Summary

Rather unexpectedly, major histocompatibility complex class II-deficient mice have a significant population of peripheral CD4+ T lymphocytes. We have investigated these cells at the population and clonal levels. CD4+ T lymphocytes from class II-deficient animals are thymically derived, appear early in ontogeny, exhibit the phenotype of resting memory cells, are potentially functional by several criteria, and have a diverse T cell receptor repertoire. They do not include substantially elevated numbers of NK1.1+ cells. Hybridomas derived after polyclonal stimulation of the CD4+ lymphocytes from class II-deficient animals include a subset with an unusual reactivity pattern, responding to splenocytes from many mouse strains including the strain of origin. Most members of this subset recognize the major histocompatibility complex class Ib molecule CD1; their heterogeneous reactivities and T cell receptor usage further suggest the involvement of peptides and/or highly variable posttranslational modifications.

Most CD4+ T cells depend on an interaction with the MHC class II molecules expressed on thymic stroma to complete differentiation. As a consequence, mice that lack the conventional class II molecules (II- mice) have a drastic reduction in the number of mature CD4+ T cells in the thymus and peripheral lymphoid organs (1-3). Unexpectedly, though, a small population of CD4+ T cells was detected in the spleen and lymph nodes of these animals. This population did not appear to result from nonspecific "leakage" because the cells had atypical properties: they expressed markers reminiscent of memory cells and were preferentially localized in the B cell areas of lymphoid organs.

The existence of CD4+ T cells in II- mice contradicts previous dogma on positive selection, which states that CD8+ cells result from a TCR/MHC class I molecule engagement in the thymus and react in the periphery to antigen presented by class I molecules, while CD4+ cells emerge after a TCR/class II interaction and have class II-restricted antigen reactivities (reviewed in reference 4). Since the CD4+ cells in the II- mice could not have been selected on conventional class II molecules, several questions arose: When and where are these cells generated? Does their cell surface phenotype show other atypical features? Are they functional? If so, what ligands do they recognize? Here, we address these questions at the population and clonal levels. Besides determining some important general properties of the peripheral CD4+ T cell population in II- mice, we describe an interesting set of hybridomas derived from this population.

Materials and Methods

Mice. II- mice (1), originally on a mixed C57BL/6J (B6) × 129 background, were mostly from the fifth to eighth generation backcross to B6; invariant chain-deficient mice (II- (5) were on a mixed B6 × 129 background; B cell-deficient mice (6), were bred onto the nonobese diabetic (NOD) genetic background; [32]-microglobulin-deficient mice ([32m- (7) were on a B6 × 129 background. Most mice were maintained in specific pathogen-free conditions at the Centre de Selection d'Animaux de Laboratoire (Orleans, France). DBA/2J, 129, and NOD/Lt mice were bred in our animal facilities. The BALB.Mls- congenic strain carries the Mls- locus (Mtv-7) of DBA/2 mice on the BALB/c genetic background and was obtained thanks to R. Zinkernagel (Institute for Experimental Immunology, Zürich, Switzerland) and R. McDonald (Ludwig Institute for Cancer Research, Epalinges, Switzerland). Mice deficient in the transporter...
associated with antigen processing-1 (TAP-1) mice; mixed B6 × 129 background) were provided by Drs. A. Berns (The Netherlands Cancer Institute, Amsterdam, The Netherlands), H. Ploegh, and H. Van Santen (Massachusetts Institute of Technology, Cambridge, MA). BALB/cBy and C3H/He mice were purchased from IFFA-CREDO (L’Arbresle, France); B10.BR, B10.A(4R), B10.D2, B10.S, B10.M, B10, and BALB.B mice were from Harlan Olac (Oxon, England); and SJL, B6.Tla, and B6 mice were from The Jackson Laboratory (Bar Harbor, ME). The strains derived from wild mice Mus spretus (SEG), Mus spicilegus (ZRU), Mus musculus castaneus (CTA), and Mus macedonicus (XBS) were kind gifts from Dr. F. Bonhomme (Université de Montpellier II, Montpellier, France).

Flow Cytometry. Single cell suspensions were stained with mAbs of the following specificities: KT3 (CD3ε), KT4 (Vβ4), MR9-4 (Vβ5), 44.22.1 (Vβ6), KJ16 (Vβ8.1/8.2), RR3.15 (Vβ11), IM7 (CD44), H57-597 (TCRaβ), for references see (1), MR1-2 (Vβ9) [8], B21.5 (Vβ10) [9], B20.1.1 (Vα2) [10], KT50 (Vα9) [11], M17/4 (LF-A-1α) [12], YN1/1.7.4 (ICAM-1/CD54), MEL-14 (L-selectin/CD62L) [13], 16A (CD45RB) [14], PC61 (CD25) [15], PK136 (NK1.1) [16], and 12-15A-biotin (CD2) [17]. Before cells from newborn and young mice were stained, they were incubated with the 2.4G2 α-FcγR antibody (18) to block nonspecific background staining. Labeled cells were analyzed on FACScan® (Becton Dickinson & Co., Mountain View, CA), ODAM ATC 3000 (Bruker Spectrospin, Wissembourg, Germany), or Coulter Epic Elite (Coulter Corp., Hialeah, FL) flow cytometers, and sorted using the ATC or Elite.

Intrathymic FITC Injections. Intrathymic FITC injections were performed as described (19). Both thymic lobes of 5-wk-old mice were injected with 10 μl of 1 mg/ml FITC in PBS. 24–36 h later, thymus and lymph node cells were prepared and stained with α-CD4 and α-CD8 reagents. The level of FITC label on thymocytes was analyzed to evaluate the efficiency of intrathymic injection, and only successfully injected mice were analyzed further. Events of each sample were collected ungated, and gated for CD4+ and CD8+ cells (at least 50–100 × 10⁶ events per gated population), and analyzed for frequencies of FITC+ cells. Cells from noninjected or intrathorax-injected (19) mice were used as negative controls.

Bromodeoxyuridine (BrdU) Labeling. 0.8 mg BrdU in PBS was injected intraperitoneally into mice every 12 h for 2 d. 12 h after the last injection, mice were killed and lymph node cells were prepared. The cells were fixed first in 70% EtOH, then 1% paraformaldehyde, and stained with CD4-PE and CD8-biotin followed by alkaline phosphatase substrate tablets; Sigma Immunochromics, St. Louis, MO).

