Human CD4 Restores Normal T Cell Development and Function in Mice Deficient in Murine CD4

By Yuk M. Law,* Rae S. M. Yeung,† Clio Mamalaki,§ Dimitris Kioussis,§ Tak W. Mak,† and Richard A. Flavell*

From the *Section of Immunobiology, Howard Hughes Medical Institute, School of Medicine, Yale University, New Haven, Connecticut 06510; the †Departments of Immunology and Medical Biophysics, Ontario Cancer Institute, University of Toronto, Toronto, Ontario, Canada M4X 1K9; and the §National Institute of Medical Research, Mill Hill, London NW7 1AA, UK

Summary

The ability of a human coreceptor to function in mice was investigated by generating human CD4 (hCD4)-expressing transgenic mice on a mouse CD4-deficient (mCD4-/-) background. From developing thymocyte to matured T lymphocyte functions, hCD4 was shown to be physiologically active. By examining the expansion and deletion of specific V/β T cell families in mutated mice with and without hCD4, it was found that hCD4 can participate in positive and negative selection. Mature hCD4 single positive cells also were found in the periphery and they were shown to restore MHC class II-restricted alloreactive and antigen-specific T cell responses that were deficient in the mCD4 (-/-) mice. In addition, these hCD4 reconstituted mice can generate a secondary immunoglobulin G humoral response matching that of mCD4 wild-type mice. The fact that human CD4 is functional in mice and can be studied in the absence of murine CD4 should facilitate studies of human CD4 activity in general and human immunodeficiency virus 1 gp120-mediated pathogenesis in acquired immune deficiency syndrome specifically.

oc/β T cells are primarily subdivided on the basis of the CD4 or CD8 coreceptors that they express. CD8 T cells recognize MHC class I in conjunction with cytoplasmic peptide. Upon antigen recognition, these cells deliver effector function which leads to the killing of the cell that is recognized. In this way, these cytotoxic T cells play a major role to resistance to viral infection by the elimination of virally infected cells. CD4 T cells recognize MHC class II in conjunction with antigenic peptide that derives from the extracellular compartment, or from intracellular vesicles. CD4 T cells either potentiate the inflammatory response by the synthesis of the cytokines IFN-γ, TNF-α and β, and so on, whereas a second subset of CD4 T cells synthesizes the cytokines such as IL-4 and IL-5, and potentiates the humoral immune response (1, 2). The coreceptors CD4 and CD8 both interact directly with their ligands MHC class I and II, respectively. Moreover, CD4 and CD8 interact directly with the tyrosine kinase Lck via binding of this enzyme to the cytoplasmic tail of these molecules (3). In a way that is not well understood at present, this interaction contributes to the delivery of the antigen-specific signalling via the TCR-MHC interaction (4).

CD4 and CD8 are also required for the development of T cells (Kaufman-Paterson, R., L. C. Burkly, D. K. Kurasara, A. Dunlop, D. Kioussis, C. Mamalaki, R. A. Flavell, and T. H. Finkel, manuscript submitted for publication; 5-8).

Antibodies to these molecules block T cell development and recent studies using CD4 or CD8 deficient mice show defective development of the helper and cytotoxic T cell compartments, respectively (9, 10). Positive and negative selection of thymocytes was dysfunctional when a mutant class I molecule that was unable to bind CD8 was used as a restriction element (5). Moreover, in mice that are deficient for mouse CD4, it has also been shown that the alloreactivity to MHC class II antigen and the antibody response to a T cell-dependent soluble antigen are markedly diminished (10).

Despite having essentially identical functions in mice and humans, the overall homology of human CD4 (hCD4)1 to murine CD4 (mCD4) is low (11). But upon closer inspection, the amino acid sequence critical in binding to the src tyrosine kinase Lck is 100% homologous. There is also functional evidence that human or mouse CD4 binds to the MHC II of the heterologous species (12-16) although the mCD4-human MHC interaction is weaker than the corresponding heterologous hCD4-mouse MHC interaction in vitro (17-19). In fact, using different levels of surface hCD4

1 Abbreviations used in this paper: hCD4, human CD4; mCD4, murine CD4.
expressing transgenic mouse lines, Barzaga-Gilbert et al. (12) have shown that the human coreceptor is functional in alloreactive responses.

In light of these interspecific interactions intracellularly and extracellularly, and the in vivo system available from mCD4 mutants, we have tested whether hCD4 can reconstitute the cellular phenotype and function of mCD4 at the organistical level. We, therefore, generated transgenic mice expressing hCD4 specifically in lymphocytes (H-/-), and bred them onto mice homozygous for the disrupted CD4 gene (M-/-). To facilitate the analysis of T cell development and function, we also used mice transgenic for the chick OVA-specific TCR (O-/+). We show here that human CD4 can participate in negative and positive selection of T cells and reconstitute the mature T cell subsets in mice. This reconstitution also reestablishes function as illustrated by alloreactive and antigen-specific responses as well as T cell-dependent B cell antibody responses.

