V regions and migrate to other sites including the vaginal epithelium and secondary lymphoid organs.

To examine the role of class I molecules in the development of γδ T cells, we studied γδ T-cell development and TCR diversity in mice mutant for the class I light chain β_2m (21). Cells from homozygous mutant mice (−/− mice) are profoundly deficient for cell surface expression of all class I molecules examined (22, 23). The deficiency in MHC-I expression results in a blockade to the differentiation of αβ⁺CD8⁺ T cells, because of a failure in positive thymic selection (22, 23). In contrast, we report here that most γδ T cells in the mutant mice are normal with respect to their numbers, distribution, and repertoire and are functionally competent. These data argue that most γδ T cells do not require interactions with class I molecules for their maturation and that the programmed development of γδ T cells and the homogeneous TCR sequences of some subsets of γδ T cells are not due to selection by class I molecules.

MATERIALS AND METHODS

Mice. (C57BL/6 × 129)F_2 and F_3 mice (H-2^b), homozygous or heterozygous for the mutant β_2m allele (−/− and +/− mice, respectively), or wild-type mice (+/+ mice) were used.

Cells. Intraepithelial lymphocytes in the intestine (i-IELs) and s-IELs were isolated as described (24, 25). Before staining, s-IELs were incubated overnight in the presence of interleukin (IL) 2 (20 units/ml) to allow the recovery of TCR expression. Adult thymus, spleen, and lymph-node cell suspensions were enriched for γδ T cells by depleting CD4⁺ T cells, CD8⁺ T cells, and B cells with monoclonal antibodies plus complement and/or panning on Petri dishes coated with purified goat anti-mouse immunoglobulin (26). The average yields of cells from a given organ of β_2m-deficient vs. normal mice did not differ significantly, nor were fewer cells recovered after the depletion of non-γδ T cells from −/− mice (determined with a Student's *t* test).

Antibodies. Anti-γδ TCR (UC7.13D5) and anti-Vγ2 region (UC7.1DA6) antibodies were provided by J. Bluestone (University of Chicago). Other antibodies employed include those detecting γδ TCR [GL3 (27)], Vδ4 region [GL2 (27)], Vγ3 region [F536 (28)], αβ TCR [H57-597 (29)], Thy-1 [J1J (30)], CD4 [GK1.5 (31)], CD8 α chain [AD4(15) (32)], IA^b [BP 107 (33)], and the heat-stable antigen (HSA) [J11d (30)].

Immunofluorescence Staining Analysis. Cells were stained as described (34). γδ TCRs and specific V regions were detected by specific antibodies followed by goat anti-hamster

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Abbreviations: β_2m, β_2-microglobulin; TCR, T-cell receptor; MHC, major histocompatibility complex; MHC-I, MHC class I; i-IEL, intestine-associate intraepithelial lymphocyte; s-IEL, skin-associated intraepithelial lymphocyte; r-IEL, reproductive-organs-associated intraepithelial lymphocyte; IL, interleukin; HSA, heat-stable antigen; V, variable; J, joining.

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antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE; Caltag, South San Francisco, CA), or detected with biotinylated anti-γδ TCR (GL3) antibody followed by PE-streptavidin (Southern Biotechnology Associates, Birmingham, AL). Thy-1 was stained with biotinylated J1J antibody followed by PE-streptavidin and bound J11d antibody was detected with FITC-goat anti-rat IgM (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Cells were stained for CD8α with PE-conjugated 9YTS 169.4 antibody (Caltag) or FITC-conjugated 53-6.7 (Becton Dickinson). Cells were subjected to two-color analysis on Epics C (Coulter) or FACS IV (Becton Dickinson) flow cytometers. Forward and right angle light scatter were used to exclude dead and aggregated cells.