Results

A Peripheral Population of CD4+ T Cells Independent of Conventional Class I and II Molecules. In adult IIα mice, 5–15% of the usual number of CD4+ cells can be found in the spleen and lymph node (Fig. 1 A), and 90% of these express the αβ TCR (references 1–3 and data not shown). As previously discussed (1), the simplest explanation for such cells is that they were positively selected on MHC class I molecules. To test this, we crossed the IIα line with mice deficient in class I expression because of a disrupted β2m gene (7). The double mutants had a peripheral population of CD4+ cells similar in numbers and phenotype to that of IIα animals (Fig. 1 and data not shown). Such cells were also present in B cell–deficient/IIα mice (data not shown), ruling out a dependence on molecules expressed on B cells, such as lgs or superantigens, as well as in H2/Ii animals, ar-

CD1-restricted CD4+ T Cells in MHC Class II-deficient Mice
guing against their selection on a nonconventional class II-like molecule (Viville, S., C. Benoist, and D. Mathis, unpublished observation).

Population Dynamics of the CD4⁺ T Cells in II⁻ Mice. To study the ontogeny of this unusual population, we followed the appearance of CD4⁺CD3⁺ cells in the spleen and mesenteric lymph nodes of mutant and control animals from the 1st d after birth. A CD4⁺CD3⁺ population was clearly detectable in the two organs of both types of mice during the 1st wk of life. In both cases, the number of CD4⁺CD3⁺ cells increased ~10-fold during the subsequent 2 wk (quantitated in the spleen; Fig. 2). Once established, the II⁻ CD4⁺ population was maintained without changing size, even in mice >1 yr old (not shown).

We next sought to determine whether the CD4⁺ cells in II⁻ mice originate in the thymus (19). Thymocytes from 5 wk-old mutant and control mice were marked in situ by intrathymic injection of FITC. 30 h later, lymph nodes were analyzed for the presence of thymus-derived, FITC-labeled cells (Fig. 3 A). CD4⁺ and CD8⁺ cells from control animals and CD8⁺ cells from mutants were all labeled to a similar extent. FITC⁺ early thymic emigrants were also readily detectable among lymph node CD4⁺ cells in II⁻ mice, albeit at frequencies slightly lower than that of control CD4⁺ and CD8⁺ cells. The rate of export of CD4⁺ cells from the II⁻ thymus was ~1/50 of the control thymus, allowing an approximation of 1–2 × 10⁴ cells per d (19).

To determine the proportion of cycling cells in the established CD4⁺ population, we exposed control and mutant mice to BrdU continuously for 48 h. As for the control II⁺ (MHC class II-positive) mice, only a small proportion of lymph node CD4⁺ cells in II⁻ mice had incorporated BrdU during the labeling period (5–11%; see Fig. 3 B), indicating that only a minor fraction was actively dividing.

Altogether, these data indicate a stable population of CD4⁺ T cells in II⁻ mice. Its thymic input rate and proliferative turnover rate are not markedly different from those exhibited by the CD4⁺ T cells of wild-type animals.

Cell Surface Marker Profiles. The initial description of CD4⁺ T cells in II⁻ mice indicated atypical cell surface antigen profiles: reduced levels of CD3/TCR and high levels of CD44. We extended this phenotypic analysis by using several additional antibodies that detect T cell differentiation and activation markers (Fig. 4).

The cells expressed normal levels of the T cell markers CD2 and CD28 (Fig. 4), as well as CD5 and Thy-1 (not shown). In contrast to control CD4⁺ T cells, 75–80% of the CD4⁺ cells from mutant mice had a phenotype characteristic of memory cells (for review, see reference 28): CD45RBlow, CD62L⁻ and increased levels of CD54 (ICAM-1) and CD11a (LFA-1). Nevertheless, the majority of cells were in a resting state as evidenced by generally low

![Figure 1](image1.png)  ![Figure 2](image2.png)

**Figure 1.** Peripheral CD4⁺ T cells in MHC class II-deficient mice were not dependent on class I molecules. Lymph node cells from wild-type, class II-deficient (II⁻), and B2m/class II double-deficient (II⁻) littermate mice were stained for the CD4 and CD8 markers. Dot plots show typical distributions in several animals analyzed. The numbers show the percentage of CD4⁺ cells in the indicated gates. The total number of cells in spleen or lymph nodes of the two mutants did not vary significantly from the wild type.

**Figure 2.** Early appearance of CD4⁺CD3⁺ cells in the spleen of II⁻ and control II⁺ mice. On the indicated days, spleen cells were prepared from mixed litters of II⁻ and wild-type heterozygote control mice, and they were analyzed for CD4, CD8, and CD3 expression. Each dot represents pooled cells or the mean value from at least three litters of II⁻ or II⁺ phenotype.
CD69 and CD25 expression. The fraction of CD69-expressing CD4+ cells in II° mice was somewhat elevated (20–30%, compared with 5–10% in II+ CD4+ cells), but still represented only a minority of the population, consistent with the cell cycle analysis mentioned above.

An unusual population of thymocytes and peripheral T lymphocytes has recently been described. These cells are positive for the NK1.1 marker (29, 30), their selection is 132m dependent (31-34), and they preferentially express certain Vα and Vβ segments (29, 30, 35-37). The frequency of CD4+ cells expressing the NK1.1 marker was higher in II° than II+ mice: 15–25% of the CD4+ cells in spleen or lymph node in II° mice were positive, while only 2–6% of control CD4+ cells were. However, given that II° mice have 10–20-fold fewer CD4+ cells than II+ mice, the absolute number of NK1.1+ CD4+ cells in both animals was similar, a finding in agreement with previous results (32–34). We conclude, therefore, that the NK1.1+ population is only a subset of the CD4+ cells in II° mice. This point is further substantiated by the broad TCR repertoire of CD4+ cells in II° mice (see below).

Functional Potential. The next priority was to establish to what extent the CD4+ T cells from II° mice function like normal CD4 T cells. Purified CD4+ cells from both mutant and control animals proliferated vigorously in response to plate-bound anti-CD3 (Fig. 5 A) or staphylococcal enterotoxin B in the presence of splenic APCs (not shown), the II° CD4+ cells often slightly better. Anti-CD3 stimulation also led to upregulation of the early activation markers CD69 and CD25 on both mutant and control CD4+ cells (not shown).

