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Immunology

Expression of CD4 in transgenic mice alters the specificity of CD8 cells for allogeneic major histocompatibility complex

(T-cell receptor/major histocompatibility complex class specificity/alloreactivity/accessory molecules)

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ABSTRACT **We have generated a transgenic mouse line in which a CD4 transgene is expressed on a significant fraction of the mature CD8$^+$ lymphocytes but is not expressed in the thymus. This provides an opportunity to examine the functional consequences of CD4 expression in a population of class I-selected CD8$^+$ lymphocytes. CD8$^+$ lymphocytes expressing the CD4 transgene proliferate in response to allogeneic class I and class II major histocompatibility complex, whereas CD8$^+$ cells from control animals proliferate only to allogeneic class I gene products. These observations suggest that the ability of a T-cell population to react with class II allogeneic major histocompatibility complex is determined by the presence of CD4.**

T-cell recognition of major histocompatibility complex (MHC) molecules is mediated by the T-cell receptor (TCR) and CD4 or CD8. The TCR is believed to contact the polymorphic peptide-binding site of MHC, whereas CD4 and CD8 bind to nonpolymorphic regions of class II and class I MHC, respectively (1, 2). The TCR and CD4 or CD8 may function as a complex, perhaps by binding to the same MHC molecule (3, 4). The expression of CD4 or CD8 defines the two major subsets of mature T cells. CD4$^+$CD8$^-$ cells recognize antigen associated with class II MHC and are predominantly helper T cells. CD4$^-$CD8$^+$ T cells recognize antigen associated with class I MHC and are predominantly cytotoxic T cells.

In addition to their ability to respond to foreign antigen bound to self-MHC, T cells also respond to foreign MHC in the apparent absence of antigen (alloreactivity). These responses are often measured in mixed lymphocyte reactions (MLRs), in which unprimed T cells produce lymphokines and proliferate in response to foreign MHC on stimulator cells. In allogeneic MLRs, there is a correlation between CD4 or CD8 phenotype and MHC class specificity: CD4$^+$ cells proliferate only in response to foreign class II MHC, whereas CD8$^+$ cells respond to foreign class I MHC. In assays in which lymphokine production by responder T cells is not required, CD8$^+$ cells have been observed to respond to class II MHC (5–8). However, for assays in which T cells are required to produce lymphokines and initiate a response to foreign MHC, the correlation between CD4/CD8 phenotype and MHC class specificity is absolute (5, 9, 10).

There are two molecules on class II-specific T cells that recognize class II MHC: TCR and CD4. It is unclear which of these molecules is responsible for the specificity for foreign class II MHC. We have investigated this question by using a line of mice in which a CD4 transgene is expressed on a subset of mature CD8$^+$ cells but is not expressed in the thymus. We find that mature CD8$^+$ cells that express the CD4 transgene proliferate both to class I and class II allogeneic MHC. This suggests that the presence of CD4 or CD8, rather than the specificity of the TCR, determines whether a population of cells will respond to foreign class I or class II MHC.

MATERIALS AND METHODS

Production of CD4 Transgenic Mice. A 1.7-kilobase (kb) cDNA construct containing the entire coding region of mouse CD4, along with 50 base pairs (bp) of 5′ noncoding DNA and 300 bp of 3′ noncoding DNA was ligated into the *Bgl* II–*Bam*HI sites of pMHβ20 (11). The dominant control element of the human CD2 gene (12) was inserted 3′ of the transcription unit. The entire 9-kb insert was excised with *Not* I and microinjected into (C57BL/6 × CBA/Ca)F_2 embryos as described (13). Founders were backcrossed to C57BL/6. RNase protection analysis was done as described (14). Mice were obtained from The Jackson Laboratory.

Flow Cytometry. For two-color flow cytometry, lymph node cells and thymocytes were stained with phycoerythrin–anti-CD4 and fluorescein isothiocyanate–anti-CD8 (Becton Dickinson) and analyzed with a FACScan flow cytometer (Becton Dickinson). For three-color analysis, thymocytes were stained by sequential incubation with anti-CD3 (145-2C11), phycoerythrin-conjugated anti-hamster immunoglobulin (Caltag, South San Francisco, CA), fluorescein isothiocyanate–anti-CD8, biotinylated anti-CD4, and allophycocyanin–avidin (Caltag). Data were collected using a FACS 440. For TCR β-chain variable region 14 (Vβ14) analysis, B-cell-depleted lymph node cells were treated sequentially with anti-Vβ14 (14-2) (15), fluorescein isothiocyanate–goat anti-rat immunoglobulin (Southern Biotechnology Associates, Birmingham, AL), rat immunoglobulin, biotinylated anti-CD8 (Becton Dickinson), allophycocyanin–avidin, and phycoerythrin–anti-CD4.