DNA Amplification. V–J regions were amplified by PCR (35). Primers corresponding to Vγ3, Vγ4, Jγ1, and Jδ2 were those described (36). Genomic DNA (1 μg of s-IEL DNA or 5 μg of uterus/vagina DNA) was heated to 94°C for 3 min (5 min for uterus/vagina DNA) and was amplified for 35 cycles in 100 μl containing all four dNTPs (each at 50 μM), 1.5 mM $MgCl_2$, 2.5 units of Taq polymerase, and each oligonucleotide at 0.25 μM. Each cycle consisted of a 0.5-min denaturation step at 94°C, a 1-min annealing step at 55°C, and a 1-min extension step at 72°C. The extension step after the last cycle was for 10 min. Amplified DNA was cloned into M13mp19, recombinant plaques were picked randomly, and single-stranded DNA minipreps were sequenced by the dideoxynucleotide chain-termination method with Sequenase (United States Biochemical).

Proliferative Responses by γδ$^+$ T Cells. Enriched γδ$^+$ T cells from spleen, thymus, lymph nodes, or s-IELs were cultured in replicate in round-bottomed microtiter wells that had been preincubated for 2 h at 37°C with 0.5 μg of purified anti-γδ TCR antibody (GL3), anti-αβ TCR antibody (H57-597), or normal hamster IgG or with no antibody. The cultures were pulse-labeled with 1 μCi of [^{3}H]thymidine (6.7 Ci/mmol; 1 Ci = 37 GBq) for the last 18 h of culture.

RESULTS

Epidermal γδ T Cells (s-IELs) and Reproductive Tissue γδ T Cells (r-IELs) in β_2m-Deficient Mice. Preparations of s-IELs from +/− mice contained normal numbers of Thy-1$^+$ cells, most of which expressed the γδ TCR. As shown in normal mice (19, 28), ≈90% of the γδ$^+$ s-IELs from −/− mice or their +/+ littermates were stained by Vγ3-specific monoclonal antibody (Fig. 1*A* and Table 1). V–J regions of rearranged Vγ3–Jγ1 and Vδ1–Jδ2 genes from s-IELs were amplified by PCR and γ and δ junction sequences were examined. Most of the in-frame sequences (21 of 22) from −/− mice were identical to each other and to the canonical s-IEL γ sequence reported for normal mice (19). Similar results were obtained with the δ sequences, where 16 of the 18 in-frame sequences from −/− s-IELs were identical to the canonical s-IEL δ junctional sequence (Table 2).

A distinct homogeneous junctional sequence has been reported for the Vγ4–Jγ1: rearrangements found in r-IELs (37). Analysis of PCR-amplified Vγ4–Jγ1 sequences from reproductive tissue of −/− mice (Table 2) showed that 21 of 22 γ sequences were rearranged productively and that all of them were identical to each other and to the canonical sequence (20, 37). Therefore, the deficiency of class I expression in −/− mice has no discernable effect on the presence of γδ$^+$ s-IELs or on the unusually restricted repertoire of TCRs expressed by s-IELs or r-IELs.

CD8$^+$γδ T Cells but Not CD8$^+$αβ T Cells of the Intestinal Epithelium Develop Normally in β_2m-Deficient Mice. γδ T cells present in the intestinal epithelium (i-IEL) of normal mice include a high proportion of CD8$^+$ cells (60–80%) and Thy-1$^-$ cells (50–80%) (24, 38). To examine whether the differentiation of γδ$^+$ or αβ$^+$ i-IELs is altered in MHC-I-

