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Immunology

Lymphocyte proliferation in mice congenitally deficient in T-cell receptor $\alpha\beta^+$ cells

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ABSTRACT **In mice and humans, T cells are characterized on the basis of T-cell receptor (TcR) expression and divided into the major TcR$\alpha\beta^+$ and minor TcR$\gamma\delta^+$ populations. TcR$\alpha\beta^+$ cells are considered to be the primary regulators of the immune response, whereas the function of TcR$\gamma\delta^+$ cells is unclear. Mice congenitally deficient in TcR$\alpha\beta$-expressing cells provide an ideal model for analyzing the independent *in vivo* function of TcR$\gamma\delta^+$ cells in the absence of TcR$\alpha\beta^+$ cells. Here we report that lymphoid organs in TcRα mutant mice undergo substantial enlargement after being challenged by environmental antigens. This organ expansion can be attributed in part to increases in the relative proportions and absolute numbers of TcR$\gamma\delta^+$ cells, but an expansion of the recently described TcR$\beta^+\alpha^-$ population also has a role. The expansion of the TcR$\gamma\delta^+$ population is polyclonal, as evidenced by the usage of multiple γ and δ variable chain segments. Furthermore, a substantial proportion of the cells appears to be activated and these activated cells express surface activation markers. The results clearly demonstrate that TcR$\gamma\delta^+$ cells proliferate independently in response to a broad spectrum of challenges. Moreover, since the expansion of the lymphoid tissues and the TcR$\gamma\delta^+$ cell population is excessive relative to that seen in wild-type animals, one role of TcR$\alpha\beta^+$ cells is directly or indirectly to limit the responses of the other lymphoid components.**

T lymphocytes are divided into two populations on the basis of the structure of the T-cell receptor (TcR) that is expressed on the cell surface. The majority of TcR$\alpha\beta$-expressing cells are capable of performing all of the functions ascribed to T cells. In contrast, the function of a small subset of T cells expressing TcR$\gamma\delta$ remains an important but unresolved question. In order to address this question, we have generated mice congenitally deficient in the production of TcR$\alpha\beta^+$ T cells. TcR$\gamma\delta^+$ cells occur in approximately normal numbers in homozygous TcR$\alpha^{-/-}$ mutant mice when these animals are maintained under pathogen-free conditions, rather than expanding to compensate for the lack of TcR$\alpha\beta^+$ cells (1). Consequently, the major T-cell areas of the lymph nodes are sparsely populated and shriveled. We have subsequently observed that, despite an absence of TcR$\alpha\beta^+$ T cells, TcR$\alpha^{-/-}$ mutant mice removed from pathogen-free conditions are able to survive for extended periods of time before showing evidence of cachexia or mortality. To assess the ability of TcR$\alpha^{-/-}$ mutant mice to deal with exposure to environmental antigens and pathogens, they were analyzed after the conditions in which they were housed were altered. We show here that their lymphoid organs were clearly able to mount dramatic proliferative responses to broad antigenic challenges, despite the absence of TcR$\alpha\beta^+$ cells.

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MATERIALS AND METHODS

Animals. TcRα knockout mice have been described previously (1). Animals used in this study were between 6-wk- and 6-mo-old. TcR$\alpha^{-/-}$ mice were maintained in specific-pathogen-free isolators. TcR$\alpha^{+/-}$ and TcR$\alpha^{-/-}$ mice in the experimental groups were transferred from control, pathogen-free conditions to open cages in the animal facility and exposed to a broad antigenic challenge for up to 4 wk before being sacrificed.

Lymphocyte Isolation. Single-cell suspensions of lymph nodes, spleens (SPLs), and Peyer's patches were made by teasing the dissected tissues apart with forceps followed by gentle Dounce homogenization. Cell yields and viability were determined by trypan blue dye exclusion.

Monoclonal Antibodies (mAbs). The mAbs to murine T-cell surface antigens used in this study were as follows: phycoerythrin-conjugated anti-Cβ TcR (H57), phycoerythrin-conjugated anti-Cδ TcR (GL3), fluorescein isothiocyanate (FITC)-conjugated anti-CD4 (GK1.5), FITC-conjugated anti-CD8 (53.6.72), and FITC-conjugated anti-CD69 (H1.2F3). All antibodies were from PharMingen (San Diego).