Stimulation with anti-CD3 induced the CD4+ cells from

Figure 4. CD4+ T cells in class II° animals display the phenotype of resting memory cells. Lymph node cells from adult II+ and II° mice were stained for CD4 and CD8 in combination with various cell surface markers as detailed in Materials and Methods. Histograms, representative of several experiments, show the expression of the indicated markers on gated CD4+ cells from both types of mice. A typical negative control is overlayed in the first panel on the left.
II\(^o\) mice to produce similar amounts of IL-2 (Fig. 5 B), and more IL-3 (not shown), IL-4, and IFN-γ (Fig. 5 C) than CD4\(^+\) cells from control mice. The main difference concerning IL-4, quite abundant in mutant CD4\(^+\) cells but very low to undetectable in wild-type cells, as expected for naïve cells. Mutant-derived CD4\(^+\) cells secreted cytokines already at 4 h; in both cases, the levels of IL-4 and IFN-γ in the supernatant increased until day 2 (Fig. 5 C). Strikingly, the IL-4/IFN-γ ratio produced by the CD4\(^+\) cells derived from II\(^o\) animals was several-fold higher than that of control CD4\(^+\) cells.

Furthermore, when Th activity of II\(^o\) and control CD4\(^+\) cells was compared in a nonspecific B cell help assay in vitro, purified CD4\(^+\) cells from mutant and control mice induced B cell proliferation to similar levels (not shown).

Together, these findings argue that the CD4\(^+\) T cells in II\(^o\) mice are functionally similar to control CD4\(^+\) cells but they secrete different ratios of lymphokines when activated.

**TCR Repertoire.** The TCR repertoire displayed by CD4\(^+\) cells from II\(^o\) mice was first investigated by quantitating expression of various VBs and V\(\alpha\)s on lymph node cells. Our initial studies (1), using conventionally housed mice at earlier stages of the backcross to B6, had shown some variability in VB usage between individual II\(^o\) mice. This variability was not confirmed in the present study (Table 1). CD4\(^+\) cells in II\(^o\) mice made broad use of VB segments, exhibiting an overall pattern comparable with that of CD4\(^+\) cells in control littermates. Some differences did emerge, however. VB5 and VB9 are much rarer in CD4\(^+\) than CD8\(^+\) cells of normal mice (Table 1 and reference 38); these VBs were found in greater proportions in CD4\(^+\) cells of II\(^o\) animals. Conversely VB4 is overrepresented in CD4\(^+\) vs CD8\(^+\) cells of normal mice (Table 1 and reference 39), and was used at a low frequency in CD4\(^+\) cells from II\(^o\) animals. There are also a few peculiarities specific

**Figure 5.** Responsiveness of purified CD4\(^+\) cells from class II\(^o\) and control mice. Sorted CD4\(^+\) cells were activated with plate-bound anti-CD3. (A) Proliferation. Incorporation of [\(\text{H}\)]Tdr was measured on days 2–3. (B) IL-2 production. After 24 h of culture, supernatants were harvested and IL-2 production was estimated. IL-2 production is presented as [\(\text{H}\)]Tdr incorporation of the CTLL indicator cells. (C) Kinetics of IL-4 and IFN-\(\gamma\) production. After 4, 18, and 53 h of stimulation, supernatants were harvested and analyzed for IL-4 and IFN-\(\gamma\) content by ELISA. Relative amounts of cytokine are displayed as the read OD value for undiluted supernatant.

### Table 1. TCR V Region Usage in II\(^o\) and Control Lymph Node T Cells

| VB4 | VB5 | VB6 | VB8 | VB9 | VB10 | VB11 | V\(\alpha\)2 | V\(\alpha\)8 |
|-----|-----|-----|-----|-----|------|------|-------------|-------------|
| CD4 |     |     |     |     |      |      |             |             |
| II\(^+\) | 7.3 | 6.7 | 2.3 | 2.8 | 8.4  | 8.8  | 18.2 16.8   | 0.8 1.1     |
|     | 7.0 | 6.9 | 3.2 | --- | 8.5  | 8.1  | 17.6 17.2   | 0.8 ---     |
| II\(^o\) | 2.2 | 2.7 | 7.0 | 8.9 | 5.8  | 6.1  | 19.9 17.6   | 2.1 3.2     |
|     | 2.6 | 4.2 | 6.1 | --- | 5.3  | 7.1  | 20.8 21.2   | 2.3 ---     |
| CD8 |     |     |     |     |      |      |             |             |
| II\(^+\) | 3.8 | 3.4 | 14.7| 14.2| 6.7  | 7.6  | 16.8 16.8   | 2.7 2.3     |
|     | 3.6 | 3.6 | 15.9| --- | 7.1  | 6.7  | 16.4 16.0   | 2.4 ---     |
| II\(^o\) | 4.3 | 4.3 | 17.8| 17.2| 8.3  | 8.2  | 15.7 15.8   | 1.9 2.1     |
|     | 4.3 | 4.7 | 17.9| --- | 8.6  | 9.2  | 16.5 16.1   | 1.9 ---     |

Lymph node cells from II\(^o\) and II\(^+\) littermate control mice were stained and analyzed by flow cytometry for expression of the indicated TCR V segments. The percentage of positive cells within gated CD4\(^+\) and CD8\(^+\) populations of individual II\(^+\) and II\(^o\) animals are shown. ---, not done. Values in bold indicate ≥2.5-fold difference in V segment representation (mean value) between II\(^+\) and II\(^o\) CD4\(^+\) cells.
to the II\(^{a}\) CD4\(^{+}\) population, notably the low V\(\alpha 2\) usage. Overall, the pattern of V segment usage by CD4\(^{+}\) cells from mutant mice was subtly different from that exhibited by conventional MHC class I- or class II-restricted cells.

Additional evidence that the II\(^{a}\) CD4\(^{+}\) cells display a broad repertoire was obtained by sequencing VDJ regions from TCR-\(\beta\) chains (Bogue, M., C. Benoist, and D. Mathis, unpublished results). VB8.2 chains showed a high diversity in the VDJ joining region, displaying the usual spectrum of N nucleotides, J region usage, distribution of CDR3 lengths, and amino acid composition in the CDR3 loop.

**High Frequency of Autoreactive Specificities among Hybridomas from II\(^{a}\) Mice.** Since the CD4\(^{+}\) cells from II\(^{a}\) mice represent a diverse population of potentially functional cells, it was of interest to identify their restricting (and possibly selecting) elements. We tried to derive allo- or autoreactive hybridomas on the grounds that such reactivities might give us a clue to their natural restricting elements, much as MHC class II-directed allo- and autoreactive cells from normal mice reflect the class II restriction of the bulk of the CD4\(^{+}\) cells. To generate a panel of hybridomas, we purified CD4\(^{+}\) T cells from several II\(^{a}\) and II\(^{b}\) littersmates, stimulated them with anti-CD3, and fused them with TCR-\(\alpha-\beta\)-BW5147 cells. We then challenged the hybridomas with splenocytes from several mouse strains, differing at MHC, minor histocompatibility, and/or endogenous murine tumor virus loci.