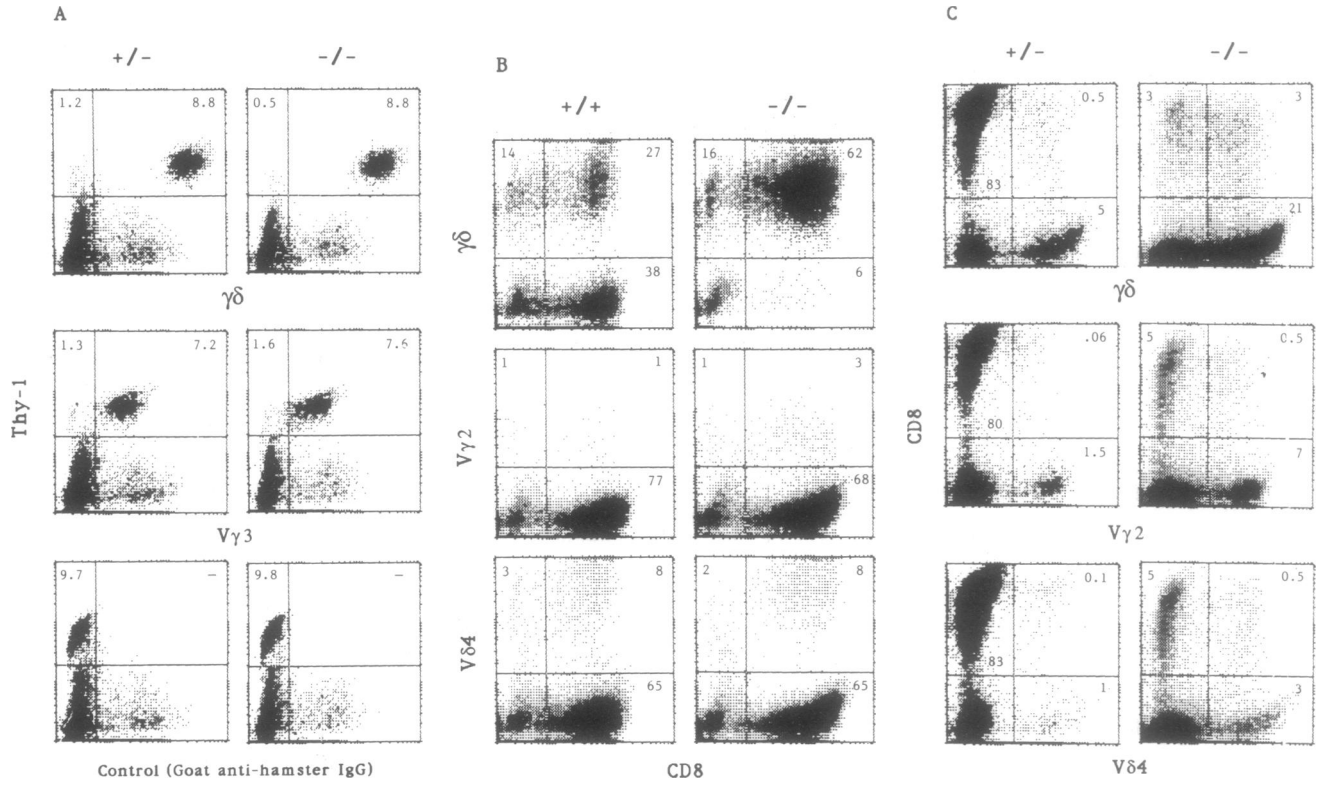

FIG. 1. V gene usage and marker expression by γδ T cells among s-IELs (*A*), i-IELs (*B*), and lymph node cells (depleted of CD4$^+$ cells) (*C*) in β_2m-mutant mice. Cell populations were analyzed by two-color flow cytometry for surface expression of γδ TCR or specific V regions vs. Thy-1 (*A*) or CD8α (*B* and *C*). In *A* and *B*, 20,000 cells and in *C* 50,000 cells were analyzed on an Epics C flow cytometer. The numbers in the quadrants indicate the percent stained cells after subtraction of the percent cells stained with no primary antibody.

Table 1. V gene and marker expression by mutant and wild-type γδ T cells in various tissues

Tissue	β_2m genotype	% cells			% γδ⁺ T cells				
		γδ TCR	αβ TCR	αβ⁺CD8⁺	Thy-1	Vγ3	Vγ2	Vδ4	CD8
s-IELs	+/+ or +/−	11 ± 5¶	0*	0*	>90¶	85 ± 14¶	5 ± 5‡	0*	0*
	−/−	9 ± 2¶	0*	0*	>90¶	88 ± 9¶	3 ± 3‡	0*	0*
i-IELs	+/+ or +/−	37 ± 8§	36, 31†	33, 27†	37 ± 6‡	0*	6 ± 2‡	20 ± 4‡	72 ± 7‡
	−/−	74 ± 9	6, 5†	1, 1†	31 ± 11‡	0*	10 ± 8‡	20 ± 8‡	84 ± 5‡
Fetal thymus (E17)	+/+ or +/−	3 ± 0.5‖	ND	ND	ND	38 ± 6‖	ND	11 ± 3‖	ND
	−/−	3 ± 0.3‡	ND	ND	ND	47 ± 12‡	ND	9 ± 2‡	ND
CD4⁻CD8⁻ thymus	+/+	7 ± 1¶	ND	ND	ND	0‡	33 ± 6‡	20 ± 0.5‡	—
	−/−	11 ± 3¶	ND	ND	ND	0‡	22 ± 8‡	21 ± 7‡	—
CD4⁻IA⁻ spleen	+/−	4 ± 2‡	ND	ND	ND	2 ± 1‡	15 ± 2‡	27 ± 1‡	7 ± 2‡
	−/−	12 ± 2‡	ND	ND	ND	0 ± 0‡	21 ± 4‡	18 ± 4‡	8 ± 4‡
CD4⁻IA⁻ lymph node	+/−	4 ± 1‡	ND	ND	ND	0 ± 0‡	37 ± 7‡	20 ± 2‡	8 ± 1‡
	−/−	16 ± 10‡	ND	ND	ND	0 ± 0‡	36 ± 4‡	19 ± 3‡	14 ± 3‡