Fluorescence-Activated Cell Sorter (FACS) Analysis. Isolated cells were incubated with the appropriate mAb at 4°C for 30–60 min. Labeled cells were analyzed by flow cytometry using a Becton-Dickinson FACScan. A total of 10^4 viable cells that bound each mAb were collected.

PCR Analysis. PCR analysis of splenic TcR$\gamma\delta$ gene expression was performed as previously described (2) on cDNA derived from splenocytes taken from TcR$\alpha^{-/-}$ mice.

Proliferation Analysis. Splenocytes isolated from mice were cultured at a density of 2×10^5 cells per well in 96-well tissue culture plates for 72 h in the presence or absence of Con A. Proliferating cells were labeled by the addition of 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) 16 h prior to harvesting. Results are expressed as the mean cpm of triplicate cultures.

RESULTS

Expansion of Lymphoid Organs in Exposed TcR$\alpha^{-/-}$ Mice. To assess the extent of lymphoid organ expansion in TcR$\alpha^{-/-}$ mutant mice challenged by exposure to environmental antigens compared with the extent of organ expansion routinely induced in wild-type mice, an experimental group of 41 TcR$\alpha^{-/-}$ mutant mice and 12 age-matched TcR$\alpha^{+/-}$ heterozygous animals were transferred from specific-pathogen-free conditions to open cages within the animal facility. The control subset of 17 TcR$\alpha^{-/-}$ mice remained in specific-pathogen-free isolators. By gross morphology and total cell yield the amount of lymphoid tissue of the antigen-

Abbreviations: FACS, fluorescence-activated cell sorter; TcR, T-cell receptor; SPL, spleen; MLN, mesenteric lymph node; PPL, Peyer's patch lymph node; PLN, pooled peripheral lymph nodes; mAb, monoclonal antibody.

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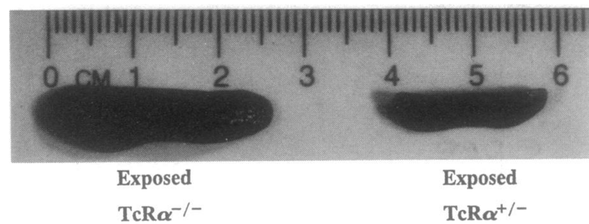

FIG. 1. Lymphoid organ expansion in TcRα mutant mice after exposure to environmental antigens. An example of an enlarged SPL from an exposed $TcR\alpha^{-/-}$ mouse is shown on the left next to an SPL from an exposed $TcR\alpha^{+/-}$ control animal.

ically challenged, exposed $TcR\alpha^{-/-}$ mice was greater than that of either control unchallenged $TcR\alpha^{-/-}$ or exposed $TcR\alpha^{+/-}$ animals. Several exposed $TcR\alpha^{-/-}$ animals had exceptionally large spleens (Fig. 1), with one $TcR\alpha^{-/-}$ spleen containing 4.7×10^8 mononuclear cells. The mean cell yield from the spleens of the 41 exposed $TcR\alpha^{-/-}$ mice (13.66×10^7 cells) was significantly higher than the yield from spleens from the 12 exposed heterozygote $TcR\alpha^{+/-}$ (6.02×10^7 cells) and the 17 control unexposed $TcR\alpha^{-/-}$ animals (4.74×10^7 cells). The mesenteric lymph nodes (MLNs) of exposed $TcR\alpha^{-/-}$ mice were similarly increased in size relative to MLNs taken from control $TcR\alpha^{-/-}$ and exposed $TcR\alpha^{+/-}$ mice (data not shown).

In a group of 36 $TcR\alpha^{-/-}$ mice, aged 16–20 wk (Fig. 2), there was wide variability between individual mice in the degree of lymphoid organ enlargement. However, there was a clear trend toward increasing cellularity in the SPL and MLN after as little as 1 wk of exposure.

