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Immunology

Defective IgE production by SJL mice is linked to the absence of CD4$^+$, NK1.1$^+$ T cells that promptly produce interleukin 4

(CD1/cytokine/interferon)

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ABSTRACT **SJL mice produce little or no IgE in response to polyclonal stimulation with anti-IgD antibody and fail to express interleukin 4 (IL-4) mRNA in the spleen 5 days after injection of anti-IgD, in contrast to other mouse strains that produce substantial amounts of IgE and IL-4. Because IL-4 is critical in IgE production, the possibility that SJL mice are poor IgE producers because their naive T cells fail to differentiate into IL-4 producers must be seriously considered. IL-4 itself is the principal factor determining that naive T cells develop into IL-4 producers. A major source of IL-4 for such differentiation is a population of CD1-specific CD4$^+$ T cells that express NK1.1. These cells produce IL-4 within 90 min of anti-CD3 injection. T cells from SJL mice fail to produce IL-4 in response to injection of anti-CD3. Similarly, SJL T cells and CD4$^+$ thymocytes do not produce IL-4 in response to acute *in vitro* stimulation. SJL T cells show a marked deficiency in CD4$^+$ cells that express the surface receptors associated with the NK1.1$^+$ T-cell phenotype. This result indicates that the SJL defect in IgE and IL-4 production is associated with, and may be due to, the absence of the CD4$^+$, NK1.1$^+$ T-cell population.**

The differentiation of naive CD4$^+$ T cells into cells capable of producing interleukin 4 (IL-4) or interferon γ (IFN-γ) depends upon their mode of "priming" (1). IL-4 itself, present at the time of priming, plays a critical role in the development of naive T cells into IL-4 producers (2, 3). A population of CD4$^+$, NK1.1$^+$ T cells promptly produces IL-4 in response to *in vivo* stimulation with anti-CD3 antibody or with the superantigen, staphylococcal enterotoxin B (4). These cells may be a source of IL-4 that could act at the initiation of immune responses. Such CD4$^+$, NK1.1$^+$ T cells are closely related to a set of CD4$^+$, CD8$^-$, heat-stable antigen (HSA)^{low} thymocytes, specific for CD1, that produce large amounts of cytokines (5–10).

IL-4 plays a critical role in immunoglobulin class switching for IgE production (11, 12). SJL mice are known to produce limited amounts of IgE in response to a variety of stimulants (13, 14). Because their B cells are capable of switching to IgE production if stimulated with lipopolysaccharide and IL-4 (15) and their T cells display a limitation in IL-4 production under certain circumstances (16), we considered the possibility that their defect arose from failure of their naive CD4$^+$ T cells to develop into IL-4 producers because of an absence of IL-4 at priming. In turn, this absence might be due to deficient IL-4 production by CD4$^+$, NK1.1$^+$ T cells. Here we provide evidence that SJL mice do have a striking diminution in acute IL-4 production in response to anti-CD3 and a marked reduction in the numbers of CD4$^+$, NK1.1$^+$ T cells and thymocytes.

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MATERIALS AND METHODS

Animals and Reagents. Virus-free C57BL/6, BALB/c, C57BL/10, B10.A, CBA/J, and C3H/HeN female mice, 8–12 week of age, were obtained from the Division of Cancer Treatment, National Cancer Institute (Frederick, MD). SJL, (BALB/c × SJL)F_1, and SWR female mice were obtained from The Jackson Laboratory.

Recombinant cytokines (mouse IL-4, human IL-2, and mouse IFN-γ) and purified antibodies [anti-CD3 (2C11), rat anti-mouse IL-4 (11B11), and rat anti-mouse IFN-γ (XMG 1.2)] were prepared as described (4).