Materials and Methods

Transgenic and Gene Disrupted Mice. The generation of the human CD4 transgenic line, 313, has been described in detail elsewhere (Kaufman-Paterson, R., et al., manuscript submitted for publication). Briefly, the DNA construct for microinjection uses a 2.8-kb human CD4 cDNA fragment which is placed in an artificially produced EcoRI site of the human CD2 promoter-luxor control region cassette (20, 21). This insertion also replaces the human CD2 first exon and leaves the 5' flanking promoter and 3' flanking regulatory sequences intact. The result is an expression that is construct copy number dependent and lymphocyte specific, independent of the position of integration in the mouse genome. The linearized construct was then injected into CBA/Ca x C57BL/10 fertilized eggs in the standard manner (21).

The mouse CD4-deficient line is described in Rahemtulla et al. (10). Mice homozygous for the disrupted gene (M-/-) were bred with the hCD4 transgenic mice. The latter mice used have been backcrossed to C57BL/6J seven times before the initiation of this breeding. Additional breeding and screening of the combined heterozygous genotypes gave rise to mice that were H-/- and M-/-, MHC haplotype b/b.

The OVA-specific, MHC class II I-Aa-restricted TCR transgenic line (O-/-) has been previously described (22) and was generously provided by Drs. Dennis Loh and Kenneth Murphy (Washington University, St. Louis, MO). Briefly, this TCR transgenic construct incorporates the productive Vz13 and V8.2 chain genomic rearrangements that have been cloned from the hybridoma cell line DO11.10 (23, 24). These mice were similarly crossed with the M-/- mice and the progeny screened and bred to reach the O-/-M-/- genotype. One such mouse was then bred with H-/-M-/- mice to create the O-/-H-/-M-/- genotype and their respective transgene-negative littermates with the MHC haplotype b/d. We also bred the O-/-H-/-M-/- mice with B6 mice to generate O-/-H-/-M-/- mice to serve as controls in antigen-specific T cell activation and FACS® analysis (Becton Dickinson & Co., Mountain View, CA) experiments. As with the O-/-H-/-M-/- mice, these M-/- mice are heterozygous for CD4 and MHC class II alleles (b/d).

The transgenic 107.1 line is a mouse strain with wild-type expression of MHC II I-E generated initially on C57BL/10 mice and backcrossed onto the C57BL/6J strain (25). C57BL/6J, C57BL/10J, and CBA/ca/j mice came from The Jackson Laboratory (Bar Harbor, ME).
antigen-specific proliferation, irradiated self-splenocytes were used and chick OVA (Sigma Chemical Co., St. Louis, MO) was added to reach a final volume of 0.2 ml/well. Assays of alloreactivity were allowed to go 5 d with a 16-h [3H]thymidine pulse at the end. Antigen-specific proliferation went 72 h and pulsed for the same duration. Wells were harvested onto paper filters with a cell harvesting system, and counted in a beta counter (Betaplate 1502; Wallac, Gaithersburg, MD).

ELISA. 3-4-mo-old mice of the H- M-, H*M-, B6, H+M § background were immunized with 50 #g KLH (Calbiochem-Novabiochem Corp., San Diego, CA) in CFA intraperitoneally. A booster in IFA was given at 3 wk and serum collectr at day 10 and week 5. KLH (20 #g/ml) in PBS was allowed to coat polystyrene 96-well plates overnight at 4°C. The plates were washed in borate saline, blocked with 1% BSA, and incubated with increasing dilutions of serum for 2 h at 21°C. After washing, goat anti-mouse total IgG (H + L) horseradish peroxidase-conjugated antibody (Bethesda Research Laboratories) was applied for 1 h at 21°C. A 4-aminoantipyrine/phenol/H2O2 solution was then used as a substrate. Calorimetric readings using a spectrophotometer (Titertek Multiscan; Flow Laboratories-ICN, Costa Mesa, CA) were taken at 20-min intervals.

Results

Expression of Human CD4 in Mice. Before assessing how hCD4 may substitute for the function of mCD4, we determined the level and distribution of hCD4 expression. As predicted by previous works using the same human CD2 promoter-regulator (Kaufman-Paterson et al., manuscript submitted for publication, and 20, 21), this transgene is expressed in nearly all lymphocytes. Consistently, >95% of the T cells in our transgenic mice are hCD4 § . When further subdivided, >95% of CD8 and 99% of mCD4 + cells coexpress hCD4 (Fig. 1, A and B). Consistent with the fact that murine CD2 is also expressed in some B cells, we find that hCD4 directed by the human CD2 promoter is present in about 70-80% of the B cells (Fig. 1, C and D). This pattern of expression is maintained in the three lines of mice that carry the transgene, i.e., transgenic line 313 expressing both murine and human CD4 (H+M § ), mice that have the human CD4 transgene on a background that is homozygous deficient for murine CD4 (H+M-), and finally, the latter mice also carrying a TCR transgene specific for OVA (O) in the context of I-Aq (O+H+M § ). Hence, the presence of the OVA-TCR transgene or the absence of the mCD4 gene product does not affect the distribution or the expression of hCD4. The level of hCD4 is similar in mCD4 and CD8 positive T cells. It is interestingly to note, however, that hCD4 is expressed at a higher level in a minority of single positive T cells that lack mCD4 (CD3 + hCD4 + CD8 - ) when compared (Fig. 2, A-C and see below).