Increased Proportions of $TcR\gamma\delta^+$ Cells in Exposed $TcR\alpha^{-/-}$ Animals. To test whether T-cell population expansion contributed to the enlargement of the lymphoid organs of exposed $TcR\alpha^{-/-}$ mice, cell populations isolated from the SPL, MLN, Peyer's patch lymph nodes (PPL) and pooled peripheral lymph nodes (PLN) were stained with anti-TcRβ and anti-TcRδ antibodies and analyzed by FACS. Representative profiles from an individual experiment analyzing β and δ TcR expression are shown in Fig. 3.

Analysis of 41 exposed $TcR\alpha^{-/-}$ mice, 12 exposed $TcR\alpha^{+/-}$ mice, and 17 unexposed $TcR\alpha^{-/-}$ mice revealed increased proportions of cells expressing TcRγδ in all lymphoid compartments analyzed in exposed $TcR\alpha^{-/-}$ mice compared with either exposed $TcR\alpha^{+/-}$ or control $TcR\alpha^{-/-}$ animals (Table 1). Together with the increased size and cellularity of the lymphoid organs, these increased percentages translate into a significant increase in the absolute number of $TcR\gamma\delta^+$ cells in exposed $TcR\alpha^{-/-}$ mice (data not shown). Individual mice did not consistently show similar increases in $TcR\gamma\delta^+$ cells in different lymphoid compartments (Figs. 3 and 4), suggesting that local proliferation of γδ T cells can occur.

As shown in Fig. 5, a substantial proportion of γδ T cells from $TcR\alpha^{-/-}$ mice were positive for the activation marker CD69, suggesting that some of the cells were activated *in vivo*. Otherwise, the expanded $TcR\gamma\delta^+$ population was mostly $CD4^-CD8^-$ or $CD4^-CD8^+$ (data not shown), the same phenotype as typical γδ T cells.

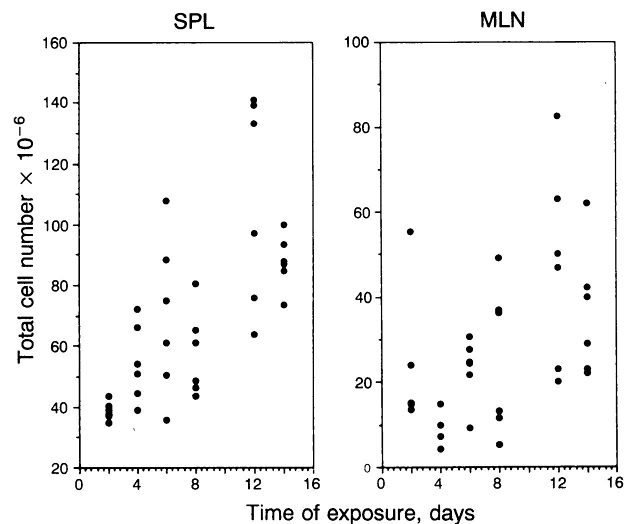

FIG. 2. Size of lymphoid organs from $TcR\alpha^{-/-}$ mice expands proportionally with time of exposure. SPL and MLN cell yields were determined from 35 $TcR\alpha^{-/-}$ mice after the animals were transferred from pathogen-free conditions and exposed to adventitious pathogens in the animal facility for up to 4 wk. The number of cells from the tissues from individual mice are shown plotted versus the length of pathogen exposure for each animal.

To assess whether there was a preferential expansion of certain $TcR\gamma\delta^+$ subpopulations, cDNA derived from $TcR\alpha^{-/-}$ splenocytes was analyzed by PCR for TcRγ and TcRδ gene usage (Table 2). A broad distribution of γ and δ gene segments was utilized.

$TcR\beta^+\alpha^-$ Cells Are More Abundant in Exposed $TcR\alpha^{-/-}$ Mice. FACS analysis of cells from $TcR\alpha^{-/-}$ mice also revealed a population of cells reactive to the anti-Cβ TcR antibody, H57. This cell population was present in low numbers in unexposed $TcR\alpha^{-/-}$ mice but was significantly elevated in the SPLs and MLNs of a large proportion of exposed $TcR\alpha^{-/-}$ mice analyzed (Fig. 6). These cells were also observed in the Peyer's patches and PLN of exposed mice. Thus, in the experiment shown in Fig. 3, $TcR\beta^+\alpha^-$ cells made up 1.7%, 5.6%, 4.6%, and 9.2% of the total cells in SPL, MLN, Peyer's patches, and PLN, respectively. The profiles shown in Fig. 3 also indicate that the staining intensity of these cells is considerably less than for $TcR\alpha\beta^+$ cells. Splenic $TcR\beta^+\alpha^-$ cells comprise both $CD4^+$ and $CD4^-$ subsets and did not coexpress the TcRδ chain (data not shown).