MLRs. Responders were lymph node cells that were depleted of B cells by passage over plates coated with goat anti-mouse IgM (16). CD8$^+$ responders were prepared by panning with anti-CD8 antibody (53-6.7) and anti-rat immunoglobulin-coated plates (16). CD8$^-$ cells were prepared by treating the nonadherent cells from CD8 plates with anti-CD8 antibody and complement. Responder T cells from transgenic mice were further purified into CD4$^+$ and CD4$^-$ subsets by an additional panning step on plates coated with purified anti-CD4 antibodies (H129.19). MLRs were done as described (9). Stimulators were spleen cells from C57BL/6 mice or from bm1, or bm12 mutant mice (from The Jackson

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Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; MLR, mixed lymphocyte reaction; Vβ14, TCR β-chain variable region 14.

Laboratory) that had been depleted of T cells with anti-Thy-1 and complement. Stimulators were irradiated (1500 rads; 1 rad = 0.01 Gy) and plated at a concentration of 5×10^5 cells per well. Responder T cells were added at the concentrations indicated in flat-bottom 96-well plates. Cultures were treated with [^{3}H]thymidine after 4 days of culture and harvested 12 hr later. Data are the average of triplicates. Blocking antibodies included in cultures were as follows: anti-class II, Y3P antibody-dialyzed ascites at 1:100; and anti-CD4, H129.19 culture supernatant at 1:50.

RESULTS

Constitutive Expression of CD4 in Transgenic Mice. We have generated a series of mouse lines expressing a CD4 transgene under control of the T-cell-specific CD2 regulatory element. Ligation of the human CD2 regulatory element to a β-globin structural gene (βCD2-A) has been previously shown to result in the expression of β-globin in both immature and mature T lymphocytes, such that the expression levels correlate with the transgene copy number (12). We have introduced a murine CD4 cDNA into a variant of βCD2-A (Fig. 1*a*), such that CD4 expression should be directed by the β-globin promoter under control of the CD2 regulatory sequences. Transgenic animals were generated by microinjection of this construct into (C57BL/6 × CBA/Ca) F_2 embryos. In this study, we characterize the function of T cells derived from one mouse line (β4BE-11) that contains ≈50 copies of the CD4 transgene.

Flow cytometric analysis of lymph node cells from control animals with antibodies directed against CD4 or CD8 revealed the expected pattern of single-positive $CD4^+CD8^-$ and $CD4^-CD8^+$ cells; only 0.5% of cells are double-positive ($CD4^+CD8^+$) (Fig. 2*a*). In contrast, in the transgenic mice, 4% of the lymph node cells are double-positive (Fig. 2*b*). The presence of CD4 on 25% of the $CD8^+$ T cells is presumably a consequence of expression of the CD4 transgene.

Analysis of expression of the CD4 transgene in the thymus is more difficult because most thymocytes express endogenous CD4. The frequency of double-positive cells is 79% in both the control and transgenic thymus (Fig. 2 *c* and *d*), and the level of CD4 expression in the $CD4^+CD8^+$ thymic T cells is similar in control and transgenic animals. Approximately 10% of the thymic lymphocytes from normal mice are single-positive cells that express high levels of TCR and resemble peripheral T cells. If the CD4 transgene were expressed in this thymic population, we would anticipate that a significant fraction of $CD8^+$ cells would also express the CD4 transgene. The pattern of CD4 and CD8 expression on thymic lymphocytes expressing high levels of TCR was examined using three-color flow cytometry (Fig. 2 *e* and *f*). The frequency of double-positive cells in this population is roughly equivalent in control (9.6%) and transgenic (9.1%) animals.