Affinity-purified goat anti-IgD antibody, goat IgG, and goat anti-IgD antiserum were provided by Fred Finkelman (Uniformed Services University of the Health Sciences, Bethesda). Rat anti-mouse monoclonal IgE antibody (23G3), affinity-purified goat anti-mouse IgG1 antibody, and biotin-conjugated, affinity-purified goat anti-mouse IgG1 antibody were purchased from Southern Biotechnology Associates. Biotin-conjugated monoclonal rat anti-mouse IgE antibody (R35118) was purchased from PharMingen. Fluorescein isothiocyanate (FITC)–rat anti-mouse B220 (RA3-6B2), FITC–rat anti-mouse I-A^d (AMS 32.1), FITC–rat anti-mouse I-A^s (10-3.6), FITC–rat anti-mouse CD8 (53-6.7), FITC–anti-mouse NK1.1 (PK136), FITC–rat anti-mouse Ly6C (AL-21), and phycoerythrin (PE)–rat anti-mouse IL-2 receptor β chain (TMβ-1) were purchased from PharMingen. RED613-rat anti-mouse CD4 was purchased from GIBCO/BRL.

***In Vivo* Treatment of Mice.** Mice were injected i.v. with anti-CD3 (1.33 μg per mouse), 1.33 μg of hamster IgG, or Hanks' balanced salt solution (HBSS). Spleens were removed at specified times after injection for RNA extraction and cell culture. Goat anti-IgD antibody (200 μg) plus normal goat IgG (600 μg) or goat anti-IgD antiserum (100 μl) was injected i.v. or s.c., respectively. Six to 8 days later serum IgE and IgG1 content was measured by using an avidin–biotin microtiter ELISA (17).

Cell Preparation. T cells were purified from lymph node cell suspensions (2 × 10^7/ml in RPMI 1640 medium/5 mM EDTA/5% fetal bovine serum). The cell suspension was incubated with FITC–anti-B220, FITC–anti-I-A^d, or FITC–I-A^s antibodies each at 10 μg/ml for 30 min at 4°C on a turning wheel. After two washes, the cells were resuspended with magnetic beads coated with sheep anti-FITC antibody (Advanced Magnetics, Cambridge, MA) and exposed twice to a magnetic field. The residual cells were used as purified T cells.

Mature mouse CD8$^-$ thymocytes were purified after one-step killing at 37°C with anti-HSA (J11d.2) and anti-CD8 (3.155) monoclonal antibodies plus low-toxic M rabbit com-

Abbreviations: IL-4, interleukin 4; IFN-γ, interferon γ; FITC, fluorescein isothiocyanate; HSA, heat-stable antigen; APCs, antigen-presenting cells; Vβ, variable region β subunit; RT, reverse transcription; PE, phycoerythrin.

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plement (Cedarlane Laboratories) followed by centrifugation over a density gradient.

Antigen-presenting cells (APCs) were prepared by treating splenocytes with anti-Thy-1.2 (HO13.4), anti-CD4 (RL172), and anti-CD8 (3.155) antibodies plus low-toxic M rabbit complement. The remaining cells were then layered onto a discontinuous Percoll (Pharmacia) gradient and centrifuged for 15 min at 1000 × *g*. Cells in the 50–60% fraction were collected and used as APCs.

FACS Analysis of Thymic T-Cell Subsets. Fluorescence staining was done at 4°C in 50 μl containing 2×10^6 $CD8^-$, HSA^{low} thymocytes with Red 613–anti-CD4, PE–anti-IL-2 receptor β chain, and FITC–anti-NK1.1 or FITC–anti-Ly6C in phosphate-buffered saline/0.1% bovine serum albumin/0.5% NaN_3. Fluorescence analysis was done on a FACScan flow cytometer (Becton Dickinson).