Independent Proliferation of $\gamma\delta^+$ and $\beta^+\alpha^-$ T Cells. To determine whether parallel expansion of $TcR\gamma\delta^+$ and $TcR\beta^+$ cells had occurred, we assessed the relative proportions of the cells within an individual tissue sample. The amount of expansion of TcRγδ and TcRβ cell populations in different tissues was not similar, with the ratio of $TcR\gamma\delta^+$ cells to

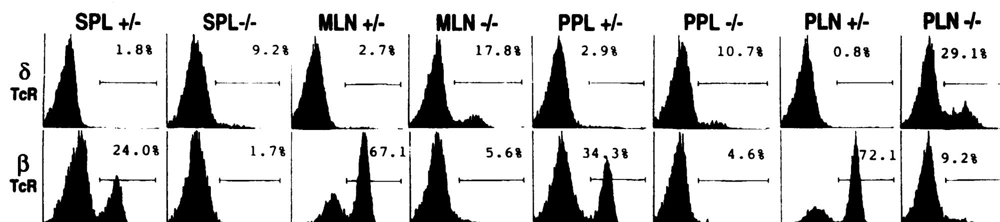

FIG. 3. Analysis of TcR expression in lymphoid tissues of exposed animals. Cells isolated from SPL, MLN, PPL, and PLN from an exposed 16-wk-old $TcR\alpha^{-/-}$ mouse and an age-matched exposed $TcR\alpha^{+/-}$ mouse were stained with antibodies directed against β and δ TcR subunits. The percentage of positively stained cells is indicated for each histogram.

Table 1. $\gamma\delta$ T cells in exposed and control mice

Organ source	% TcR$\gamma\delta^+$ cells in various lymphoid organs			
	SPL	MLN	PPL	PLN
Exposed				
TcR$\alpha^{+/-}$	2.5 ± 1.3	1.8 ± 0.7	1.7 ± 0.7	1.3 ± 0.2
Unexposed				
TcR$\alpha^{-/-}$	5.4 ± 2.2	7.1 ± 3.1	3.0 ± 1.2	2.1 ± 0.8
Exposed				
TcR$\alpha^{-/-}$	10.9 ± 3.7	17.3 ± 5.8	15.4 ± 5.1	17.5 ± 8.2

Increased percentages of TcR$\gamma\delta^+$ cells in exposed TcR$\alpha^{-/-}$ mice compared to exposed TcR$\alpha^{+/-}$ and control unexposed TcR$\alpha^{-/-}$ animals. Cells isolated from SPL, MLN, PPL, and PLN of 41 exposed TcR$\alpha^{-/-}$, 12 exposed TcR$\alpha^{+/-}$ and 17 unexposed TcR$\alpha^{-/-}$ mice were stained with the anti-CδTcR mAb and analyzed by FACS. Data are presented as the mean ± 1 SD proportion of stained cells as a percentage of the total cells in a given lymphoid organ preparation.

TcRβ^+ cells in different samples ranging between 1.2 and 45. The ratios also differed dramatically both between different tissues of an individual mouse and between individual mice, suggesting that $\gamma\delta^+$ T cells and $\beta^+\alpha^-$ T cells proliferated, at least to some extent, independently. Among a total of 128 paired measurements derived from all the TcR$\alpha^{-/-}$ mice used in this study, the number of TcR$\beta^+\alpha^-$ cells in a particular organ rarely exceeded the number of TcR$\gamma\delta^+$ cells.