RNA transcripts derived from the transgenic and endogenous CD4 genes can be distinguished by RNase protection analysis. An antisense RNA probe was prepared from the transgene. RNase protection analysis (Fig. 1*b*) of RNA from peripheral lymphocytes from transgenic animals reveals two protected fragments consistent with the expression of both the transgenic (465 bp) and endogenous (440 bp) CD4 genes. A single protected fragment derived from the endogenous CD4 gene is observed with RNA from peripheral lymphocytes from control animals. Analysis of RNA from thymic lymphocytes from transgenic mice reveals a protected fragment reflecting expression of endogenous CD4 and a barely detectable fragment corresponding to the expression of trans-

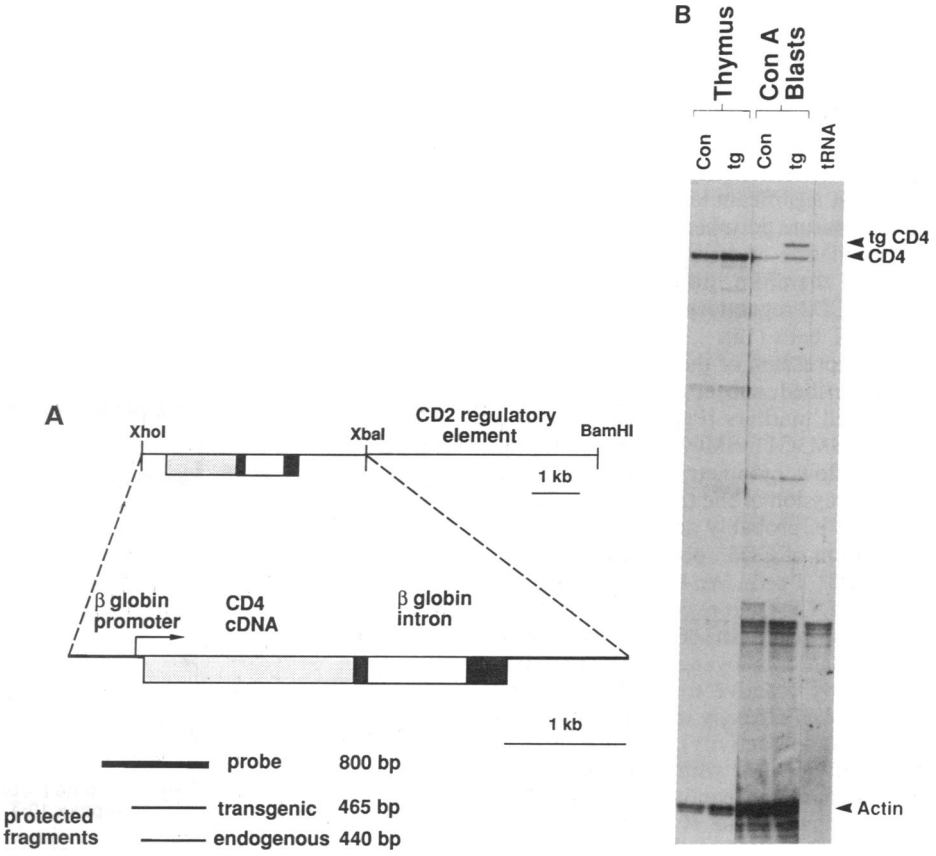

FIG. 1. (*A*) CD4 expression vector used for generation of transgenic mice. The probe used for RNase protection analysis is indicated. (*B*) RNase protection analysis of RNA from thymocytes and Con A blasts from transgenic (tg) and control (Con) mice. RNA fragments derived from the endogenous and transgenic transcripts are indicated. Human γ-actin probe was included for quantitative comparison.