Finally, we also compared the expression level of the trans-
gene to its natural endogenous counterpart in human PBMC. Fig. 2A shows that surface hCD4 expression is at least five times higher in human cells than in transgenic mouse cells.

**Reconstitution of the T-lymphocyte Subsets.** As reported by Rahemtulla (10), 90% of the T cells in the periphery of the M- mice are CD8+ and 10% are CD3+CD4-CD8-. This disproportionate shift of single positive cells to the CD8+ phenotype is corrected by hCD4. Fig. 3 shows the reconstitution of hCD4 single positive cells. In the H+M- mice, the CD3+ cells are composed of 56% CD8+ cells and >40% hCD4 single positive cells (Fig. 3A). Human CD4, therefore, permits approximately fourfold more T cells to be selected for MHC class II recognition. As we will show below, these hCD4+ T cells are also functional in T cell assays. To confirm this observation and because B cell contamination can increase the apparent number of hCD4+CD8- cells, we performed three-color staining using anti-CD8, B220, and hCD4 antibodies with gating on the CD8-/B220- population; this showed the same percentage of hCD4+ cells (data not shown). The rescue of CD4 T cells by hCD4 transgene expression is still greater in the TCR transgenic mice. Most CD3+ cells (>70%) are hCD4+CD8-, and 25% are CD3+CD8+ in the O+H-M- mice, as shown by Fig. 3B. Likewise, in the OVA-TCR transgenic with mCD4, the percentage of CD4 cells is even higher, reaching 82% of all CD3+ cells (Fig. 3C). It is also to be noted that the gross appearance of the thymus, LNs, and spleen was normal in these various lines of transgenic and/or mutated mice. The total number of cells in the spleen and its absolute number of T cells (CD3+) and B cells (B220+) was not altered significantly as compared with B6 mice of a similar age (data not shown).

**Human CD4 Redirects Positive and Negative Selection.** To examine whether positive and negative selection ensues from the interaction between immature thymocytes and thymic epithelium (MHC II+) when hCD4 is expressed in place of mCD4, we studied the Vβ profiles of littermates (O-M- and either H- or H+) from the H-M- (MHC I-A+ I-E-) and O-H-M- (I-A-I-E+) cross.

To compare the quantitative effectiveness of hCD4 in positive and negative selection in mice that are deficient in mCD4, we focused specifically on the mature peripheral CD3+CD8- subset. In mice expressing human or mouse CD4 coreceptors, these cells are all CD3+CD4+. In the O-H-M- mice, however, these cells (CD3+CD4-CD8-) had no coreceptors and consisted of only 10-15% of the CD3+ cells. They were compared with the CD3+HCD4+CD3- cells of the O+H-M- mice. In the O-H-M- mice, this is the hCD4 single positive subset which increased to 40-45% of the CD3+ cells. Additionally, we used mCD4 single positive cells from B6 mice (I-Eb negative) and B6 mice expressing an I-E transgene (transgenic line 107.1, I-E+) as controls.

Vβ6+ CD4 T cells are positively selected by I-E and as a result show an elevated percentage in I-E+ mouse strains (31). Vβ6+ CD4 cells (see Fig. 5B) account for 7.85% in B6 mice that lack the I-E selecting element but increase to 11.45% of the CD3+mCD4+ subpopulation in the syngeneic I-E+ 107.1 mice. This number falls to 7.79% in the O-H-M- mice lacking CD4 (the same level seen without I-E in the control B6 mice), despite the presence of I-E. HCD4 reconstitutes this Vβ6 population to 10.35% in the O-H-M- mice, showing that as determined by this assay, hCD4 can function equally well in positive selection as the endogenous murine CD4 gene.

We also examined the positive selection of OVA-TCR cells in the mCD4-deficient background. The selection of the OVA-TCR clonotype does not absolutely require CD4. Thus, in the CD8- subset (which is also CD4-) of the O+H-M- mice which comprises 10-15% of the CD3+ cells, 52% express the OVA-TCR clonotype (Fig. 4C). Of the 85-90% of the remaining CD3+ cells that are CD4-CD8+, 50% are...
Figure 4. The selection for the MHC class II-restricted OVA TCR cells in various experimental mouse lines is illustrated in these profiles of CD3+CD8- gated cells and their staining by KJ1-26, the clonotype-specific antibody. In mice with CD4 (A, B, and D), the gated cells are essentially all CD4+. In the O+H-M- line (C), the gated cells lack coreceptors. In the presence of human or mouse CD4 and OVA TCR (A and B), the percentage of clonotype-selected cells increased above the level seen in the O+H-M- line consistently, and the profiles shown are representative of a minimum of five mice from each line studied.

clonotypic (data not shown). However, the presence of either human or mouse CD4 not only increases the size of the CD3+CD8- subset itself but also that of the CD8- subpopulation (which is CD4+) which is clonotypic. For example, the percentage of CD4 single positive cells increased from ~60% (data not shown) to 82% in the O+H-M+ mice (Fig. 3 C), and from 44% (O-M-M-) to 75% in the O+H-M- mice (Fig. 3, A and B). Furthermore, within this CD8 negative or CD4 single positive population, >70 and 80% (vs 52% in mice lacking CD4) are clonotypic in the O+H-M+ and O+H-M- mice, respectively (Fig. 4, A and B).