Functional Capability of Cells *in Vitro*. Cells isolated from the SPL and MLN of exposed TcR$\alpha^{-/-}$ mice were analyzed for their ability to respond to a mitogenic challenge *in vitro*. Cells from TcR$\alpha^{-/-}$ mice were able to proliferate after stimulation with the mitogen Con A (Table 3), although the mitogenic response was less than that seen in heterozygous animals (data not shown). In addition, $\gamma\delta$ T cells from exposed TcR$\alpha^{-/-}$ mice were able to mount a large proliferative response after stimulation with purified protein derivative (PPD) (3). Together, these data demonstrate that the enlarged lymphoid organs of exposed TcR$\alpha^{-/-}$ mice are composed of functionally competent polyclonal cells.

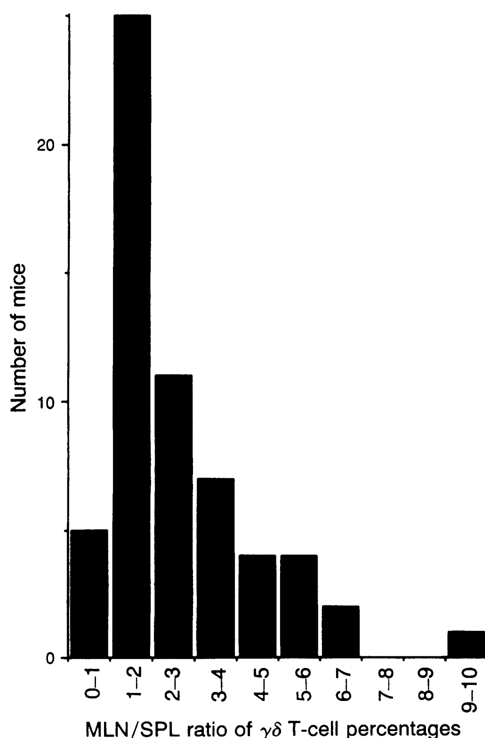

FIG. 4. Independent expansion of lymphoid organs in exposed TcR$\alpha^{-/-}$ mice. The ratio of $\gamma\delta$ T cells was determined from paired measurements of the percentages found in the MLN versus the SPL of 60 mice. These ratios were divided into ranges and the number of mice within each range was plotted.

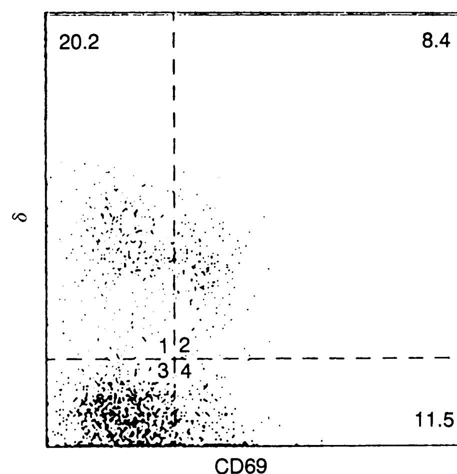

FIG. 5. $\gamma\delta$ T cells from exposed TcR$\alpha^{-/-}$ mice express activation markers. Cells isolated from the SPL of TcR$\alpha^{-/-}$ mice were stained with antibodies to CD69 and TcRδ and analyzed by FACS. The proportion of positively stained cells is indicated as a percentage in each of the quadrants. A FACS profile from a single mouse is shown.

DISCUSSION

We demonstrate that mutant mice lacking TcR$\alpha\beta^+$ cells respond to an adventitious pathogenic challenge by increasing the size of lymphoid organs and by a major proliferative expansion of T cells, particularly TcR$\gamma\delta^+$ cells. Moreover, since $\gamma\delta$ T cells are preferentially localized to epithelial surfaces (2, 4–6), the increased cellularity in the lymphoid organs analyzed in this study revealed a conspicuous presence of $\gamma\delta$ T cells in sites where they are usually rare. In some cases, $\gamma\delta$ T cells approached the numbers of $\alpha\beta$ T cells that would colonize the equivalent site in wild-type mice.