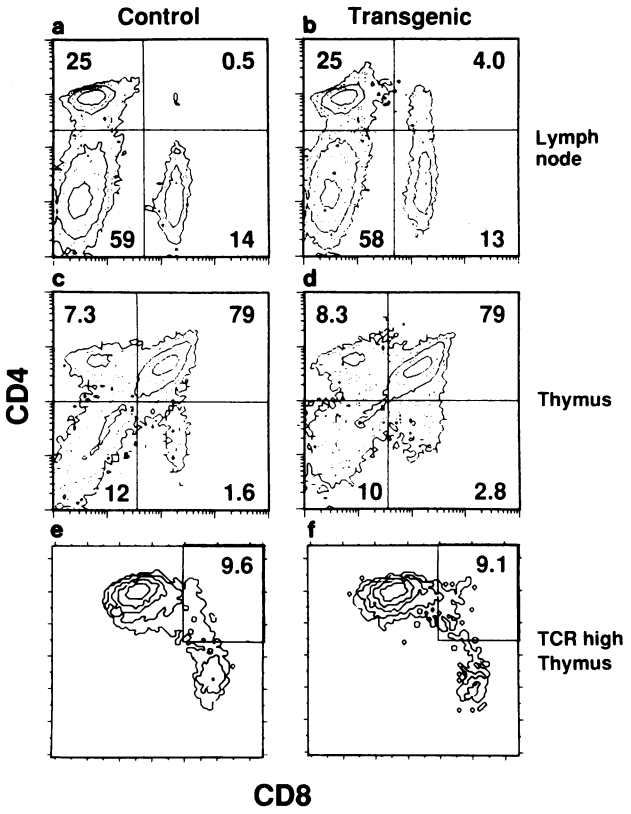

FIG. 2. CD4 and CD8 expression on T cells from normal and transgenic mice. (*a* and *b*) Lymph node cells. (*c* and *d*) Thymocytes. (*e* and *f*) Thymocytes gated for high levels of TCR expression.

genic CD4. In addition, a high-molecular-weight band is also present that presumably reflects low levels of inappropriately initiated transcripts from the transgene. These transcripts initiate in 5′ upstream sequences of β-globin and contain multiple start and stop codons that will inhibit translation. These RNA protection analyses, taken together with the fluorescence-activated cell sorter data suggest that the CD4 transgene is not expressed at significant levels in the thymus but is active in a fraction of mature peripheral T cells resulting in the appearance of a population of double-positive cells in the periphery. Our results, therefore, differ from previous studies that show that the CD2 regulatory element is active in thymocytes and mature T cells (12).

To determine whether expression of the transgene correlates with a previously described, subset of $CD8^+$ cells, we examined a variety of T-cell markers [Pgp-1, interleukin 2 receptor, CD45R-16A (17), SM3G11, SM6C10 (18), Qa2, Jlld, and CD3] by three-color flow cytometry. None of these markers correlate with expression of the transgene, suggesting that the CD4 transgene is probably activated or inactivated in a random population of $CD8^+$ peripheral cells.

Alloreactivity of $CD4^+CD8^+$ T cells. We have examined the functional consequences of CD4 expression in peripheral $CD8^+$ cells by analyzing the reactivity of $CD8^+$ cells from transgenic and control animals to allogeneic MHC proteins. Peripheral T cells are poorly reactive to self-MHC in the absence of antigen but respond strongly to MHC molecules from other strains of mice (alloreactivity). The use of stimulator cells from strains of B6 mice with mutations in the MHC locus allows us to examine the reactivity of T cells from transgenic mice to both class I and II allogeneic MHC molecules. For example, spleen cells from bm12 mice mutant in a class II gene (A_β) will stimulate a proliferative response from $CD4^+$ cells but not from $CD8^+$ cells from a B6 mouse; whereas spleen cells from the bm1 strain, mutant in a class I

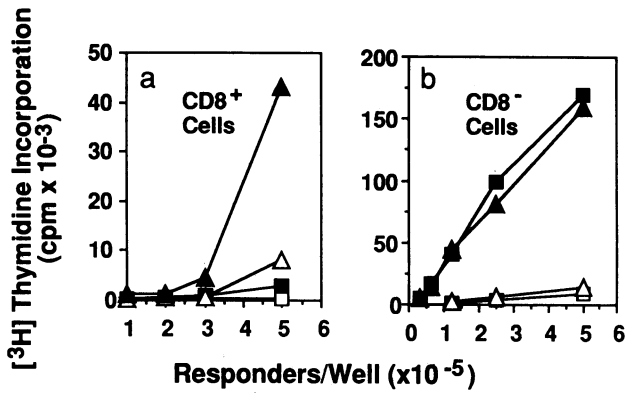

FIG. 3. Proliferation of T cells from normal and transgenic mice in response to allogeneic spleen cells. Responder titration of $CD8^+$ (*a*) and $CD8^-$ (*b*) T cells from normal (□, ■) and transgenic (△, ▲) mice to bm12 (■, ▲) and B6 (□, △) stimulator cells. Control $CD8^+$ cells were >95% $CD4^-CD8^+$, whereas transgenic $CD8^+$ cells were 71% $CD8^-CD8^+$ and 24% $CD4^+CD8^+$. Data are the average of triplicates, and SDs are <25%.