As indicated in Fig. 6, very few of the hybridomas from II\(^{a}\) littersmates responded to any spleen cell stimuli, save for a scattered few whose reactivities correlated with H-2 haplotype (e.g., XII44 and XIV18, reactive to H-2\(^{b}\)) or with endogenous murine tumor virus genes (e.g., XII49 and XIV50, Mls\(^{a}\)); only one (X17) was autoreactive (A\(^{b}\)-reactive). The reactivity pattern of the hybridomas derived from II\(^{a}\) mice was very different: while there was no activation when they were cultured alone, >10% responded to II\(^{a}\) APCs. This suggested a nonpolymorphic class I-like molecule is recognized by the autoreactive hybridomas from II\(^{a}\) mice.

**Recognition of Class I-like Ligands by the Autoreactive Hybridomas.** To investigate the nature of the nonconventional ligand(s) recognized by the polyreactive hybridomas derived from mutant mice, we selected 11 for a further analysis. Although a population of CD4\(^{+}\) T cells was present in \(\beta 2m^{a}/\beta 2m^{b}\) double-mutant animals, this did not entirely rule out peripheral reactivity to MHC molecules, and the nonpolymorphic nature of the ligands was reminiscent of nonclassical MHC molecules. We therefore considered the possibility that such molecules, either class II- or class I-like, might serve as ligands. Their expression might well depend, for the former, on the II involved in trafficking of conventional MHC class II molecules, or for the latter, on \(\beta 2m\). We thus tested the panel of hybridomas with spleen cells from mutant II\(^{a}\) or \(\beta 2m^{a}\) mice (5, 7). All of the hybridomas were well stimulated by II-deficient APCs (not shown), while II\(^{a}\) APCs were not recognized by control A\(^{b}\)-reactive hybridomas. On the other hand, 6 of the 11 hybridomas did not respond to splenocytes from \(\beta 2m^{a}\) mice (Fig. 7). This encouraged us to pursue the idea that nonpolymorphic class I-like molecules are recognized by the autoreactive hybridomas from II\(^{a}\) mice.

**Qa-1 Recognized by One of the Autoreactive Hybridomas.** The reactivity of the \(\beta 2m\)-dependent hybridoma VIII111 appeared to correlate with the expression of the b allele of the class Ib molecule Qa-1 (Fischer-Lindahl, K., personal communication). For example, as shown in Fig. 6, splenocytes from the strains C3H/He (H-2\(^{b}\)) and B10.S (H-2\(^{b}\)) both express Qa-1\(^{b}\) and elicited a response, while those from B10.BR (H-2\(^{b}\)) and SJL (H-2\(^{b}\)) show Qa-1\(^{a}\) and did not. This correlation was extended by using a pair of congenic strains. B6 mice express the b allele of Qa-1, while B6.Tla\(^{a}\) animals are congenic for the T region, including the Qa-1 encoding T23 gene, and carry the Qa-1\(^{a}\) allele. B6 but not B6.Tla\(^{a}\) spleen cells were well recognized by the VIII111.1 hybridoma (not shown). Finally, we directly tested whether Qa-1 was being recognized by stimulating the VIII111.1 hybridoma with L cells transfected with the T23\(^{b}\) gene (24). The hybridoma responded specifically to transfectants expressing Qa-1\(^{b}\), but not to mock transfected cells nor to cells transfected with other Ib genes (Fig. 8).

**CD1 Recognized by the Majority of the Autoreactive Hybridomas.** For the other autoreactive hybridomas, the reactivities depicted in Fig. 6 were too broad to give much clue as to the targets involved. We could exclude the class Ib molecule Qa-2, as the stimulating ligand(s), was found to be present on splenocytes of B10.M mice (not shown) that carry a deletion encompassing the genes encoding surface-bound Qa-2 (40). Therefore, we tested the panel against a set of transfectants expressing other class Ib molecules: the T region-encoded antigens TL (26), T22, and T10 (25), and a molecule encoded outside the MHC, CD1 (Teitell, M., et al., manuscript submitted for publication). Both the \(\beta 2m\)-dependent and -independent hybridomas were included in the screening because there is at least one example of a class Ib molecule that shows variable dependence on \(\beta 2m\) for surface expression (41). The results are presented in Fig. 8. Strikingly, seven hybridomas, including two \(\beta 2m\)-independent, specifically recognized transfectants expressing CD1.1; these reactivities could be detected with CD1.1-transfected RMA or L cells (not shown).

**TAP-independent Trafficking of the Target Ligands.** Peptide loading and surface expression of classical MHC class I molecules are generally dependent on the TAP molecule (see reference 42 and references therein), but this is not always the case for nonclassical class I molecules (43-45). To determine whether the ligands recognized by our unusual CD4\(^{+}\) T hybridomas depend on the TAP gene for expression, we stimulated the panel with spleen APC from mice carrying a debilitating mutation in the TAP-1 gene (42). While the Qa-1 ligand seen by the VIII1111 hybridoma required TAP to be expressed, none of the ligands recog-
nized by the CD1-reactive hybridomas were TAP dependent (representative examples are shown in Fig. 9). This was true whether or not their expression was dependent on β2m.

**Heterogeneity of the CD1-reactive Hybridomas.** Given the high frequency of CD1-reactive clones among the autoreactive CD4⁺ cells in II° mice, two questions arose. (a) Did these cells display a monomorphic specificity, perhaps accompanied by a constrained TCR repertoire? (b) Is the CD1 target recognized by itself or in conjunction with other molecules: peptides such as classical MHC molecules, or lipids as recently reported (46)?

**Figure 6.** Allostimulation of CD4⁺ hybridomas derived from II⁺ and II° mice. Hybridomas were challenged with splenic APC from the indicated mouse strains. After 15–20 h of culture, supernatants were harvested and release of IL-2 was evaluated. Anti-CD3 stimulation was used as positive control for hybridoma function (not shown). Black boxes indicate positive reaction, - indicates a negative reaction, and empty boxes were not done. None of the hybridomas secreted IL-2 in the absence of APC. The presented results are a compilation of several experiments. Correlating loci are indicated on the right. The Aβ specificity of VII75 and X17 has been confirmed by blocking their reactivities with specific mAbs, while the Mls-1 reactivity of X17 is caused by VB6 expression (not shown).
The heterogeneity of the hybridomas was evaluated by a number of criteria, as summarized in Fig. 10. TCR V region sequencing showed diverse usage of VB and Vα regions, with variable CDR3 lengths and amino acid compositions (not shown). It may be worth emphasizing that none of our CD1-reactive hybridomas expressed the invariant VB14-Jβ15 sequence characteristic of NK1.1 CD4+ cells (37), as determined by reverse transcription PCR with VB14-specific primers (not shown).