It is clear that the expansion of $\gamma\delta$ T cells in TcR$\alpha^{-/-}$ animals does not require $\alpha\beta$ T-cell help. Although many studies have reported antigen reactivity for murine $\gamma\delta$ T cells *in vitro* (7–10), it is important to elucidate the differences between *in vivo* $\gamma\delta$ T-cell reactivity *per se* and $\gamma\delta$ T-cell reactivity as a bystander effect of TcR$\alpha\beta$ cell activation. In the majority of *in vivo* studies to date, it is difficult to determine conclusively whether the observed increased reactivity or proliferation of TcR$\gamma\delta$-expressing cells is due to specific $\gamma\delta$ T-cell activation without $\alpha\beta$ T-cell help (11–17). Consistent with the observations presented here, a recent report on another strain of TcR$\alpha^{-/-}$ mutant mice has also demonstrated that TcR$\gamma\delta$-expressing cells may have a role in regulatory immunity in the absence of $\alpha\beta$ T cells, particularly with respect to the control of bacterial infections (18).

Table 2. γ and δ TcR gene expression in TcR$\alpha^{-/-}$ mice

Primer	Band	Size, bp	Primer	Band	Size, bp
Vγ1–Jγ4	++	426	Vδ1–Jδ1	+	170
Vγ4–Cγ1	+	225	Vδ3–Jδ1	+	170
Vγ6–Cγ1	+	350	Vδ5–Jδ1	++	250
Vγ7–Cγ1	+	235	Vδ6–Jδ1	++	150

γ and δ TcR gene usage in TcR$\alpha^{-/-}$ mice was determined by PCR analysis performed on cDNA from the SPL. A + indicates that a DNA fragment of the appropriate size was amplified from splenocyte cDNA. Although PCR is essentially qualitative, a ++ indicates that the signal obtained relative to a β-tubulin control was reproducibly stronger than most of the other TcR signals. V, variable domain; J, joining segment; and C, constant domain.

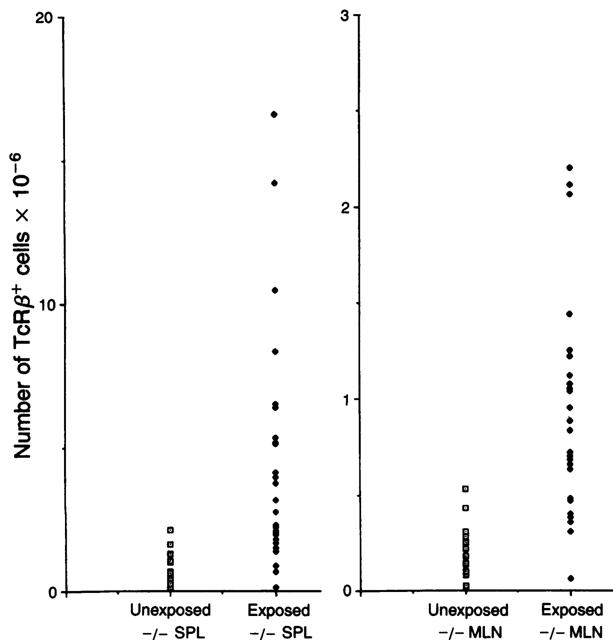

FIG. 6. Increases in $TcR\beta^{+}\alpha^{-}$ cells in exposed $TcR\alpha^{-/-}$ mice compared to control $TcR\alpha^{-/-}$ animals housed in pathogen-free isolators. Cells isolated from the SPL and MLN of 29 exposed and 15 control $TcR\alpha^{-/-}$ mutant mice were stained with the anti-$C\beta$ TcR mAb and analyzed by FACS. The percentage of cells which stained positively for $TcR\beta$ was recorded. Absolute numbers of $TcR\beta^{+}\alpha^{-}$-expressing cells were determined from the cell yields together with the relative proportion of cells that were stained for each individual mouse.

The origin of $TcR\beta^{+}\alpha^{-}$ cells, described previously in another $TcR\alpha^{-/-}$ strain (19), is unknown, although the population comprises both $CD4^{-}$ cells, like progenitor thymocytes, and $CD4^{+}$ cells, like mature $TcR\alpha\beta^{+}$ cells in wild-type mice. It therefore remains possible that $TcR\beta^{+}\alpha^{-}$ cells represent an uncharacterized mature peripheral T-cell population, although biochemical evidence suggests that mature T cells cannot express stable cell surface $TcR\beta$ chains in the absence of $TcR\alpha$ chains (20). The data presented in this study suggest that this unique population is able to expand, together with, but independently of, $\gamma\delta$ T cells.