Cell Cultures. RPMI 1640 medium/10% fetal bovine serum/50 μM 2-mercaptoethanol/2 mM L-glutamine/penicillin at 100 units/ml/streptomycin at 100 μg/ml/1 mM sodium pyruvate was used as culture medium. IL-4-producing capacity was measured by culturing 10^6 lymph node T cells or $CD8^-$ thymocytes *in vitro* with soluble anti-CD3 at 3 μg/ml, 10^5 APCs, and human IL-2 at 10 units/ml in a total 1-ml vol in 24-well plates for 48 hr. Supernatants were harvested to measure IL-4 content. For total splenocytes, 10^6 splenocytes were cultured under the same conditions but without addition of APCs.

To determine whether naive T cells could be primed to become IL-4 producers, 5×10^6 lymph node T cells were cultured in individual wells of 6-well plates in a total 3-ml vol with soluble anti-CD3 antibody (1 μg/ml), IL-2 at 10 units/ml, 5×10^5 APCs in the presence of IL-4 (1000 units/ml), or anti-IL-4 antibody (10 μg/ml). After 3 days, cells were washed three times, and 10^6 cells were restimulated with 10^5 fresh APCs, soluble anti-CD3 at 1 μg/ml, and IL-2 at 10 units/ml in a total 1-ml vol in 24-well plates. IL-4 and IFN-γ in supernatants were measured at 48 hr.

Cytokine Assays. IL-4 content was measured with the CT.4S cell line (18), using serial dilutions of supernatants and comparing responses to those elicited by known amounts of murine IL-4. IFN-γ was assayed with a specific two-site ELISA, with reference standard curve prepared using known amounts of recombinant IFN-γ.

Analysis of Expression of IL-4 mRNA. Total spleen RNA was prepared by using the guanidinium method as described (4). As positive controls, mRNA extracted from the IL-4 producing cell line LT-1 (19) was used. For analysis of expression of IL-4 mRNA, mRNAs were amplified by a modified standard reverse transcription (RT)-PCR amplification procedure as described (4). Primer sequences were as follows: IL-4: 5′ primer, GAATGTACCAGCAGCCATATC, and 3′ primer, CTCAGTACTACGAGTAATCCA; β-actin: 5′ primer, GATGACGATATCGCTGCGCTG, and 3′ primer, GTACGACCAGAGGCATACAGG. Some PCR products were analyzed by Southern blotting with ^{32}P-labeled IL-4-specific cDNA probe.

RESULTS

SJL Mice Fail to Produce IgE in Response to *in Vivo* Treatment with Anti-IgD. BALB/c and C57BL/6 mice demonstrate striking increases in serum IgE levels 7 or 8 days after injection of 100 μl of goat anti-mouse IgD serum, but SJL mice make a barely detectable response, 10- to 100-fold less than those of the other strains (Fig. 1*A*). The IgG1 response of SJL mice is similar to that of C57BL/6 mice, although markedly less than that of BALB/c mice.

Spleen cells from SJL mice injected with anti-IgD 5 days earlier had little or no IL-4 mRNA, whereas spleen cells from similarly injected BALB/c and C57BL/6 mice expressed easily detectable IL-4 mRNA (Fig. 1*B*). This result is consistent with the poor IgE production by SJL mice being due to their failure to produce detectable amounts of IL-4 at the time required to induce class switching. CD40-L mRNA was induced to a comparable degree in each strain of mouse (data not shown), indicating that the failure of SJL mice to induce IL-4 mRNA expression is not from an inability of anti-IgD to stimulate their lymphocytes.

SJL Mice Fail to Promptly Produce IL-4 in Response to *in Vivo* Injection with Anti-CD3. To determine whether the poor IL-4 and IgE production of SJL mice in response to anti-IgD might be explained by a failure in acute IL-4 production, we injected anti-CD3 antibody into SJL mice (Fig. 2). SJL mice failed to produce either IL-4 mRNA or protein at 90 min, whereas C57BL/6, BALB/c, SWR, and (BALB/c × SJL)F_1 mice did so. In addition, C57BL/10, B10.A, CBA/J, and C3H/HeN mice all responded to injection of anti-CD3 with prompt IL-4 production (data not shown).