Vβ5 and Vβ11 T cells are deleted in mice that express a functional I-E molecule in conjunction with the endogenous retroviruses MMTV (mouse mammary tumor virus) 6, 8, and 9 (32, 33). To address whether hCD4 can participate in and influence negative selection, we therefore determined the percentage of Vβ5 and Vβ11 T cells in the periphery (Fig. 5, D and F). The percentage of Vβ5/CD8- or CD4+ and Vβ11/CD8- or CD4+ cells in I-E+ 107.1 transgenic mice was 0.64 and 0.39%, respectively, in the presence of mCD4. In the O+H-M- mice, hCD4 maintains this deletion at 0.75% (Vβ5) and 1.5% (Vβ11). In the O+H-M- mice, we used the CD3+CD4-CD8- population as the corresponding subset to compare with the mouse or human CD4 single positive subset. For Vβ5, it consisted of 2.12%, and for Vβ11 3.56% of the double negative peripheral T cells despite the presence of I-E. In the absence of I-E but in the presence of mCD4 as in the B6 mouse strain (which is syngeneic to strain 107.1), it is 3.58 and 4.98% for Vβ5 and Vβ11, respectively. The intermediate level seen in the O+H-M- mice suggests that although CD4 enhances the negative selection of these Vβ families in the presence of I-E, it is not mandatory. It is possible that the affinity of the interaction between some Vβ TCRs and their class II ligand is such that coreceptor assistance is not necessary in the deletion process. It is interesting to note that consistent with this observation, deletion of both Vβ11 and more strikingly, Vβ5, also occurs in the CD8 T cells of mice without human and mouse CD4 (O+H-M-). The deletion of these cells was thought to be due to the interaction of CD4 with MHC class II during the double positive stage of the thymocyte development (34, 35). It is 3.05% for Vβ5 and 4.57% for Vβ11, a level significantly lower than that seen in the B6 strain (12.55 and 7.15%, respectively), and nearly matches the percentages seen in the transgenic 107.1 and O+H-M- strains (Fig. 5, C and E). This finding again suggests that there is not an absolute requirement for CD4 for the recognition of superantigen during T cell development.

Human CD4 Participates in Alloreactivity and Antigen-specific Response. In attempting to define the role of hCD4 in the mouse T cell response, we first set up a primary mixed lymphocyte response disparate in a single MHC class II I-E antigen. This was achieved by using the I-Ea transgenic 107.1 line bred onto the B6 background. Serially diluted responder cells from 88-95% T cell-enriched preparations were set up with irradiated stimulator cells provided by 107.1 or B6 splenocytes (Fig. 6). The responsiveness of the H+M- cells matched that of the T cells from the B6 and transgenic 313 line (H+M+), whereas that of the H-M- cells is markedly diminished. Similarly, Killeen et al. (36) also showed restoration of alloreactivity to an MHC class II antigen (H-2bmi2) when a human CD4 transgene was expressed in a different mouse CD4-deficient line (see Discussion).

Since CD4 should also potentiate T cell activation to processed soluble antigen presented by MHC II molecules, we used the O+H-M- mice to test whether T cell proliferation to antigen is restored. When irradiated splenocytes were
Figure 5. These graphs depict the percentages of Vβ lymphocytes analyzed within the T cell subsets. In the B6, TG 107.1 and O-H-M- mouse lines, the CD8+ subset (A, C, and E) is of the CD3+CD8+ phenotype. In the O-H-M- line, the CD8 subset is of the CD3+CD8+bCD4+ phenotype. For the CD4 or CD8- subset (B, D, and F) in the B6 and TG 107.1 lines, this consists of the CD3+CD8-mCD4+ population. In the O-H-M- line, this consists of the CD3+CD8-bCD4+ population and in the O-H-M- line, this consists of the CD3+CD8-CD4- population (*). Three-color staining was done using the designated anti-Vβ antibodies, anti-CD3, and anti-CD8 on T enriched cells. The CD3+CD8- cells were further...
fed OVA and incubated with T-enriched unprimed cells from either O−H+M+, O−H−M+ (product of the B6/O+M− cross), O+H+M− or O+H−M− mice, there was again a marked enhancement of proliferation in the OVA-TCR hCD4+ and mCD4+ cells (Fig. 7). When a fixed number of cells were incubated with increasing amounts of antigen, a similar pattern of difference in responsiveness was also seen (data not shown).