T-cell proliferation in exposed $TcR\alpha^{-/-}$ mice is accompanied by B-cell maturation and high levels of secretion of immunoglobulin classes traditionally regarded as being T-cell dependent (3). Increased levels of serum immunoglobulin G have also been observed in mice deficient in interleukin 2 (21), while, in contrast, antibody production has clearly been demonstrated to be deficient in mice lacking $TcR\alpha\beta^{+}$ $CD4^{+}$ cells (22–25). It is possible, therefore, that some of the $\gamma\delta$ T cells proliferating in the $TcR\alpha^{-/-}$ mice may help immunoglobulin production (3). Although immunoglobulin isotype switching is generally considered to be dependent on $TcR\alpha\beta^{+}$ cells, there is already some evidence of Qa-1-restricted $\gamma\delta$ T cells providing B-cell help (26).

Table 3. Con A mitogenic activation of splenic $\gamma\delta$ T cells in $TcR\alpha^{-/-}$ mice

	[3H]Thymidine incorporation, cpm*	
Con A, μg/ml	SPL	MLN
0	3,033	5,903
1.25	16,385	19,277
2.5	40,428	38,200
5	42,176	48,322

*All SEM were less than 15%.

Studies such as those reported here are important for assessing the extent to which the different $TcR\alpha\beta$, $TcR\gamma\delta$, and B-cell immune compartments are interdependent. It is widely accepted that $TcR\alpha\beta^{+}$ cells play a critical role in the initiation and regulation of many aspects of the immune response (27–30). Therefore, it is interesting that, although no antigen-specific responses have been demonstrated, the immune response in $TcR\alpha^{-/-}$ mice is not totally paralyzed by the absence of $TcR\alpha\beta$-expressing cells, but rather is unregulated. The formal demonstration of the expansion of $TcR\gamma\delta^{+}$ cells in the absence of $TcR\alpha\beta^{+}$ cells emphasizes the importance of considering the different possible mechanisms that may normally control immune regulation. This study highlights the importance of $\alpha\beta$ T cells in a different, and equally important aspect of regulation, namely the control of the extent of the immune response. Our data suggest that the capacity to regulate local T-cell and B-cell proliferation may be lost in the absence of $\alpha\beta$ T cells. This regulation may be directly mediated by a specific population of $\alpha\beta$ T cells, or indirectly mediated by activating the clearance mechanisms for the removal of bulk antigen.

The data presented here suggest an explanation for the observation that $TcR\alpha^{-/-}$ mice develop intestinal pathology, described as inflammatory bowel disease (31). Any antigenic challenge, perhaps generated by normally nonpathogenic microorganisms, will initiate local expansion of lymphoid tissues that may generate inflammatory responses resulting in an inflammatory bowel disease-like pathology. Thus, relative cleanliness of animal facilities may govern the pathology of these and other mutant mouse strains.