Heterogeneity was more evident in the responses to stimulation by APCs from different mutant or inbred mouse strains. As mentioned above (Fig. 7), two of the seven CD1-reactive hybridomas were capable of responding to APCs from β2m deficiency. The mMT knockout mutation, which abrogates B cell development (6), prevented splenic APCs from stimulating two other hybridomas, indicating that B cells are necessary presenting cells in these instances. As already indicated in Fig. 6, most of the hybridomas responded to all of the nonmutant common laboratory strains. Clone VIII82, however, reacted very poorly to APCs from BALB/c mice, even though B6 and BALB/c mice have essentially identical CD1.1 molecules and CD1.2 molecules that differ only by one amino acid (47, 48). Finally, we tested the hybridomas against splenic APCs from various subspecies of Mus (Fig. 10). Here again, the responses led to splits. These results clearly indicate that the CD1-specific hybridomas are heterogeneous and do not recognize one simple ligand.

Furthermore, the two seemed to have comparable functional potentials: they were stimulated to about the same degree with polyclonal reagents, secreted lymphokines, and they proliferated upon activation and provided "help" to B cells. Finally, both populations displayed a diverse repertoire of TCRs.

Nevertheless, the CD4+ population from II° mice displayed some special features. These cells localized preferentially to the B rather than T cell areas in the peripheral lymphoid organs (1). Perhaps, most strikingly, the majority of them expressed cell surface markers reminiscent of resting memory cells, while in normal mice, only 10–20% of the CD4+ T lymphocytes show this phenotype. Also atypical was their capacity to produce high amounts of lymphokines, particularly IL-4, upon activation. Lastly, although the II° CD4+ cells, like their II+ counterparts, displayed a diverse TCR repertoire, this repertoire did exhibit some particularities, notably altered levels of certain VBs and a dearth of Vα2+ receptors.

Altogether, one is left with the impression of a population sharing many features with the normal CD4+ T lymphocyte population, yet not identical to it. We do not formally know whether these cells are a peculiarity of II° mice. They have not so far been identified in normal mice but could just be masked by the bulk CD4+ T cell population.

The CD4+ cells from II° mice were also distinct from the unusual population of NK1.1+ mature CD4+8- and CD4+8- thymocytes and peripheral T lymphocytes discovered several years ago (35, 36). An enriched proportion of these cells displays VB8+ TCRs, and almost all of them exhibit an invariant TCR-α chain, VB14-J15 (29, 30, 35–37). The majority shows a cell surface phenotype similar to memory cells, particularly high levels of CD44+ expression (29). NK1.1+ lymphocytes are the major lymphokine-producing cells in the thymus (49–51), and peripheral NK1.1+ cells have recently been recognized as major producers of
IL-4 (52). The NK1.1+ cells do not depend on MHC class II molecules for selection/expansion, but rather on expression of β2m (or MHC class I molecules) on bone marrow-derived cells (31–34). The CD4+ population from II° mice does contain a component of NK1.1+ cells; however, this is relatively minor, ~20–30%, with no enrichment in numbers. The bulk of the population has some features like NK1.1+ cells, notably a memory phenotype and high production of lymphokines upon stimulation, but the differences between the two remain evident: a diverse vs restricted TCR repertoire and independence of β dependence on expression of β2m. It is possible that the NK1.1+ CD4+ cells are a peculiar subset of the larger CD4+ population we have defined in II° animals, perhaps expanded in response to a specific stimulus.

Recognition of MHC Class Ib Molecules. To study the reactivities of the CD4+ lymphocytes from II° mice, we turned to an analysis at the clonal level. Initially, we attempted to derive antigen-specific hybridomas (not shown).

II° mice were immunized with various proteins in complete Freund's adjuvant and sets of hybridomas derived from purified CD4+ cells reactivated in vitro. The hybridomas were challenged with the protein antigen and with bacterial antigens such as heat shock protein 60 and purified protein derivative from mycobacteria, and preparations of heat-killed mycobacteria and listeria. No response to any of these challenges was elicited from the hybridomas. We

Figure 8. Activation by CD1 of most of the autoreactive hybridomas. Hybridomas were stimulated with transfected cell lines expressing the indicated class Ib molecules and the respective untransfected controls. The stimulation obtained with B6 splenocytes was used as a reference value, and the responses to the transfected cells are expressed as percentages of the response to B6 APC. The background value (−) obtained with the non-transfected control APC is shown.

Figure 9. Differential TAP dependence of the class Ib ligands. Hybridoma cells were stimulated with splenic APC from TAP-1− and TAP-1+ littermate mice. Splenic APCs were added as described in Fig. 7. While the Qa-1 recognized by VII11 was dependent on the presence of TAP-1 in the APCs, all CD1-reactive hybridomas were TAP independent; only a representative three are shown here.

Figure 10. Heterogeneity of the CD1-specific hybridomas. Results were compiled from several sets of experiments. Expression of TCR V segments was determined by sequencing of the TCR-α and -β chains and/or staining. For two hybridomas, two in-frame α chain sequences were found. A second TCR-α sequence was found for VII49 with an out-of-frame rearrangement. Surface expression of the V segment (β chain for VII24 and VII82, β chain for VII19, VII68, and VII57) was verified by staining with V region-specific antibodies. VB9 expression of VII49 was determined by staining only. The VII57 Vα sequence is so far undescribed, but was tentatively assigned Vα since it had the highest homology (~80%) to members of this Vα family. The CD1-specific hybridomas were stimulated with splenic APCs from β2m−/− B cell-deficient mice (μmt−), wild-type BALB/c mice, or several subspecies of Mus. Black boxes, positive reactions; empty boxes, no reaction; nd, not determined.
altered our strategy, therefore, this time preparing a large number of hybridomas after in vitro anti-CD3 activation of purified CD4+ cells from IIα and II+ mice, and challenging them with splenocytes from a panel of mouse strains. We could identify hybridomas derived from both types of mice that exhibited anti-MHC or anti-Mls reactivities. Only in the case of IIα mice, however, did we observe a significant percentage of hybridomas that were both auto- and polyreactive.