**Human CD4 Provides B Cell Help.** CD4 T cell help is required for T cell–dependent IgG responses. To determine whether hCD4 can function as a coreceptor in this T-B cell interaction, mice were immunized with KLH, a T cell–dependent antigen, boosted, and the secondary antibody response analyzed by an antibody capture ELISA (Fig. 8). Although the mice without CD4 still mounted a modest response, the control B6 and experimental H+M− mice produced a specific IgG response at least three times larger. Another experiment using two mice from each group and including the 313 transgenic line (H+M+), produced similar results with the 313 response matching that of the B6 and H+M− mice (data not shown). These observations are consistent with studies of Killeen et al. (36), who showed that

![Diagram](image-url)

**Figure 6.** An MHC class II disparate primary MLR using T-enriched cells from mice with or without human CD4 and/or mouse CD4. Experiment shown here was done in triplicate wells and is representative of two other experiments done using additional mice. See Materials and Methods for experimental details.

![Diagram](image-url)

**Figure 7.** The response of various OVA TCR transgenic lines to OVA. The response of serially diluted unprimed T-enriched cells to OVA was assessed by proliferation or thymidine incorporation after incubation with irradiated O− syngeneic splenocytes and 100 μg/ml of antigen for 3 d. Cells were incubated in triplicate wells and the data shown here is representative of three other experiments using additional mice. When the concentration of antigen was serially diluted and the stimulators and responders were kept constant, a similar pattern and difference in response was seen.

![Diagram](image-url)

**Figure 8.** An antibody capture ELISA was performed to measure the specific total serum IgG titer 5 wk after the primary immunization. The values are from the means of three mice from each group. Another experiment using two mice from each group showed a similar difference between the H−M− and the H+M− mice and, with the B6 response equivalent to the H+M− response.

checked for mCD4 or hCD4 expression by separately staining for these three markers. CD4 expression was seen in >97% of these cells in all the experiments performed. B6 is I−E− and the other three mouse lines are I−E+. Each symbol plotted represents a single mouse analyzed. (N) Number of mice analyzed; (M) mean; (S) standard error of the mean.
primary antibody responses to TNP-KLH are reconstituted in CD4-deficient mice carrying the human CD4 transgene.

**Discussion**

In this paper we present evidence that the human T cell coreceptor, hCD4, is functional in T cell development and T cell function in mice. In mice that are deficient in mCD4, CD8+ lymphocytes occupy 90% of the peripheral T cell compartment. The expression of hCD4 in these mice restores the CD4 single positive population to 40–45%, facilitates positive and negative selection in the thymus, and hCD4 functions in T cell activation in vitro and T cell help in vivo. Using a mCD4 enhancer and the human CD4 promoter, Killeen et al. (36) recently observed a restoration of 70% when hCD4 is expressed as a transgene in mCD4-deficient mice. The larger percentage (70 vs 40–45%) of restored single positive CD4 T cells in the latter system is most likely due to the higher expression level of hCD4. Despite the fact that the expression level is somewhat lower in our system, functional reconstitution is obtained.

To establish functionality of hCD4 in our mouse system, we first looked at thymic selection. HDC4 participates in negative selection of Vβ5 and Vβ11 cells that are deleted in the context of MHC II I-E and “self” superantigen. This process is defective in the H-M- thymus, but is restored for the CD4 lymphocytes of the Vβ5 and Vβ11 families in the H-M+ mice, since the percentages of these Vβ clones in the periphery of the reconstituted mice (O-H-M-) nearly match those of the I-E-expressing M+ transgenic mice (line 107.1). In addition, this effect carries over to the CD8 subset because hCD4 is also present in the CD8+ cells in the O-H-M- transgenic mice which causes a further deletion of the CD8+ Vβ5 and Vβ11 cells. Moreover, deletion of CD8+ Vβ5 and Vβ11 T cells also occurs to some extent in the H-M- thymus despite the absence of CD4. Previously it was considered that the deletion of these CD8 T cells by MHC class II presented superantigen occurred at the double positive CD4+CD8+ stage and was therefore mediated by CD4 (34, 35). Our finding here confirms the observation of Wallace et al. (37) who also examined the negative selection of CD8 cells with other strains of mice and different Vβ families. That is, it is possible that CD4 may not be absolutely necessary for deletion of some Vβ groups. Alternatively, this deletion can occur at a stage other than the double positive cell stage at which CD4 can exert an effect. Hence, negative selection of certain Vβ families may not absolutely require CD4 but is enhanced in the presence of CD4. For positive selection, we examined Vβ6 T cells selected by I-E, and the OVA-specific TCR transgenic T cells selected by I-A*. Again, we observed the percentage of Vβ6 CD4 cells in the O-H-M+ mice (10.35%) to be above the I-E* B6 control (7.85%), and equivalent to the I-E-expressing transgenic 107.1 control (11.45%). The OVA-specific T cells are strongly positively selected in hCD4-expressing mice comprising ~82% of peripheral hCD4 single positive T cells in O-H-M+ mice, vs. a similarly large 73% in the O-H-M- mice. The reason why a slightly higher percentage is actually seen in the O-H-M- mice is not clear.