gene (K), will stimulate only $CD8^+$ cells to proliferate (5, 9). The transgenic mice used in these experiments, from the third or fourth backcross to C57BL/6, were homozygous for $H\text{-}2^b$. In initial experiments, $CD8^+$ T cells were purified from the lymph nodes of transgenic animals and nontransgenic littermates. Twenty-four percent of the $CD8^+$ cells from transgenic animals also express CD4, whereas the $CD8^+$ cells from control animals are all $CD4^-$. Although transgenic and nontransgenic CD8 cells proliferate in response to bm1 mutant stimulator cells (data not shown), transgenic but not control $CD8^+$ cells proliferate in response to the class II mutant bm12 cells (Fig. 3).

The $CD8^+$ T cells from transgenic animals consist of two distinct subsets: a $CD4^-CD8^+$ population and a $CD4^+CD8^+$ population. To determine which subset is responsible for class II alloreactivity, we fractionated the $CD8^+$ cells from transgenic mice into $CD4^+CD8^+$ and $CD4^-CD8^+$ subpopulations. Reactivity against allogeneic class II is only seen with $CD4^+CD8^+$ cells (Fig. 4*a*), whereas a response to allogeneic class I MHC is seen with both $CD4^+CD8^+$ and $CD4^-CD8^+$ subpopulations (Fig. 4*b*). In addition, reactivity of transgenic

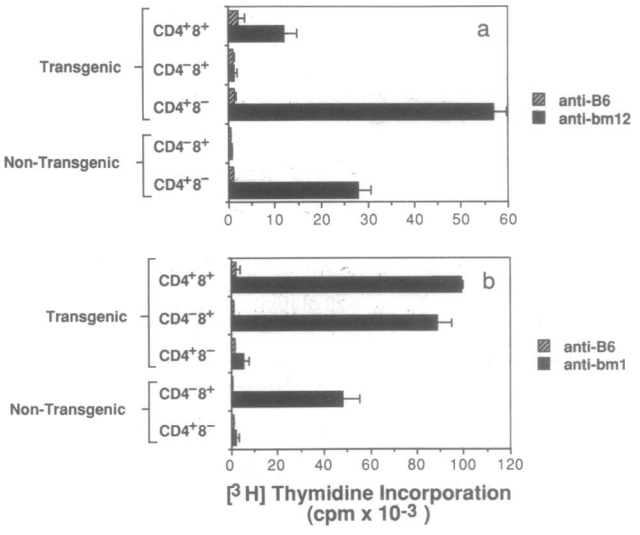

FIG. 4. Proliferation of separated $CD4^+CD8^+$, $CD4^-CD8^+$, and $CD4^+CD8^-$ lymph node T cells to bm12 and B6 (3×10^5 responders per well) (*a*) and bm1 (1×10^5 responders per well) (*b*). $CD8^+$ responders were >99% $CD8^+$ by flow cytometry.

$CD8^+$ T cells with allogeneic class II MHC is inhibited by antibodies directed to either class II or CD4 (Fig. 5). These antibodies also inhibit the response of normal $CD4^+CD8^-$ cells to bm12 stimulator cells but fail to inhibit the response of $CD4^-CD8^+$ cells to the class I mutant bm1 stimulator cells. These experiments indicate that the presence of CD4 on $CD8^+$ cells renders these cells responsive to allogeneic class II molecules.

The level of reactivity of the transgenic $CD4^+CD8^+$ cells to bm12 cells is somewhat lower than that of the $CD4^+CD8^-$ cells. This difference may, in part, be due to a lower frequency of T cells responsive to bm12 cells in the $CD4^+CD8^+$ population than in the $CD4^+$ $CD8^-$ population. Alternatively, the lower response of $CD4^+CD8^+$ cells to bm12 cells may reflect poorer lymphokine production by mature $CD8^+$ cells relative to $CD4^+$ cells. Limiting-dilution analysis to determine the responder frequency in the two populations will be necessary to test these possibilities. It is also possible that the presence of CD8 on a $CD4^+$ cell inhibits the reactivity to class II molecules (19).