Focusing on II with this unusual pattern of reactivity, we explored their specificities using splenocytes from congenic and mutant mouse strains and cells transfected with MHC class I molecules. Many of the hybridomas reacted specifically to class Ib molecules: one recognized Qa-1, seven saw CD1, and three recognized unidentified ligands, all expressed independently of β2m.

One is led to ask whether reactivity to nonclassical class I molecules reflects the restricting specificity of the majority of the CD4+ cells in IIα mice, or is just a feature of a minor component of the population. Clearly, a large percentage of the autoreactive hybridomas recognized class Ib molecules. But most of them did not show reactivity to any of the APCs with which they were challenged. It is certainly possible that most of the CD4+ cells in IIα mice are restricted by class Ib molecules, including CD1, but that they react to these molecules in forms that are not present on splenocytes, e.g., in association with different ligands like peptides or lipids. The autoreactive clones could just represent the higher affinity fraction of a population of Ib-restricted cells, much like rare class II-autoreactive cells in wild-type mice betray the large class II-restricted population.

CD1 Recognition. Several recent findings have provoked interest in the class Ib molecule CD1. It is the only antigen-presenting Ib molecule known to have been conserved through evolution, and the homology between species is high (53). Interestingly, CD1 exhibits features similar to both class I and class II MHC molecules. On the one hand, the intron/exon structure of the CD1 gene and the corresponding domain structure of the protein are like those of standard class I genes and molecules, and there is sequence homology between the α3 domain of CD1 and the same domain of classical class I molecules (54). On the other hand, the α3 domain of CD1 has a similar degree of sequence homology with the β2 domain of class II molecules (54), and CD1's cell-type distribution (41, 53, 55) and antigen presentation pathway appear more characteristic of class II molecules (44). While murine CD1-restricted T cells have not yet been described, several human T cell clones recognizing CD1 have been isolated (44, 56-59); they are CD4−CD8+ cells expressing either an αβ or γδ TCR. In one case, the presented antigen was of a novel type, mycolic acid, a unique lipid component of the mycobacterial cell wall (46). This observation reopens the question of whether CD1 can bind peptides at all, a question previously raised because sequence information suggested a very different structure in the ligand-binding region (60) and because surface expression of CD1 appeared independent of TAP molecules (44, 45). Indeed, TAP independence of murine CD1 was confirmed, at the functional level in our assay, and it has recently been demonstrated in transfectants (Teitell, M., et al., manuscript submitted for publication).

Our findings add several new features to the emerging portrait of this intriguing class Ib molecule. First of all, we found that CD4+ T cells can also show CD1 reactivity. This may indicate that CD4 can serve as a coreceptor for CD1 recognition by direct CD1−CD4 contact. It has been demonstrated that CD8 can bind CD1 (Teitell, M., et al., manuscript submitted for publication), but the CD1 α3 domain similarity to both class I and II molecules may allow promiscuous binding to either coreceptor molecule. Secondly, we observed significant heterogeneity in the reactivity patterns and TCR usages of the different CD1-reactive hybridomas. This suggests that the target ligand may be composed of more than just the CD1 molecule itself, perhaps including peptides, lipids (46), or other posttranslational modifications. It seems unlikely, though, that a lipid moiety would be recognized by our hybridomas because the polymorphism we detect between very related Mus subspecies would be difficult to account for in this way. Finally, since the CD1-reactive hybridomas derive from the small CD4+ population in IIα mice, we have the means to dissect their function in vivo. Already, we know that they do not mount a response to several multiepitope protein antigens and are incapable of providing full B cell help, evidenced by a total lack of germinal centers in IIα mice, no matter how or how much one stimulates them (1). We are left with several pressing questions: How are these autoreactive cells rendered and maintained innocuous in mice? What agents are capable of provoking them? What is the functional correlate of their impressive capacity to produce IL-4? What cells and molecules are responsible for their selection/expansion?

We thank A. Orth, H. Ploegh, H. Van Santen, A. Berns, and K. Rajewsky for generously providing mice; S. Tourne for sharing mice, the staff of the CSAL/CNRS for maintaining the specific pathogen-free colony; M. Bogue and S. Viville for sharing unpublished results; M. Kosco-Vilbois for help with B helper assays; R. Ceredig, T. Shirai, K. Hafn, A. Livingstone, and J. Langhome for antibodies; S. Tonegawa, M. Cochet, L. Van Kaer, and M. Testell for cell lines; Kirsten Fischer-Lindahl and E. Jouvin-Marche for sharing information, and S. Kauffman for bacterial antigens.
This work was supported by funds from the INSERM, the CNRS, the Centre Hospitalier Universitaire Régional, and a grant from the Human Frontier Science Program Organization to D. Mathis and C. Benoist, and a European Molecular Biology Organization fellowship and a grant from the Swedish Natural Science Research Council to S. Cardell.

Address correspondence to Diane Mathis, INSERM-CNRS-ULP, BP 163, 67404 Illkirch, Cedex, CU de Strasbourg, France.

Received for publication 17 February 1993 and in revised form 27 April 1995.