SJL Spleen Cells, Purified T Cells, and Thymocytes Fail to Produce IL-4 Acutely in Response to *in Vitro* Culture with Anti-CD3 Plus IL-2. Spleen cells and purified T cells from SJL mice produced 4- to 77-fold less IL-4 in response to *in vitro*

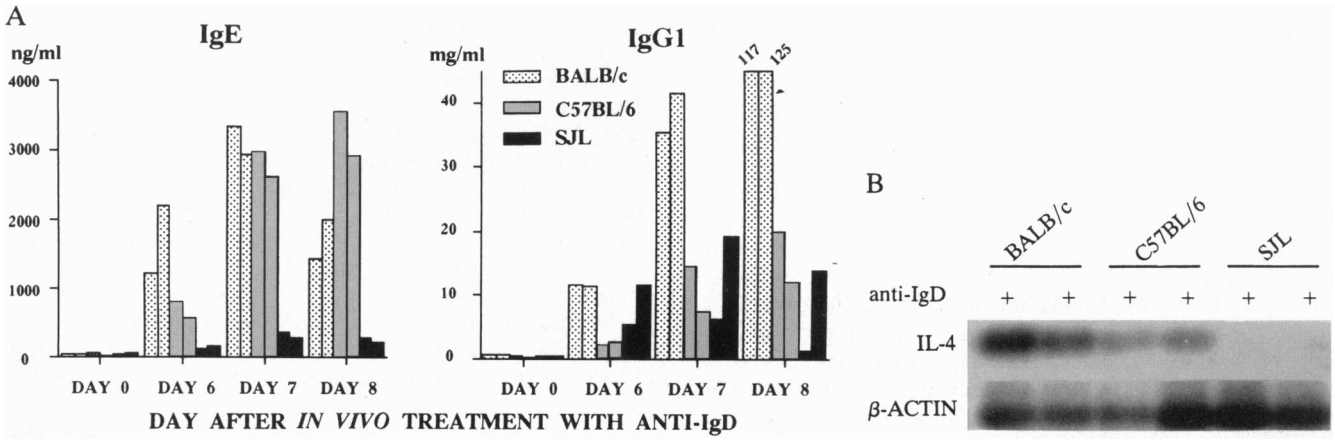

FIG. 1. IgE and IgG1 production and expression of IL-4 mRNA by BALB/c, C57BL/6, and SJL mice in response to injection of anti-mouse IgD antiserum. (*A*) Individual mice were injected with 100 μl of goat anti-IgD antiserum s.c. They were bled 6, 7, and 8 days later, and serum IgE and IgG1 concentrations were determined. (*B*) RNA was extracted from spleen cells of mice treated with 100 μl of anti-IgD antiserum 5 days after treatment. Expression of IL-4 and β-actin mRNA was measured by RT-PCR with Southern blot analysis.

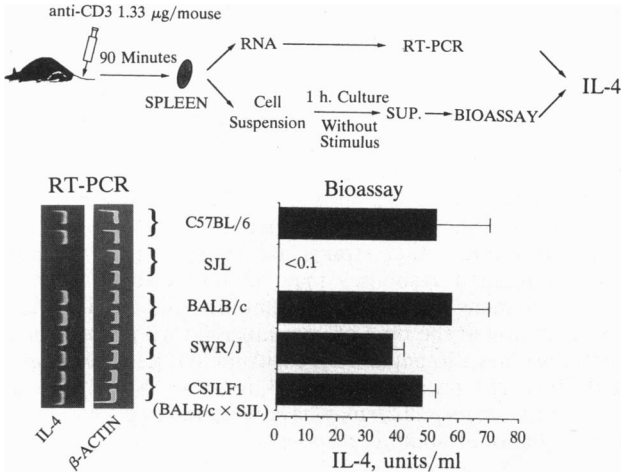

FIG. 2. SJL mice fail to produce IL-4 in response to injection with anti-CD3 antibody. C57BL/6, SJL, BALB/c, SWR/J, and (BALB/c × SJL)F_1 (CSJLF1) mice were injected with 1.33 μg of anti-CD3 antibody i.v. Spleens were harvested 90 min later for RNA extraction and for lymphokine production. Isolated mRNA was analyzed for expression of IL-4 and β-actin message using RT-PCR. To determine lymphokine production, 5×10^6 spleen cells were cultured immediately in individual wells of 24-well plates for 1 hr without additional stimulus. Culture supernatants were harvested and tested for IL-4 production by bioassay.