Cell populations, enriched for γδ T cells by elimination of CD4⁺, CD8⁺, and/or IA⁺ cells or not, as indicated, were analyzed for marker expression and V gene usage by flow cytometry. When less than three determinations were made, each is presented; otherwise results are expressed as the mean ± SD. The number of determinations are as follows. *n*: *, 1; †, 2; ‡, 3; §, 4; ¶, 5; ‖, 11. All tissues were from mice of both sexes between 6 and 21 weeks of age. No effect of age or sex on the results was observed. Cell yields were not less in −/− compared to +/+ or +/− mice. In comparing repertoire data from −/− vs. +/+ or +/− animals by a Student's *t* test, only the difference in staining Vδ4⁺ cells among γδ⁺ spleen cells was significant at the 5% level; this difference was not significant at the 2% level. Preliminary findings that γδ⁺ T cells are present in fetal and adult thymus of β_2m-deficient mice were reported in ref. 22. E17, 17th day of gestation; ND, not done.

deficient mice, i-IELs were prepared from −/− and control +/− or +/+ mice. A striking difference was observed in the abundance of CD8α⁺αβ⁺ T cells, which were ≈3.3% as frequent in −/− mice as in +/− or +/+ control mice (Table 1). In contrast, in −/− mice the frequency of γδ⁺CD8α⁺ i-IELs was not decreased but was instead higher than in +/+ mice (Fig. 1*B* and Table 1), perhaps in part because of the decreased proportion of CD8⁺αβ⁺ T cells.

Thymic Development of γδ T Cells in β_2m-Deficient Mice. To examine whether MHC-I deficiency affects thymic development of γδ⁺ T cells, thymocytes from fetal and adult mice were analyzed (Table 1). In the embryonic-day-17 thymus, the abundance of γδ⁺ T cells was similar in −/− and +/+ mice, as was the usage of Vγ3 and Vδ4 regions (≈40% and 10%, respectively). In the adult thymus of both −/− and +/+ mice, Vγ3⁺ cells were undetectable, and the usage of Vγ2 and Vδ4 was similar, ≈25% and 20%, respectively. The frequency of γδ T cells in the adult thymus was not decreased in −/− mice compared to +/+ mice. Rather, the frequency of γδ⁺ T cells was consistently higher in −/− mice (11.1 ± 2.7% vs. 6.5 ± 0.9% of CD4⁻CD8⁻ thymocytes, significantly different by Student's *t* test; $P < 0.02$).

γδ T Cells in Spleen and Lymph Nodes of β_2m-Deficient Mice. As a proportion of CD4⁻CD8⁻ splenocytes, the numbers of γδ T cells were not decreased in −/− mice compared to +/+ or +/− mice (Table 1). Less than 10% of the γδ⁺ spleen cells from −/− mice were CD8⁺, similar to those in normal mice. The repertoire of γδ⁺ splenocytes, with respect to usage of Vγ2, Vγ3, and Vδ4, was also similar in −/− and +/− mice. The γδ⁺ lymph-node cell population (Fig. 1*C*) was similar to that in spleen, except that a larger fraction of the γδ⁺ T cells expressed Vγ2 (36% vs. 15–21%, Table 1).