References

1. Cosgrove, D., D. Gray, A. Dierich, J. Kaufman, M. Lemeur, C. Benoist, and D. Mathis. 1991. Mice lacking MHC class II molecules. Cell. 66:1051–1066.
2. Grusby, M.J., R.S. Johnson, V.E. Papaioannou, and L.H. Glimcher. 1991. Depletion of CD4+ T cells in major histocompatibility complex class II-deficient mice. Science (Wash. DC). 253:1417–1420.
3. Köntgen, F., G. Süs, C. Stewart, M. Steinmetz, and H. Bluethmann. 1993. Targeted disruption of the MHC class II Aa gene in C57BL/6 mice. Int. Immunol. 5:957–964.
4. Möller, G. 1993. Positive T-cell selection in the thymus. Immunol. Rev. 135:1–279.
5. Viville, S., J. Neefjes, V. Lotteau, A. Dierich, M. Lemeur, H. Ploegh, C. Benoist, and D. Mathis. 1993. Mice lacking the MHC class II-associated invariant chain. Cell. 72:635–648.
6. Kitamura, D., J. Roes, R. Kühn, and K. Rajewsky. 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin µ chain gene. Nature (Lond.). 350:423–426.
7. Koller, B.H., P. Marrack, J.W. Kappler, and O. Smithies. 1990. Normal development of mice deficient in β2M, MHC class I proteins, and CD8+ T cells. Science (Wash. DC). 248:1227–1229.
8. Utsunomiya, Y., H. Kosaka, and O. Kanagawa. 1991. Differential reactivity of VB9 T cells to minor lymphocyte stimulating antigen in vitro and in vivo. Eur. J. Immunol. 21:1007–1011.
9. Necker, A., N. Rebai, M. Matthes, E. Jouvin-Marche, P. Cazenave, P. Swannworawong, E. Palmer, H.R. MacDonald, and B. Malissen. 1991. Monoclonal antibodies raised against engineered soluble mouse T cell receptors and specific for Vβ8-, Vβ2- or Vβ10-bearing T cells. Eur. J. Immunol. 21:3035–3040.
10. Gregoire, C., N. Rebai, F. Schweiguth, A. Necker, G. Mazza, N. Auphan, A. Millward, A-M. Schmitt-Verhulst, and B. Malissen. 1991. Engineered secreted T-cell epitope αβ heterodimers. Proc. Natl. Acad. Sci. USA. 88:8077–8081.
11. Tomonari, K., E. Lovering, S. Fairchild, and S. Spencer. 1989. Two monoclonal antibodies specific for the T cell receptor Vα8. Eur. J. Immunol. 19:1131–1135.
12. Sanchez-Madrid, F., P. Simon, S. Thompson, and T.A. Springer. 1983. Mapping of antigenic and functional epitopes on the α and β subunits of two related mouse glycoproteins involved in cell interactions, LFA-1 and MAC-1. J. Exp. Med. 158:586–602.
13. Gallatin, W.M., I.L. Weissman, and E.C. Butcher. 1983. A cell-surface molecule involved in organ-specific homing of lymphocytes. Nature (Lond.). 304:30–33.
14. Bottomly, K., M. Luqman, L. Greenbaum, S. Carding, J. West, T. Pasqualini, and D.B. Murphy. 1989. A monoclonal antibody to murine CD45R distinguishes CD4 T cell populations that produce different cytokines. Eur. J. Immunol. 19:617–623.
15. Zubler, R.H., J.W. Lowenthal, F. Erard, N. Hashimoto, R. Devos, and H.R. MacDonald. 1984. Activated B cells express receptors for, and proliferate in response to, pure interleukin 2. J. Exp. Med. 160:1170–1183.
16. Koo, G.C., and J.R. Peppard. 1984. Establishment of monoclonal anti-NK-1.1 antibody. Hybridoma. 3:301–303.
17. Guckel, B., C. Berek, M. Lutz, P. Altevogt, V. Schirrmacher, and B.A. Kyewski. 1991. Anti-CD2 antibodies induce T cell unresponsiveness in vivo. J. Exp. Med. 174:957–967.
18. Unkeless, J.C. 1979. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. J. Exp. Med 150:580–587.
19. Scolay, R., E. Butcher, and I. Weissman. 1980. Thymus cell migration: quantitative studies on the rate of migration of cells from the thymus to the periphery in mice. Eur. J. Immunol. 10:210–215.
20. Tough, D.F., and J. Sprent. 1994. Turnover of naive and memory phenotype T cells. J. Exp. Med. 179:1127–1135.
21. Carayon, P., and A. Bord. 1992. Identification of DNA-replicating lymphocyte subtypes using a new method to label the bromo-deoxyuridine incorporated into the DNA. J. Immunol. Methods. 147:225–230.
22. White, J., M. Blackman, J. Bill, J. Kappler, P. Marrack, D.P. Gold, and W. Born. 1989. Two better cell lines for making hybridomas expressing specific T cell receptors. J. Immunol. 143:1822–1825.
23. Kruisbeek, A.M. 1991. Production of mouse T cell hybridomas. In Current Protocols in Immunology. J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, and W. Strober, editors. John Wiley & Sons, Inc., New York. pp. 14.1–14.11.
24. Cochet, M., A. Casrouge, A.-M. Dumont, C. Transy, F. Baleux, W.L. Maloy, J.E. Coligan, P. Cazenave, and P. Kourilsky. 1989. A new cell surface molecule closely related to mouse class I transplantation antigens. Eur. J. Immunol. 19:1927–1931.
25. Ito, K., L. Van Kaer, M. Bonneville, S. Hsu, D.B. Murphy, and S. Tonegawa. 1990. Recognition of the product of a novel MHC TL region gene (27b) by a mouse γδ T cell receptor. Cell. 62:549–561.
26. Holcombe, H.R., A.R. Castano, H. Cheronutre, M. Teitell, J.K. Maher, P.A. Peterson, and M. Kronenberg. 1995. Nonclassical behavior of the thymus leukemia antigen: peptide transporter independent expression of a nonclassical class I molecule. J. Exp. Med. 181:1433–1443.
27. Candela, S., J. Katz, C. Benoist, D. Mathis, and K. Haskins. 1991. Islet-specific T-cell clones from nonobese diabetic mice...
express heterogeneous T-cell receptors. Proc. Natl. Acad. Sci. USA. 88:6167–6170.

28. Bradley, L.M., M. Croft, and S.L. Swain. 1993. T-cell memory: new perspectives. Immunol. Today. 14:197–199.

29. Ballas, Z.K., and W. Rasmussen. 1990. NK1.1+ thymocytes. Adult murine CD4+ CD8+ thymocytes contain an NK1.1+, CD3+, CD5+, CD44hi, TCR-Vβ8+ subset. J. Immunol. 145: 1039–1045.

30. Levitsky, H.I., P.T. Golumbek, and D.M. Pardoll. 1991. The fate of CD4+8+ T cell receptor-αβ+ thymocytes. J. Immunol. 146:1113–1117.

31. Bix, M., M. Coles, and D. Raulet. 1993. Positive selection of Vβ8+CD8+ thymocytes by class I molecules expressed by hematopoietic cells. J. Exp. Med. 178:901–908.

32. Bendelac, A., N. Killeen, D.R. Litman, and R.H. Schwartz. 1994. A subset of CD4+ thymocytes selected by MHC class I molecules. Science (Wash. DC). 263:1774–1778.

33. Coles, M.C., and D.H. Raulet. 1994. Class I dependence of the development of CD4+CD8–NK1.1+ thymocytes. J. Exp. Med. 180:395–399.

34. Ohteki, T., and H.R. MacDonald. 1994. Major histocompatibility complex class I-related molecules control the development of CD4+8+ and CD4–8– subsets of natural killer 1.1+ T cell receptor-αβ+ cells in the liver of mice. J. Exp. Med. 180:699–704.