culture with anti-CD3 plus IL-2 than did cells from mice of each of the other strains tested (Table 1). Similarly, $CD8^-$ thymocytes from SJL mice also produce very little IL-4 in response to culture with anti-CD3 plus IL-2, in the presence of T-cell-depleted spleen cells as APCs. By contrast, $CD8^-$ thymocytes from C57BL/6 mice produced substantial amounts of IL-4 in response to such stimulation (Table 1 $CD4^+$ $CD8^-$ thymocytes).

The poor production of IL-4 by peripheral $CD4^+$ T cells and thymocytes could be explained either by a defect in the capacity of the $CD4^+$, $NK1.1^+$ population from these mice to produce IL-4 or by diminished numbers of such cells. We initially examined the frequency of $CD4^+$, $NK1.1^+$ cells in the thymus because other markers are coexpressed on these cells, which aid in their enumeration. Among the markers coexpressed with NK1.1 on $CD4^+$, $CD8^-$ thymocytes that express

Table 1. Acute IL-4 production from spleen cells, purified T cells, and $CD4^+$, $CD8^-$ thymocytes of different strains cultured with anti-CD3 or anti-CD3 plus IL-2 for 48 hr

Strain	IL-4, units/ml *in vitro* culture: anti-CD3	anti-CD3 + IL-2
Unseparated spleen cells		
BALB/c	839	967
C57BL/6	40	114
C57BL/10	71	125
B10.A	21	76
CBA/J	771	1461
C3H/HeN	299	718
SJL	7	19
Purified T cells		
BALB/c	261	443
C57BL/6	38	58
SJL	4	6
$CD4^+$, $CD8^-$ thymocytes		
C57BL/6	704	882
SJL	1	12

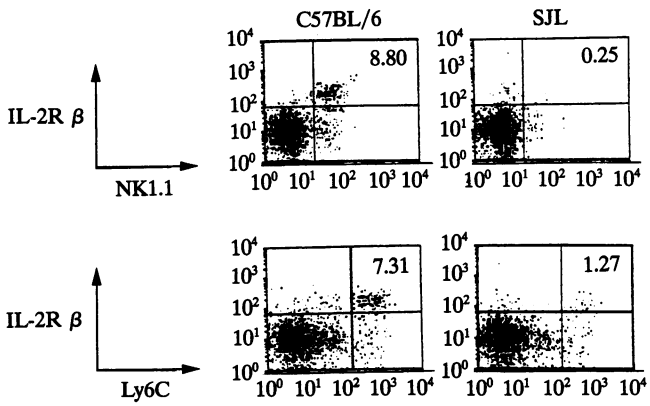

FIG. 3. Frequency of $Ly6C^+$ and $NK1.1^+$ cells among IL-2 receptor β $chain^+$, $CD4^+$, $CD8^-$, HSA^{low} thymocytes from SJL mice. C57BL/6 and SJL thymocytes that lacked CD8 and had low levels of HSA were stained with Red 613–anti-CD4, PE–anti-IL-2 receptor β chain, and FITC–anti-NK1.1 or FITC–anti-Ly6C in phosphate-buffered saline/0.1% bovine serum albumin/0.5% NaN_3. Dot-plots represent relative fluorescence for IL-2 receptor β (IL-2R β) chain and NK1.1 (*Upper*) or IL-2 receptor β chain and Ly6C (*Lower*) among cells gated for CD4 expression. Percentages of $NK1.1^+$ or $Ly6C^+$ cells expressing the IL-2 receptor β $chain^+$ among $CD4^+$, $CD8^-$, HSA^{low} thymocytes are indicated.