Table 2. V–J junctional diversity of rearranged γ and δ genes from s-IELs and r-IELs in β_2m-mutant mice

Rearrangement	Genotype	Frequency of sequences in-frame	Frequency of canonical sequences
Vγ3–Jγ1 (skin)	+/+	5/6	4/6
	−/−	22/24	21/24
Vδ1–Jδ2 (skin)	+/−	5/5	4/5
	−/−	18/20	16/20
Vγ4–Jγ1 (uterus)	+/+	6/6	6/6
	−/−	21/22	21/22

Genomic DNA was obtained from epidermal cells enriched for s-IELs or from intact uterus and vagina tissue of mice between 6.5 and 10 weeks of age. DNA fragments corresponding to the junctional regions of rearranged Vγ3–Jγ1, Vδ1–Jδ2, and Vγ4–Jγ1 genes were amplified by PCR and sequenced. The canonical junctional sequences are those defined in refs. 19 and 37.

γδ T Cells in β_2m-Deficient Mice Are Functionally Competent. Stimulation of the TCR with immobilized anti-TCR monoclonal antibodies mimics stimulation by antigen (39) and is often used to assess the functional competence of a polyclonal population (40). Significant proliferative responses stimulated by anti-γδ TCR antibody were observed with enriched populations from spleen, thymus, lymph node, and epidermis (s-IELs) of −/− mice (Table 3). Furthermore, the stimulated populations of enriched γδ T cells from thymus, spleen, and epidermis (s-IELs) of −/− mice produced growth factors that stimulate the CTLL-2 cell line—i.e., IL-2 and/or IL-4 (data not shown).

Surprisingly, enriched γδ⁺ T cells from +/+ control mice often responded less well than those from −/− mice, in terms of both proliferation (Table 3) and production of IL-2 and IL-4 (data not shown). The low responses by +/+ γδ T cells do not reflect inviability of the cells, since they responded well when stimulated with anti-γδ TCR antibody plus IL-2 (data not shown) or with anti-αβ TCR antibody (Table 3).

Immature αβ⁺ thymocytes express HSA, detected by the J11d antibody, whereas mature αβ⁺ T cells are HSA⁻ (30, 41). In examining normal +/+ mice, we found that >50% of γδ⁺ thymocytes (67%) are HSA⁺, whereas >90% of the γδ⁺ T cells in the lymph node (Fig. 2), spleen, and s-IELs (data not shown) are HSA⁻. Thus, γδ T cells, like αβ T cells, may have immature and mature states defined by HSA. The proportions of HSA⁻γδ⁺ cells in the thymus of −/− mice were no lower than those in +/+ mice, but in fact were higher (50.5 ± 5% vs. 33.2 ± 4%, significantly different by Student's *t* test; $P < 0.002$) (Fig. 2). These results suggest that class I expression is not required for maturation of most HSA⁻ γδ T cells. Most splenic and lymph node γδ⁺ T cells and γδ⁺ s-IELs in both −/− and +/+ mice were HSA⁻ (Fig. 2). Thus, in terms of proliferative responses, growth factor production, and HSA phenotype, no functional defect could be discerned in γδ⁺ T cells from class I-deficient mice.

DISCUSSION

The expression of functional H-2 K, D, and Qa-2 molecules is strongly diminished if not abolished in β_2m mutant mice (22, 23), as is the functional expression of a *Tl*-region-encoded class I antigen (T22^b) that serves as a target for a γδ⁺

Table 3. Proliferation of enriched $\gamma\delta^+$ T cells in response to TCR stimulation

Source of $\gamma\delta$ T cells	Experiment	β_2m genotype	% cells		Stimulation index					
					Anti-$\gamma\delta$ TCR			Anti-$\alpha\beta$ TCR		
			$\gamma\delta^+$	$\alpha\beta^+$	d3	d4	d5	d3	d4	d5
Spleen	1	+/+	5	31	1.2	1.2	1.5	62.4	66.8	59.8
		−/−	6	12	8.4	6.2	5.9	20.3	15.7	43.3
	2	+/+	4	23		4.1			15.8	
		−/−	7	15		3.3			5.4	
	3	+/+	8	15		1.9			32.1	
		−/−	16	6		56.1			3.9	
Lymph node	2	+/+	23	27		3.5			34.9	
		−/−	38	10		91.6			12.8	
	3	+/+	14	15		6.7			45.3	
		−/−	14	23		6.7			37.4	
Thymus	2	+/+	7	16		1.3			39.5	
		−/−	15	10		7.2			6.8	
	3	+/+	5	12			0.8			140.2
		−/−	8	7			11.8			23.5
s-IEL	3	+/+	9	ND		9.4			3.1	
		−/−	7	ND		6.6			1.0	