Recent work (Kaufman-Paterson et al., manuscript submitted for publication, and 38, 39) using transgenic and gene-deficient mice has provided new evidence supporting the stochastic model of positive selection. According to this model (40, 41), a developing T cell with a TCR that is class II restricted goes through a stage where either CD8 or CD4 is randomly turned off. At this intermediate stage, if CD4 is present and the TCR engages its cognate class II ligand, then this cell will have been successfully selected for maturation. Since hCD4 is constitutively expressed in the CD8+ cells in our transgenic mice, we have asked whether this affects the unusually large percentage of CD8 cells that proceeds through positive selection. For example, hCD4 may not only rescue the CD3+CD8- MHC class II-restricted T cell population but also those cells that are CD3+CD8+ that would not have been selected.

In the Vβ selection of our O-H-M- mice that are I-E+ and have CD4 on CD8- cells, small effects on the CD8- subset can be observed. First, the percentage of Vβ5 and Vβ11 CD8+ cells from these mice is lower than that in the transgenic 107.1 mice (Fig. 5, C and E), suggesting that negative selection in this population has been aided by the presence of hCD4. Similarly, the percentage of Vβ6 CD8+ cells is slightly higher in the O-H-M- than in the transgenic 107.1 mice (Fig. 5 A). The small increase of Vβ6 CD8+ cells in the O-H-M- mice could suggest a rescue effect provided by hCD4 and hence be consistent with the stochastic model of positive selection. To directly assess whether the CD4+CD8- cells were selected by MHC class II and to test the stochastic model functionally, we can take advantage of our transgenic/mutated mouse system by sorting for OVA-TCR+ CD4+CD8- cells and compare their response to OVA with OVA-TCR human or mouse CD4 single positive cells. This work is in progress.

Finally, we have also provided evidence that the restoration of the CD4 single positive phenotype is functional at the level of the mature T cell. That is, cells from H-M- mice respond markedly better to allo-MHC class II than H-M- cells in a primary class II disparate MLR and, respond better in an antigen-specific manner when the OVA-TCR mice are used. Both responses closely match that of the mCD4 wild type. In addition, T cell help is restored in H-M- mice since good levels of antibodies to KLH are obtained after immunization in both primary (36) and secondary antibody responses as shown in our study.

Mice with targeted disruptions of a given gene should not only allow the exploration of the function of the defective gene, but should also make possible refined animal models to study human physiology or disease. There is strong evidence to suggest the CD4 cell dysfunction and perhaps loss in HIV-1 infection is in part due to indirect mechanisms mediated by Env gp120–CD4 interactions (42–46). Certain autoimmune diseases may also involve the interaction between CD4 and predisposing HLA ligands. Since human CD4 is functional in transgenic mice, mice that express a physiologically active human CD4 replacing its murine counterpart may provide a useful model to study these problems.

1240 Human CD4 Replacing Murine CD4 in Mice
We thank Drs. Kenneth Murphy and Dennis Loh for the OVA TCR transgenic mice. We are also grateful to Dr. Sylvie Guerder for her input and suggestion to this work. Tom Taylor provided expert flow cytometry technical services and Frances Degrenier provided valuable assistance in the preparation of the manuscript, and we thank them both.

This work was supported in part by grant 1-ROI-AL29902 from the National Institutes of Health. Yuk M. Law is a National Institute of Child Health & Development Fellow of the Pediatric Scientist Development program. Richard A. Flavell is a Senior Investigator of the Howard Hughes Medical Institute.

Address correspondence to Richard A. Flavell, FMB 410, Immunobiology, HHMI, 310 Cedar Street, Yale University School of Medicine, New Haven, CT 06510.

Received for publication 5 August 1993 and in revised form 13 January 1994.