low levels of HSA is Ly6C (8, 9). Fig. 3 shows that among C57BL/6 $CD8^-$ thymocytes that are dull for HSA, the majority of the $NK1.1^+$ or $Ly6C^+$ cells express IL-2 receptor β chain. Such $NK1.1^+$ and $Ly6C^+$ cells expressing IL-2 receptor β chain constitute 8.8% and 7.3%, respectively, in the C57BL/6 cell population. Among the same population of thymocytes from SJL mice, none expressed NK1.1, and only 1.3% expressed Ly6C. These results indicate that SJL mice have a defect in the numbers of cells capable of promptly producing IL-4 in the thymus. Splenic $CD4^+$, $NK1.1^+$ T cells are also markedly diminished in SJL mice, constituting 0.2% of spleen cells in contrast to a frequency of 1.1% in C57BL/6 mice (data not shown).

T Cells from SJL Mice Can Be Primed *in Vitro* to Produce IL-4. If deficient acute IL-4 production is responsible for the

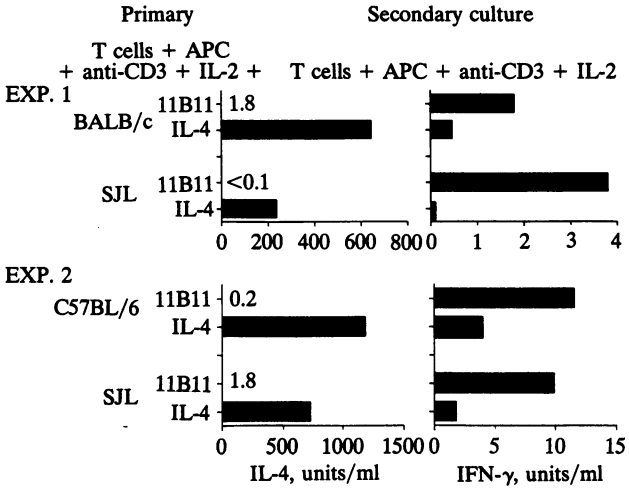

FIG. 4. T cells from SJL mice can be primed to produce IL-4. For the primary culture, 5×10^6 lymph node T cells from naive BALB/c, C57BL/6, or SJL mice were cultured in 6-well plates in a total 3-ml vol with soluble anti-CD3 at 1 μg/ml, IL-2 at 10 units/ml, 5×10^5 T-cell-depleted spleen cells, and IL-4 at 1000 units/ml or anti-IL-4 antibody (11B11) at 10 μg/ml. After 3 days of priming, cells were washed and recultured with anti-CD3 plus IL-2 in the presence of fresh T-cell-depleted spleen cells in a total 1-ml vol in 24-well plates for 48 hr. Supernatants were harvested; IL-4 and IFN-γ contents were measured.

failure of naive T cells to develop into cells capable of producing IL-4, it would be anticipated that such "conventional" T cells should be capable of being primed to become IL-4 producers if an appropriate source of IL-4 were available. Lymph node T cells from BALB/c, C57BL/6, and SJL mice were cultured with soluble anti-CD3, IL-2, and T-cell-depleted spleen cells (as APCs) in the presence of IL-4 or of anti-IL-4 antibody. After 3 days of such "priming," the cells were washed and rechallenged with anti-CD3 plus IL-2 in the presence of fresh APCs. As shown in Fig. 4, SJL T cells could be primed to become IL-4-producing cells. The level of priming observed was less than that seen in BALB/c cells but more comparable to that observed for C57BL/6 cells. Like T cells from both BALB/c and C57BL/6 mice, the priming of $CD4^+$ SJL T cells for IL-4 production depended upon the presence of IL-4 in the priming culture.