$\gamma\delta^+$ T cells from mice between 4 and 6 months old were enriched by depleting $CD4^+Ig^+$ (experiments 1–3) and $CD8^+$ (experiments 2 and 3) T cells from the indicated population (except s-IELs). Cell yields were not less in −/− compared to +/+ mice. The percent of the enriched population that are $\gamma\delta^+$ or $\alpha\beta^+$ T cells is indicated. Enriched $\gamma\delta^+$ cells (at 2×10^5 cells per culture except s-IELs, which were at 6×10^5 cells per culture) were cultured for 3, 4, or 5 days (d) in microtiter wells that had been preincubated with purified anti-$\gamma\delta$ TCR antibody, anti-$\alpha\beta$ TCR antibody, normal hamster IgG, or phosphate-buffered saline (PBS). The responses are presented as a stimulation index, the ratio of incorporated [^{3}H]thymidine in replicate cultures stimulated with TCR-specific antibody to that in cultures stimulated with normal hamster IgG (experiments 2 and 3) or with PBS (experiment 1). There were no significant responses with normal hamster IgG compared to PBS and there were no significant differences between background responses of cells from +/+ and −/− mice. ND, not done; Ig, immunoglobulin.

T-cell hybridoma (42, 48). Although there are many other poorly defined class I molecules of unknown function that associate with β_2m and are encoded by genes with an exon–intron organization similar to MHC-I genes (43), it is likely that most if not all of them are functionally deficient in the mutant animals.

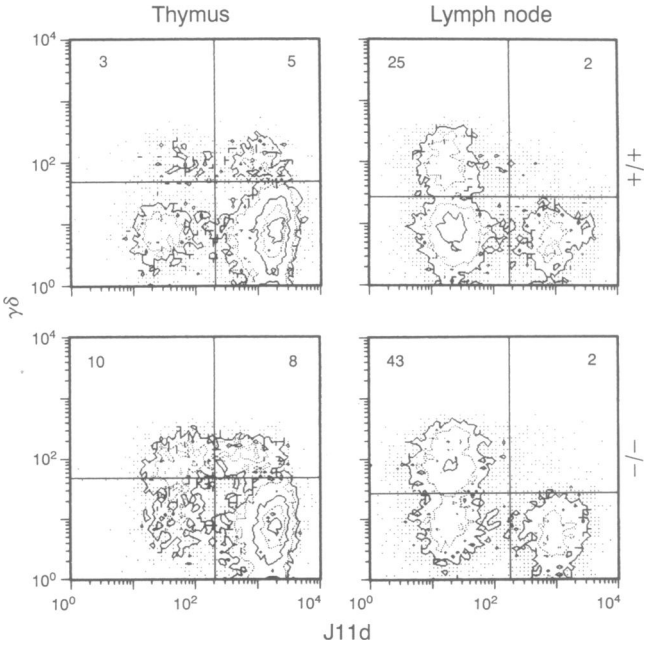

FIG. 2. Expression of J11d antigen by thymic and lymph-node $\gamma\delta$ T cells in normal and β_2m-mutant mice. Enriched populations of $\gamma\delta^+$ T cells were stained with J11d antibody vs. $\gamma\delta$-TCR-specific antibody. $\gamma\delta^+$ T cells were enriched from thymus and lymph-node cell suspensions of 4- to 6-month-old mice by depleting $CD4^+$ and $CD8^+$ cells. The lymph-node cell population was further enriched by depleting IA^+ and immunoglobulin-positive cells. Ten thousand cells were analyzed on a FACS IV flow cytometer.