$CD4^+CD8^+$ Lymph Node Cells Have a Class I-Selected TCR Repertoire. Positive selection in the thymus is thought responsible for the generation of single-positive $CD4^-CD8^+$ cells reactive with class I MHC and $CD4^+CD8^-$ cells reactive with class II MHC. One explanation for the class II alloreactivity in CD8 cells from transgenic mice is that selective events in the thymus are altered by expression of the CD4 transgene, resulting in $CD4^+CD8^+$ cells that have TCRs selected for reactivity with class II MHC. Although we could not detect significant expression of the transgene in the thymus, we cannot exclude the possibility that a small subpopulation of thymocytes expresses the transgene and gives rise to the peripheral $CD4^+CD8^+$ cells. If the population of cells expressing the CD4 transgene contains both cells that are selected for reactivity to class I and class II MHC, we would expect that $CD4^+CD8^+$ T cells would display a repertoire of TCRs intermediate between $CD4^+CD8^-$ and $CD4^-CD8^+$ populations. To examine this possibility, we compared the frequency of expression of the TCR β-chain variable region, Vβ14, in the different subsets of T cells. In H-2^b mice, the frequency of V$\beta14^+$ cells is three to four times higher in $CD4^+$ cells (7–8%) than in $CD8^+$ cells (2–3%) (15). This skewed distribution results from positive selection in the thymus; Vβ14 preferentially interacts with class II MHC, resulting in a higher frequency in $CD4^+$ cells. The frequency of V$\beta14^+$ cells in the $CD4^+CD8^+$ population from transgenic mice (2.8%) is similar to the frequency in $CD4^-CD8^+$ cells from the same transgenic mice (2.0%) or from nontransgenic littermates (2.1%), and the frequency of Vβ14 in the $CD4^+$ $CD8^-$ cells from transgenic and nontransgenic mice is 7.5% and 7.9%, respectively. In addition, the percentage of cells expressing the β-chain variable regions Vβ6, Vβ11, and Vβ8 was similar in transgenic $CD4^+CD8^+$ and $CD4^-CD8^+$ cells (data not shown). These observations provide further evidence that the $CD4^+CD8^+$ cells in transgenic mice, like control $CD8^+$ cells, undergo positive selection for class I, rather than class II, MHC recognition.

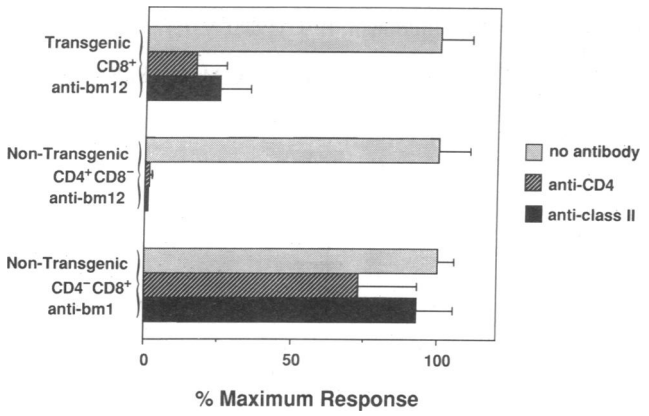

FIG. 5. Antibody blocking of transgenic $CD8^+$ anti-bm12 response. Responses were transgenic $CD8^+$ T cells at 4×10^5 responders per well with bm12 stimulators (11,000 cpm), nontransgenic $CD4^+CD8^-$ T cells at 2×10^5 responders per well with bm12 stimulators (42,000 cpm), and nontransgenic $CD4^-CD8^+$ T cells at 2×10^5 responders per well with bm1 stimulators (30,500 cpm).

DISCUSSION

T cells recognize a complex of antigen and self-MHC. The reactivity of T cells for allogeneic MHC implies that T cells also have a specificity for either class I or class II MHC. For example, a population of $CD4^+CD8^-$ cells that can respond to antigen in association with self-class II MHC also responds to allogeneic class II but not to allogeneic class I MHC. Furthermore, individual T-cell clones have been identified that react with antigen and self-MHC, as well as with allogeneic MHC. With rare exception (20), the self-MHC and allogeneic MHC recognized by a clone are of the same class (21, 22). Thus, a T cell may have a specificity, not only for a particular antigen and MHC allele, but also for class I or class II MHC.