References

1. Mossman, T.G., H. Cherwinski, M.W. Bond, M.A. Giedlin, and R.L. Coffman. 1986. Two types of murine helper T cell clone: 1. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136:2348.
2. Mossman, T.R., and R.L. Coffman. 1989. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. 7:145.
3. Barber, E.K., J.D. Dasgupta, S.F. Schlossman, J.M. Trevillyan, and C.E. Rudd. 1989. The CD4 and CD8 antigens are coupled to a protein-tyrosine kinase (p56\(\text{cdk}\)) that phosphorylates the CD3 complex. Proc. Natl. Acad. Sci. USA. 86:3277.
4. Glachenhaus, N., N. Shastri, D.R. Littman, and J.M. Turner. 1991. Requirement for association of p56\(\text{cdk}\) with CD4 in antigen-specific signal transduction in T cells. Cell. 64:511.
5. Killeen, N., A. Moriarty, H.-S. Teh, and D.R. Littman. 1992. Requirement for CD8-major histocompatibility complex class I interaction in positive and negative selection of developing T cells. J. Exp. Med. 167:89.
6. Ramsdell, F., and B.J. Fowlkes. 1989. Engagement of CD4 and CD8 accessory molecules is required for T cell maturation. J. Immunol. 143:1467.
7. Seong, R.H., J.W. Chamberlain, and J.R. Parnes. 1992. Signal for T-cell differentiation to a CD4+ cell lineage is delivered by CD4 transmembrane region and/or cytoplasmic tail. Nature (Lond.). 356:718.
8. Zütting-Pfück, J.C., L.A. Jones, D.L. Longo, and A.M. Krüisbeek. 1990. CD8 is required during positive selection of CD4+CD8+ T cells. J. Exp. Med. 171:427.
9. Fung-Leung, W.P., M.W. Schilham, A. Rahemtulla, T.M. Kundig, M. Vollenweider, J. Potter, W. van Ewijk, and T.W. Mak. 1991. CD8 is needed for development of cytotoxic T cells but not helper T cells. Cell. 65:443.
10. Rahemtulla, A., W.P. Fung-Leung, M.W. Schilham, T.M. Kundig, S.R. Sambhara, A. Narendram, A. Arabian, A. Wakeman, C.J. Paige, R.M. Zinkernagel, et al. 1991. Normal development and function of CD8+ T cells but markedly decreased helper cell activity in mice lacking CD4. Nature (Lond.). 353:180.
11. Turner, J.M., M.H. Brodsky, B.A. Irving, S.D. Levin, R.M. Perlmutter, and D.R. Littman. 1990. Interaction of the unique N-terminal region of tyrosine kinase p56\(\text{cdk}\) with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs. Cell. 60:755.
12. Barzagia-Gilbert, E., D. Grass, S.K. Lawrance, P.A. Peterson, E. Lacy, and V.H. Engelhard. 1992. Species specificity and augmentation of responses to class II major histocompatibility complex molecules in human CD4 transgenic mice. J. Exp. Med. 175:1707.
13. Fischer Lindahl, K., and F.H. Bach. 1975. Human lymphocytes recognize mouse alloantigens. Nature (Lond.). 254:607.
14. Lombardi, G., I. Barber, G. Aichinger, T. Heaton, S. Sidhu, J.R. Batchelor, and R.I. Lechler. 1991. Structural analysis of anti-DR1 allorecognition by using DR1/H-2K\(\text{d}\) hybrid molecules. Influence of the beta 2-domain correlates with CD4 dependence. J. Immunol. 147:2034.
15. von Hoegen, P., M.C. Miceli, B. Tourville, M. Schilham, and J.R. Parnes. 1989. Equivalence of human CD4 and mouse CD4 in enhancing antigen responses by a mouse class II-restricted T cell hybridomas. J. Exp. Med. 170:1879.
16. Yoshihawa, K., and A. Yano. 1984. Mouse T lymphocytes proliferative responses for human MHC products in mouse anti-human xenogeneic MLR. J. Immunol. 132:2820.
17. Konig, R., L.Y. Huang, and R.N. Germain. 1992. MHC class II interaction with CD4 mediated by a region analogous to the MHC class I binding site for CD4. Nature (Lond.). 356:798.
18. Lamarre, D., A. Ashkenazi, S. Fleury, D.H. Smith, R.P. Sekaly, and D.J. Capon. 1989. The MHC-binding and gp120-binding functions of CD4 are separable. Science (Wash. DC). 245:743.
19. Clayton, L.K., M. Sieh, D.A. Fious, and E.L. Reinherz. 1989. Identification of human CD4 residues affecting class II MHC versus HIV-1 gp120 binding. Nature (Lond.). 339:548.
20. Greaves, D.R., F.D. Wilson, D. Lang, and D. Kiossis. 1989. Human CD2 3'-flanking sequences confer high-level T cell-specific, position-independent gene expression in transgenic mice. Cell. 56:979.
21. Lang, G., D. Wotton, M.J. Owen, W.A. Sewell, M.H. Brown, D.Y. Mason, M.J. Crumpton, and D. Kiossis. 1988. The structure of the human CD22 gene and its expression in CD22 transgenic mice. EMBO (Eur. Mol. Biol. Organ.). 7:1675.
22. Murphy, K.M., A.B. Heimberger, and D.Y. Mason. 1990. Induction by antigen of intrathymic apoptosis of CD4+CD8+ TCR\(\text{b}\) thymocytes in vivo. Science (Wash. DC). 250:1720.
23. Kappler, J., R. Kubo, K. Haskins, J. White, and P. Marrack. 1983. The mouse T cell receptor: comparison of MHC-restricted receptors on two T cell hybridomas. Cell. 34:727.
24. Yaque, J., M. Blackman, W. Born, P. Marrack, J. Kappler, and E. Palmer. 1988. The structure of V-alphal and J-alpha segments in the mouse. Nucleic Acids Res. 16:11355.
25. Widera, G., L.C. Burky, C.A. Pinkert, E.C. Bottger, C. Cowing, R.L. Palmiter, R.L. Brinster, and R.A. Flavell. 1987.
Transgenic mice selectively lacking MHC class II (I-E) antigen expression on B cells: an in vivo approach to investigate Ia gene function. Cell. 51:175.