DISCUSSION

The defect of SJL mice in IgE production in response to certain stimuli has been rather enigmatic. Their B cells produce IgE when stimulated *in vitro* with lipopolysaccharide and IL-4 (15), implying that a B-cell defect was not responsible for their limited IgE production. Similarly, stimulation of T cells can lead to the development of IL-4-producing cells (16), suggesting that no intrinsic defect prevents T cells from becoming IL-4 producers. These observations are consistent with active inhibition in IL-4 production or IgE secretion by a suppressor mechanism, as proposed (13). An alternative possibility is that the defect in SJL mice lies in an inability to produce sufficient IL-4 for priming of conventional T cells for IL-4 production. This result would lead to a dominance in IFN-γ-producing T cells. Because IFN-γ can inhibit switching to IgE production (15, 20), such cells could act as suppressors, further limiting IgE production.

Here we showed that SJL mice have a striking defect in both IgE and IL-4 responses to polyclonal stimulation with anti-IgD antibody. This failure to develop IL-4-producing cells correlates well with their failure to secrete IL-4 or express IL-4 mRNA in response to anti-CD3, suggesting that an absence of IL-4 at the time of priming might be responsible for the failure of differentiation of naive cells into IL-4 producers and the failure of production of IgE. Enumerating $CD8^-$, HSA^{low} thymocytes that express NK1.1, Ly6.C, and IL-2Rβ as well as $CD4^+$, $NK1.1^+$ cells in the spleen reveals a profound defect in these cells in SJL mice that could account for the lack of acutely produced IL-4.

$CD4^+$, $NK1.1^+$ thymocytes express a very limited range of T-cell receptors, most of which utilize variable region subunit α (Vα)14 associated with variable region subunit β (Vβ)8, -7, or -2 (8, 9, 21). These receptors are specific for CD1 (10). SJL mice might have a defect in the numbers of these cells because they lack Vβ8 as a result of a large deletion in their Vβ genetic region (22). However, SWR mice, which have an independent but more extensive Vβ deletion also involving Vβ8 (23), have no such defect in producing IL-4 in response to injection of anti-CD3 (Fig. 2). An analysis of six (BALB/c × SJL) recombinant inbred strains reveals that all expressed the SJL allele in the Vβ region (24), although only one strain failed to produce IL-4 in response to anti-CD3. This strain made the poorest IgE response to injection of anti-IgD (data not shown). Thus, by itself, the deletion in Vβ cannot explain the defect of SJL mice, although it could contribute to this defect. On the other hand, SJL mice have decreased expression of the CD1.2 isoform of CD1 (25). Recognition of CD1 may be essential for activating IL-4 production by $NK1.1^+$ T cells (T.Y., A.B., and W.E.P., unpublished work). Such a defect in CD1 expression might partially explain the genetic basis of the poor IgE response and the defect in numbers of $CD4^+$, $NK1.1^+$ T cells in SJL mice. In addition, SJL mice possess limited numbers of NK cells (26). Whether the limitation in expression of this set of $NK1.1^+$ cells is in any way related to the limited expression of $CD4^+$, $NK1.1^+$ cells is unknown.

The difference between SJL and other strains of mice in IL-4 production may very well account for differences in other types of immune responses, most notably in the development of forms of autoimmunity in which tissue damage is prominent. It is well-known that SJL mice are particularly susceptible to experimental allergic encephalomyelitis, whereas BALB/c mice and several other strains are resistant (27). Because IFN-γ-dominated responses may be responsible for tissue damage in many types of autoimmunity and because IL-4 administration at the time of immunization with myelin basic protein is known to ameliorate experimental allergic encephalomyelitis (28), production of IL-4 by $CD4^+$, $NK1.1^+$ T cells at the outset of responses may have wide-ranging significance for the quality of immune responses.

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