Both the TCR and CD4 or CD8 interact with MHC on antigen-presenting cells. The specific reactivity of a T cell for a particular antigen and self-MHC is determined by the TCR; transfection of rearranged TCR genes from a T-cell clone reactive for a particular antigen and self-MHC pair will render the recipient responsive to this antigen and MHC complex (23, 24). An efficient response to antigen and MHC also requires the presence of CD4 or CD8 (25–28). The relative contribution of the TCR and CD4 or CD8 to the specificity for allogeneic class I or class II is not clear. For example, the failure of $CD4^-CD8^+$ cells to proliferate to class II allogeneic MHC could result if the TCRs that are selected for reactivity to antigen and self-class I MHC can only recognize allogeneic class I MHC. Alternatively, the presence of CD8 and the absence of CD4 may be responsible for the specific reactivity of this population with allogeneic class I MHC.

If the specificity of a T cell for class I or class II MHC is imparted by CD4 or CD8 and not the TCR, we predict that the introduction of CD4 into a $CD8^+$ class I-reactive cell would result in new reactivities to allogeneic class II MHC. We have, therefore, generated transgenic mice that have peripheral $CD8^+$ T cells also expressing CD4. This procedure permits us to examine the alloreactivity of a population of T cells that have been selected in the thymus for recognition of class I MHC but that express both CD4 and CD8. We find that these cells react with both class I and class II allogeneic MHC. This result suggests that the ability of a T cell to respond to allogeneic class II does not depend upon thymic selection for recognition of self-class II MHC but rather depends on the expression of CD4.

Although proliferative responses of $CD4^-CD8^+$ cells to bm12 cells are consistently not observed (9, 10), previous experiments (5) have shown that $CD8^+$ cells respond to target cells bearing the class II mutant bm12 in a primary cytotoxic T-lymphocyte assay. These cytotoxic T-lymphocyte responses were only seen when cultures were supplemented with Con A supernatants containing high concentrations of lymphokines. In contrast, we show that $CD8^+$ cells that coexpress CD4 can proliferate to bm12 cells in the absence of added lymphokines. It is interesting that for anti-bm12 responses of $CD8^+$ cells, lymphokine production, but not cytotoxic T-lymphocyte induction, requires CD4. CD4 may provide intracellular signals or increased avidity for stimu-

lator cells that are required for lymphokine production but not for cytotoxic T-lymphocyte induction.

Our results, as well as previous experiments (5, 6, 8), raise an apparent paradox: TCRs selected for recognition of self-class I MHC may react with either class I or class II allogeneic MHC molecules. An explanation for this paradox emerges from a consideration of the predicted similarities between the structures of MHC class I and class II. Although class I and class II MHC molecules are structurally distinct, the peptide-binding sites of the two classes of MHC molecules are predicted to share common structural features and common dimensions (29). Furthermore, the TCR has been suggested to only contact bound peptide and the regions of MHC immediately flanking the peptide pocket (30, 31). Because these regions of MHC are highly polymorphic, the peptide-binding sites of class I molecules from different individuals may be as distinct from one another as they are from the peptide-binding sites of class II MHC molecules. Thus, a TCR selected for recognition of self-class I may be as likely to react with foreign class II as with foreign class I MHC. The observation that normal $CD8^+$ cells fail to proliferate to allogeneic class II MHC could, therefore, be a consequence of the absence of CD4 on these cells.

Our data do not exclude the possibility that there is some bias for recognition of allogeneic class I MHC inherent in the TCRs of a class I-selected population. To address this question it will be necessary to determine the responder frequencies for a number of different foreign class II molecules. Even if such a bias exists, our data indicate that T cells selected for class I recognition can react with allogeneic class II MHC at a frequency sufficiently high to be readily measured in a primary MLR. These data suggest a model in which the TCR $\alpha\beta$ heterodimer determines the specificity for antigen and MHC allele, whereas CD4 and CD8 primarily determine the MHC class specificity of the T-cell response.