26. Tomonari, K. 1988. A rat antibody against a structure functionally related to the mouse T-cell receptor/T3 complex. Immunogenetics. 28:455.

27. Bill, J., O. Kanagawa, D. Woodland, and E. Palmer. 1989. The MHC molecule I-E is necessary but not sufficient for the clonal deletion of V811-bearing T cells. J. Exp. Med. 169:1405.

28. Marrack, P., R. Shimonkevitz, C. Hannum, K. Haskins, and J. Kappler. 1983. The major histocompatibility complex-restricted antigen receptor on T cells. IV. An antidiotopic antibody predicts both antigen and I-specificity. J. Exp. Med. 158:1635.

29. Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Marrack. 1983. The major histocompatibility complex-restricted antigen receptor on T cells: I. Isolation with a monoclonal antibody. J. Exp. Med. 157:1149.

30. Landais, D., B.N. Beck, J. Buerstedde, S. DeGraw, D. Klein, N. Koch, D.B. Murphy, M. Pierres, D.H. Sachs, T. Tada, et al. 1986. The assignment of chain specificities for anti-Ia monoclonal antibodies using I cell transfectants. J. Immunol. 137:3002.

31. MacDonald, H.R., R.K. Lee, R. Schneider, R.M. Zinkernagel, and H. Hengartner. 1988. Positive selection of CD4+ thymocytes controlled by MHC class II gene products. Nature (Lond.). 336:471.

32. Acha-Orbea, H., W. Held, G.A. Waanders, A.N. Shakow, L. Scarpellino, R.K. Lee, and H.R. MacDonald. 1993. Exogenous and endogenous mouse mammary tumor virus superantigens. Immunol. Rev. 131:5.

33. Simpson, E., P.J. Dyson, A.M. Knight, P.J. Robinson, J.I. Elliot, and D.M. Altman. 1993. T-cell receptor repertoire selection by mouse mammary tumor viruses and MHC molecules. Immunol. Rev. 131:93.

34. Fowlkes, B.J., R.H. Schwartz, and D.M. Pardoll. 1988. Deletion of self-reactive thymocytes occurs at a CD4+CD8+ precursor stage. Nature (Lond.). 334:520.

35. MacDonald, H.R., A.L. Glasebrook, R. Schneider, R.K. Lee, H. Pircher, T. Pedrazzini, O. Kanagawa, J.F. Nicolas, R.C. Howe, and R.M. Zinkernagel. 1989. T-cell reactivity and tolerance to Mlsa-encoded antigens. Immunol. Rev. 107:89.

36. Killeen, N., S. Sawada, and D.R. Littman. 1993. Regulated expression of human CD4 rescues helper T cell development in mice lacking expression of endogenous CD4. EMBO (Eur. Mol. Biol. Organ.) J. 12:1547.

37. Wallace, V.A., A. Rahemtulla, E. Timms, J. Penninger, and T.W Mak. 1992. CD4 expression is differentially required for deletion of Mls-1a-reactive T cells. J. Exp. Med. 176:1459.

38. Davis, C.B., N. Killeen, M.E. Case Crooks, D. Raulet, and D. Littman. 1993. Evidence for a stochastic mechanism in the differentiation of mature subsets of T lymphocytes. Cell. 73:237.

39. Chan, S.H., D. Cosgrove, C. Wältzinger, C. Beuquiot, and D. Mathis. 1993. Another view of the selective model of thymocyte selection. Cell. 73:225.

40. Borgulya, P., H. Kishi, U. Muller, J. Kirberg, and H. von Boehmer. 1991. Development of the CD4 and CD8 lineage T cells: instruction versus selection. EMBO (Eur. Mol. Biol. Organ.) J. 10:913.

41. von Boehmer, H., and P. Kieneslov. 1993. Lymphocyte lineage commitment: instruction versus selection. Cell. 73:207.

42. Pantaleo, G., C. Grazier, and A.S. Fauci. 1993. The immunopathogenesis of human immunodeficiency virus infection. N. Engl. J. Med. 328:327.

43. Banda, N.K., J. Bernier, D.K. Kurahara, R. Kurkle, N. Haigwood, R.P. Sekaly, and T.H. Finkel. 1992. Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. J. Exp. Med. 176:1099.

44. Meyard, L., S.A. Otto, R.R. Jonker, M.J. Minijster, R.P.M. Keet, and F. Meijemra. 1992. Programmed death of T cells in HIV-1 infection. Science (Wash. DC). 257:217.

45. Groux, H., G. Torpier, D. Monté, Y. Mouton, A. Capron, and J.C Amiesien. 1992. Activation-induced death by apoptosis in CD4+ T cells from human immunodeficiency virus-infected asymptomatic individuals. J. Exp. Med. 175:531.

46. Siliciano, R.F., T. Lawton, C. Knall, R.W. Karr, P. Berman, T. Gregory, and E.L. Reinherz. 1988. Analysis of host-virus in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for CD4+ T cell depletion. Cell. 54:561.