35. Fowlkes, B.J., A.M. Kruisbeek, H. Ton-That, M.A. Weston, J.E. Coligan, R.H. Schwartz, and D.M. Pardoll. 1987. A novel population of T-cell receptor αβ-bearing thymocytes which predominantly expresses a single Vβ gene family. Nature (Lond.). 329:251–254.

36. Rudd, R.C., G.C. Miescher, R.C. Howe, R.K. Lees, C. Bron, and H.R. MacDonald. 1987. Developmentally regulated expression of T cell receptor β chain variable domains in immature thymocytes. J. Exp. Med. 166:577–582.

37. Lantz, O., and A. Bendelac. 1994. An invariant T cell receptor α chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4–8– T cells in mice and humans. J. Exp. Med. 180:1097–1106.

38. Bill, J., O. Kanagawa, J. Linten, Y. Utsunomiya, and E. Palmer. 1990. Class I and class II MHC Gene products differ in mice and humans. J. Mol. Cell. Immunol. 4:269–280.

39. Tomonari, K., E. Lovering, and S. Spencer. 1990. Correlation between the Vβ4+CD4+ T cell population and the H-2d haplotype. Immunogenetics. 31:333–339.

40. O'Neil, A.E., K. Reid, J.C. Garberi, M. Karl, and L. Flaherty. 1986. Extensive deletions in the Q region of the mouse major histocompatibility complex. Immunogenetics. 24:368–373.

41. Balk, S.P., S. Burke, J.E. Polischuk, M.E. Frantz, L. Yang, S. Porcelli, S.P. Colgan, and R.S. Blumberg. 1994. β2-Microglobulin-independent-MHC class Ib molecule expressed by human intestinal epithelium. Science (Wash. DC). 265:259–262.

42. van Kaer, L., P.G. Ashton-Rickardt, H.L. Ploegh, and S. Tonegawa. 1992. TAP1 mutant mice are deficient in antigen presentation, surface class I molecules, and CD4–8– T cells. Cell. 71:1205–1214.

43. Aldrich, C.J., R. Waltrip, E. Hermal, M. Ataya, K. Fischer Lindahl, J.J. Monaco, and J. Forman. 1992. T cell recognition of Qa1b antigens on cells lacking a function TAP-2 transporter. J. Immunol. 149:3773–3777.

44. Porcelli, S., C.T. Morita, and M.B. Brenner. 1992. CD1b restricts the response of human CD4+8– T lymphocytes to a microbial antigen. Nature (Lond.). 360:593–597.

45. de la Salle, H., D. Hanau, D. Fricker, A. Urracher, A. Kelly, J. Salamero, S.H. Powis, L. Donato, H. Baustinger, M. Lafontet, et al. 1994. Homozygous human TAP peptide transporter mutation in HLA class I deficiency. Science (Wash. DC). 265:237–241.

46. Beckman, E.M., S.A. Porcelli, C.T. Morita, S.M. Behar, S.T. Furlong, and M.B. Brenner. 1994. Recognition of a lipid antigen by CD1-restricted αβ+ T cells. Nature (Lond.). 372:691–694.

47. Balk, S., P.A. Bleicher, and C. Terhorst. 1991. Isolation and expression of cDNA encoding the murine homologues of CD1. J. Immunol. 146:768–727.

48. Bradbury, A., K.T. Belt, T.M. Neri, C. Milstein, and F. Calabi. 1988. Mouse CD1 is distinct from and co-exists with TL in the same thymus. EMBO (Eur. Mol. Biol. Organ.) J. 7: 3081–3086.

49. Arase, H., N. Arase, K. Nakagawa, R.A. Good, and K. Onoe. 1993. NK1.1+ CD4+ CD8– thymocytes with specific lymphokine secretion. Eur. J. Immunol. 23:307–310.

50. Bendelac, A., P. Matzinger, R.A. Seder, W.E. Paul, and R.H. Schwartz. 1992. Activation events during thymic selection. J. Exp. Med. 175:731–742.

51. Hayakawa, K., B.T. Lin, and R.R. Hardy. 1992. Murine thymic CD4+ T cell subsets: a subset (Thy0) that secretes diverse cytokines and overexpresses the Vβ8 T cell receptor gene family. J. Exp. Med. 176:269–274.

52. Yoshimoto, T., and W.E. Paul. 1994. CD4+ NK1.1+ T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3. J. Exp. Med. 179:1285–1295.

53. Calabi, F., C.Y. Yu, C.A.G. Bisland, and C. Milstein. 1991. CD1: from structure to function. In Immunogenetics of the Major Histocompatibility Complex. R. Shrivastava, B.P. Rams, and P. Tyle, editors. VHC Publishers, New York. pp. 215–243.

54. Calabi, F., and C. Milstein. 1986. A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. Nature (Lond.). 323:540–543.

55. Lacasse, J., and I.H. Martin. 1992. Detection of CD1 mRNA in Paneth cells of the mouse intestine by in situ hybridization. J. Histochem. Cytochem. 40:1527–1534.

56. Porcelli, S., M.B. Brenner, J.L. Greenstein, S.P. Balk, C. Terhorst, and P.A. Bleicher. 1989. Recognition of cluster of differentiation 1 antigens by human CD4+CD8– cytolytic T lymphocytes. Nature (Lond.). 341:447–450.

57. Fauré, F., S. Itsumaki, C. Miossec, and T. Herceg. 1990. CD1c as a target recognition structure for human T lymphocytes: analysis with peripheral blood gamma/δelta cells. Eur. J. Immunol. 20:703–706.

58. Balk, S.P., E.C. Ebert, R.L. Blumenthal, F.V. McDermott, K.W. Wucherpfennig, S.B. Landau, and R.S. Blumberg. 1991. Oligoclonal expansion and CD1 recognition by human intestinal intraepithelial lymphocytes. Science (Wash. DC). 253:1411–1415.

59. Dellabona, P., G. Casorati, B. Friedli, L. Angman, F. Sallusto, A. Tunnalifflie, A. Roosneck, and A. Lanzevecchia. 1994. In vivo persistence of expanded clones specific for bacterial antigens within the human T cell receptor αβ CD4+8– subset. J. Exp. Med. 177:1763–1771.

60. Calabi, F., J.M. Jarvis, L. Martin, and C. Milstein. 1989. Two classes of CD1 genes. Eur. J. Immunol. 19:285–292.