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1. Doyle, C. & Strominger, J. L. (1987) *Nature (London)* **330,** 256–259.
2. Norment, A. M., Slater, R. D., Parham, P., Engelhard, V. H. & Littman, D. R. (1988) *Nature (London)* **336,** 79–81.
3. Emmrich, F., Strittmatter, U. & Eichmann, K. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8298–8302.
4. Salter, R. D., Benjamin, R. J., Wesley, P. K., Buston, S. E., Garrett, T. P. J., Clayberger, C., Krensky, A., Norment, A. M., Littman, D. R. & Parham, P. (1990) *Nature (London)* **345,** 41–46.
5. Golding, H., Mizuochi, T., McCarthy, S. A., Cleveland, C. A. & Singer, A. (1987) *J. Immunol.* **138,** 10–17.
6. MacPhail, S. & Stutman, O. (1987) *J. Immunol.* **139,** 4007–4015.
7. Sprent, J. & Webb, S. R. (1987) *Adv. Immunol.* **41,** 39–133.
8. Gao, E.-K., Kanagawa, O. & Sprent, J. (1989) *J. Exp. Med.* **170,** 1947–1957.
9. Sprent, J. & Schaefer, M. (1985) *J. Exp. Med.* **162,** 2068–2088.
10. Heeg, K., Steeg, C., Schmitt, J. & Wagner, H. (1987) *J. Immunol.* **138,** 4121–4127.
11. Charnay, P., Mellon, P. & Maniatis, T. (1985) *Mol. Cell. Biol.* **5,** 1498–1511.
12. Greaves, D. R., Wilson, F. D., Lang, G. & Kioussis, D. (1989) *Cell* **56,** 979–986.
13. Hogan, B., Costantini, F. & Lacy, E. (1986) in *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
14. MacDonald, P. M., Ingham, P. W. & Struhl, G. (1986) *Cell* **47,** 721–724.
15. Liao, N. S., Maltzman, J. & Raulet, D. H. (1989) *J. Exp. Med.* **170,** 135–143.
16. Mage, M. G. (1984) *Methods Enzymol.* **108,** 118–124.
17. Bottomly, K., Luqman, M., Greenbaum, L., Carding, S., West, J., Pasqualini, T. & Murphy, D. B. (1989) *Eur. J. Immunol.* **19,** 617–624.
18. Hayakawa, K. & Hardy, R. R. (1988) *J. Exp. Med.* **168,** 1825–1838.
19. Kanagawa, O. & Maki, R. (1989) *J. Exp. Med.* **170,** 901–912.
20. Schilham, M. W., Lang, R., Benner, R., Zinkernagel, R. M. & Hengartner, H. (1986) *J. Immunol.* **137,** 2748–2754.
21. Sredni, B. & Schwartz, R. H. (1980) *Nature (London)* **287,** 855–857.
22. von Boehmer, H., Hengartner, H., Nabholz, M., Lernhardt, W., Schreier, M. H. & Haas, W. (1979) *Eur. J. Immunol.* **9,** 592–597.
23. Dembic, Z., Haas, W., Zamoyska, R., Parnes, J., Steinmetz, M. & von Boehmer, H. (1987) *Nature (London)* **326,** 510–511.
24. Saito, T., Weiss, A., Miller, J., Norcross, M. A. & Germain, R. N. (1987) *Nature (London)* **325,** 125–130.
25. Gabert, J., Langlet, C., Zamoyska, R., Parnes, J. R., Schmitt-Verhulst, A.-M. & Malissen, B. (1987) *Cell* **50,** 545–554.
26. Dembic, Z., Haas, W., Weiss, W., McCubrey, J., Kiefer, H., von Boehmer, H. & Steinmetz, M. (1986) *Nature (London)* **320,** 232–238.
27. Gay, D., Maddon, P., Sekaly, R., Talle, M. A., Godfrey, M., Long, E., Goldstein, G., Chess, L., Axel, R., Kappler, J. & Marrack, P. (1987) *Nature (London)* **328,** 626–629.
28. Sleckman, B. P., Peterson, A., Jones, W. K., Foran, J. A., Greenstein, J. L., Seed, B. & Burakoff, S. J. (1987) *Nature (London)* **328,** 351–353.
29. Brown, J. H., Jardetzky, T., Saper, M. A., Samraoui, B., Bjorkman, P. J. & Wiley, D. C. (1988) *Nature (London)* **332,** 845–850.
30. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **335,** 744.
31. Clothia, C., Boswell, D. R. & Lesk, A. M. (1988) *EMBO J.* **7,** 3745–3755.