That $\gamma\delta$ T cells may generally recognize class I antigens is based on the isolation of several $\gamma\delta^+$ T-cell lines that recognize foreign or even self class I molecules. On the other hand, the frequency of allo-MHC-reactive T cells is reportedly considerably lower among $\gamma\delta^+$ T cells than $\alpha\beta^+$ T cells (3). Attempts to demonstrate the participation of class I molecules as restricting elements in the reactivity of $\gamma\delta$ cells to heat shock proteins, mycobacterial antigens, or a recently described keratinocyte antigen have thus far failed (3, 5, 9). Although technical issues may account for the failure to demonstrate class I restriction of these cells, it is also possible that their recognition is not restricted by class I molecules or is not restricted at all.

We could discern no alteration in $\gamma\delta^+$ T-cell development in the β_2m-mutant mice, in contrast to the ≈50- to 100-fold reduction in $\alpha\beta^+CD8^+$ T cells. Analysis of cell numbers, marker expression, repertoire, and functional responses failed to reveal any defect in $\gamma\delta^+$ T cells in β_2m-mutant mice. Our analysis included $\gamma\delta^+$ T cells thought most likely to recognize MHC-I—i.e., those that express CD8. $\alpha\beta^+CD8^+$ i-IELs were nearly absent in the mutant mice whereas the $\gamma\delta^+CD8^+$ i-IELs were present in normal numbers. The absence of $\alpha\beta^+CD8^+$ i-IELs argues that these cells are class I-dependent, despite the evidence that a substantial fraction of them are thymus-independent (44). $\gamma\delta^+CD8^+$ i-IELs are apparently neither thymus-dependent (44, 45) nor class I-dependent; a recent study suggests that class II MHC molecules influence the repertoire of $\gamma\delta^+$ i-IELs (46). The present analysis also included $\gamma\delta^+$ T cells thought most likely to require developmental selection (i.e., the s-IELs and r-IELs) that have homogeneous V–diversity–J junctional sequences. The data show that MHC-I molecules do not participate in selection of these invariant $\gamma\delta$ T cells, although they may be selected by some other ligands (47). Although our data argue against a requirement for class I MHC recognition for development of most $\gamma\delta$ T cells, they do not directly address whether these cells, once mature, recognize class I molecules.

Interestingly, the −/− mice had significantly higher frequencies of $\gamma\delta$ T cells in lymphoid organs than did +/+ mice

(Tables 1 and 3), and these cells from −/− mice usually exhibited greater responsiveness to stimulation with anti-γδ TCR antibody (Table 3). This increased frequency of γδ T cells may be due to a partial replacement of $\alpha\beta^+CD8^+$ T cells by γδ T cells in −/− mice. It is also conceivable that $\alpha\beta^+CD8^+$ T cells or MHC-I molecules have an inhibitory effect on the development and functional maturation of $\gamma\delta^+$ T cells.

In recent studies, the β_2m mutation was bred into mice that express TCR γ and δ transgenes that confer reactivity to a *Tl*-region-encoded class I molecule (42, 48). It was found that γδ-transgene-expressing T cells developed within the thymus of these mice, but these cells were unresponsive to antigen or to stimulation with anti-γδTCR antibodies and failed to significantly populate the spleen. These results suggest a requirement for some developing γδ cells to interact with class I molecules to attain functional competence.

The apparent discrepancy between the results with TCR-transgenic and nontransgenic β_2m-deficient mice may be explained if a subset of $\gamma\delta^+$ T cells, represented by the donor cell of the γ and δ transgenes, requires interactions with class I molecules for functional maturation. The γδ T cells with homogeneous TCR usage (i.e., s-IELs and r-IELs) are clearly not of this type as they are unaltered in class I-deficient mice. The putative class I-dependent subset could be relatively small based on the low frequency of allo-MHC reactive γδ T-cell clones (3) and our failure to observe a decrease in $\gamma\delta^+$ T cells from β_2m-mutant mice. However, we cannot exclude the possibility that the loss of a major class I-dependent subset results in their replacement by other $\gamma\delta^+$ T cells.

The class I-independent subset may be selected by interactions with non-class-I ligands or may not require selection. In this regard, it is interesting that many thymic $\gamma\delta^+$ T cells are HSA^+, as these cells may represent the "preselected repertoire" of γδ T cells. Alternatively, the mature HSA^- phenotype may be acquired without selection.

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