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1. Philpott, K. L., Viney, J. L., Kay, G., Rastan, S., Gardiner, E. M., Chae, S., Hayday, A. C. & Owen, M. J. (1992) *Science* **256**, 1448–1452.
2. Kyes, S., Carew, E., Carding, S., Janeway, C. A. & Hayday, A. C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5527–5531.
3. Wen, L., Roberts, S. R., Viney, J. L., Wong, F. S., Mallick, C., Findly, R. C., Peng, Q., Craft, J. E., Owen, M. J. & Hayday, A. C. (1994) *Nature (London)* **369**, 654–658.
4. Raulet, D. H. (1989) *Annu. Rev. Immunol.* **7**, 175–207.
5. Itohara, S., Farr, A. G., Lafaille, J. J., Bonneville, M., Takagaki, Y., Haas, W. & Tonegawa, S. (1990) *Nature (London)* **342**, 754–757.
6. Allison, J. P. & Havran, W. L. (1991) *Annu. Rev. Immunol.* **9**, 679–705.
7. Janis, E., Kaufman, S., Schwartz, R. & Pardoll, D. (1989) *Science* **244**, 713–716.
8. O'Brien, R., Happ, M. P., Dallas, A., Palmer, E., Kubo, R. & Born, W. K. (1989) *Cell* **57**, 667–674.
9. Rajasekar, R., Sim, G. & Augustin, A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1767–1771.
10. Born, W., Hall, L., Dallas, A., Boymel, J., Shinnick, T., Young, D., Brennan, P. & O'Brien, R. (1990) *Science* **249**, 67–69.
11. Ericsson, P. O., Hansson, J., Widegan, B., Dohlsten, M., Sjogren, H. O. & Hedland, G. (1991) *Eur. J. Immunol.* **21**, 2797–2802.
12. Carding, S. R., Allan, W., Kyes, S. A., Hayday, A., Bottomly, K. & Doherty, P. C. (1990) *J. Exp. Med.* **172**, 122–129.
13. Hiromatsu, K., Yoshikai, Y., Matsuzaki, G., Ohga, S., Muramori, K., Matsumoto, K., Bluestone, J. A. & Nomoto, K. (1992) *J. Exp. Med.* **175**, 49–56.
14. Skeen, M. J. & Ziegler, H. K. (1993) *J. Exp. Med.* **178**, 985–996.
15. Ichikawa, Y., Shimizu, H., Yoshida, M., Takaya, M. & Arimori, S. (1991) *Clin. Exp. Rheumatol.* **9**, 603–609.
16. Kjeidsen-Kragh, J., Quayle, A. J., Skalhegg, B. S., Sioud, M. & Forre, O. (1993) *Eur. J. Immunol.* **22**, 2092–2099.

17. Arstila, T. P., Toivanen, P. & Lassila, O. (1993) *Eur. J. Immunol.* **23**, 2034–2037.
18. Mombaerts, P., Arnoldi, J., Russ, F., Tonegawa, S. & Kaufman, S. H. E. (1993) *Nature (London)* **365**, 53–56.
19. Mombaerts, P., Clarke, A. R., Rudnicki, M. A., Iacomini, J., Itohara, S., Lafaille, J. J., Wang, L., Ichikawa, Y., Jaenisch, R., Hooper, M. L. & Tonegawa, S. (1992) *Nature (London)* **360**, 225–231.
20. Groettrup, M., Baron, A., Griffiths, G., Palacios, R. & von Boehmer, H. (1992) *EMBO J.* **7**, 2735–2739.
21. Schorle, H., Hotschke, T., Hunig, T., Schimpl, A. & Horak, I. (1991) *Nature (London)* **352**, 621–624.
22. Cosgrove, D., Gray, D., Dierich, A., Kaufman, J., Lemeur, M., Benoist, C. & Mathis, D. (1991) *Cell* **66**, 1051–1066.
23. Grusby, M. J., Johnson, R. S., Papaioannou, V. E. & Glimcher, L. H. (1991) *Science* **253**, 1417–1420.
24. Rahemtulla, A., Fung-Leung, W. P., Schillham, M. W., Kundig, T. M., Sambhara, S. R., Narendran, A., Arabian, A., Wakeham, A., Paige, C. J., Zinkernagel, R. M., Miller, R. G. & Mak, T. W. (1991) *Nature (London)* **353**, 180–184.
25. Carbone, A., Harbeck, R., Dallas, A., Nemazee, D., Finkel, T., O'Brien, R., Kubo, R. & Born, W. (1991) *Immunol. Rev.* **120**, 35–50.
26. Vidovic, D. & Dembic, Z. (1991) *Curr. Top. Microbiol. Immunol.* **173**, 239–244.
27. Guerder, S. & Matzinger, P. (1992) *J. Exp. Med.* **176**, 553–564.
28. Cassell, D. & Forman, J. (1991) *J. Immunol.* **146**, 3–10.
29. Fayolle, C., Deriaud, E. & Leclerc, C. (1991) *J. Immunol.* **147**, 4069–4073.
30. Cobbold, S., Qin, S., Leong, L. Y. N., Martin, G. & Waldmann, H. (1991) *Immunol. Rev.* **129**, 165.
31. Mombaerts, P., Mizoguchi, E., Grusby, M. J., Glimcher, L. H., Bhan, A. K. & Tonegawa, S. (1993) *Cell* **75**, 275–282.