CD4 Promotes Breadth in the TCR Repertoire
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A diverse population of MHC class II-restricted CD4 lineage T cells develops in mice that lack expression of the CD4 molecule. In this study, we show that the TCR repertoire selected in the absence of CD4 is distinct, but still overlapping in its properties with that selected in the presence of CD4. Immunization of mice lacking CD4 caused the clonal expansion of T cells that showed less breadth in the range of Ag-binding properties exhibited by their TCRs. Specifically, the CD4-deficient Ag-specific TCR repertoire was depleted of TCRs that demonstrated low-affinity binding to their ligands. The data thus suggest a key role for CD4 in broadening the TCR repertoire by potentiating productive TCR signaling and clonal expansion in response to the engagement of low-affinity antigenic ligands. The Journal of Immunology, 2001, 167: 4311–4320.
there is a substantial population of Th cells that completes development in the thymus and emigrates to secondary lymphoid tissue where it can engage in immune responses. These CD4-deficient T cells account for protective immunity against protozoal and viral challenges and they also direct the elaboration of diverse T cell-dependent Ab responses (33–36). Interestingly, although these cells have the capacity to differentiate into Th1 effector cells, they are significantly compromised in Th2 differentiation in multiple experimental settings (38–40).

In this paper, we have taken several approaches to determine how the CD4 molecule regulates the selection of the TCR repertoire in the CD4 lineage, both within and beyond the thymus. Using αβ and β TCR-transgenic mice, we have found that despite substantial overlap between the Ag-binding properties of TCRs selected with or without CD4, the absence of CD4 leads to a constriction of the TCR repertoire such that it is markedly deficient in TCRs that bind with weak affinity to their ligands. The data thus reveal the significance of CD4 in promoting breadth in the TCR repertoire and facilitating the recognition of low-affinity ligands.

Materials and Methods

**Mice, Abs, LACK/I-A^K, Fc, and flow cytometry**

CD4-deficient (Cd4^{−/−}) mice (33) were backcrossed onto the C57BL/6 background (H-2b) for 13 generations. C57BL/6 Cd4^{−/−} mice were subsequently crossed to B10.D2 mice and then intercrossed to produce homozygous H-2b offspring. The αβ Tva-transgenic mice were generated from B6/CBA F_{1} eggs by pronuclear injection of a modified form of transgene b (41) in which the human CD2 cDNA was replaced with a Tva cDNA (42). Transgenic founders and their offspring were screened for the presence of the transgene by Southern blot and/or FACS analysis. Founders were bred onto the C57BL/6 background and then crossed to H-2b Cd4^{−/−} mice. Tva-transgenic mice were mated with Cd4^{−/−} Tva-transgenic mice. TC-RI−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed in the Par

Abbreviations were purchased from BD PharMingen (San Diego, CA) and Caltag Laboratories (Burlingame, CA) or were purified from supernatant fluids of hybridoma cell lines. Single-cell suspensions of lymphocytes (0.2–1×10^6 cells) were incubated for 30 min on ice in a final volume of 25–50 μL of PBS containing 0.3% BSA, 0.01% NaN_3, and diluted Abs. The cells were washed once in the same buffer, stained with secondary reagents as necessary, washed again, and then analyzed using a BD Biosciences FACScan flow cytometer and CellQuest software (Mountain View, CA). Expression of the Tva protein was detected using an envelope-rabbit IgG fusion protein, SuA-rabbit IgG (43), and donkey anti-rabbit Ig-GFTC secondary Ab (The Jackson Laboratory). For TCR repertoire analysis, pooled lymph node cells were stained with FITC-anti-Thy1.2, TC-anti-CD8a, TC-anti-B220, TC-anti-Mac-1, and biotinylated anti-αβ or anti-β Abs. LACK/I-A^K Fc-expressing LMS2 Drosophila cells (44) were grown in Drosophila SFM medium (Life Technologies, Rockville, MD). The LACK/I-A^K fusion protein was purified from cell culture supernatants using an anti-I-A^K (clone 5S114) affinity column followed by a Mono-Q ion exchange column (Amersham-Pharmacia Biotech, Piscataway, NJ). To stain 2×10^6 hybridoma or primary T cells, 4 μg of purified LACK/I-A^K Fc protein was mixed with 1 μL of 0.5 mg/ml protein A-Alexa 488 (Molecular Probes, Eugene, OR) at room temperature for 30 min. Immediately before staining, the above mixture was combined with 0.5 μL of normal mouse serum and 0.5 μL of normal rat serum. Hybridoma cells were stained at 37°C for 1–3 h and primary T cells were stained on ice for 1 h with intermitting mix.

**LACK-specific T cell hybridomas**

WT and Cd4^{−/−} H-2b mice were immunized in their footpads with 30 μL of 0.5 mg/ml recombinant LACK protein in CFA. Polymt lymph nodes were harvested 10 days after immunization. Lymph node cells were activated in vitro with syngeneic irradiated (2000 rad) spleen cells and 20 μg/ml LACK^{156–171} peptide (ICFSPSLEHPVVSQWD). Three to 4 days later, 2×10^4 hybridoma cells were fused to BW5147 cells to generate hybridomas following standard protocols (45). The resulting hybridomas were screened for CD3ε and CD4 expression by FACS analysis and tested for IL-2 secretion in response to stimulation with the LACK peptide. CD4 loss variants of WT hybridomas were generated by depletion with anti-mouse CD4 magnetic beads (Dynal Biotech, Great Neck, NY). CD4-expressing variants of hybridomas derived from CD4-deficient mice were generated by infection with a CD4 retroviral vector. The retroviral particles were generated by cotransfection of 293-T cells with SVPS-E-MLV and pBabe-Puro-CD4 as previously described (10). The CD4^{−} and CD4^{+} variants of wild-type (designated WT and WT-4 respectively) and CD4-deficient hybridomas (designated KO+4 and KO, respectively) were sorted for equivalent surface TCR and CD4 expression using a BD Biosciences FACS Calibur sorting machine.

Briefly, 1×10^9 hybridoma cells were stimulated with 0–15 μg/ml LACK peptide or an irrelevant peptide OVA^{323–336} (ISQAVHAAHAEINE) in flat-bottom 96-well plates. Either 1×10^5 syngenic irradiated (2000 rad) spleen cells, or 1×10^5 I-A^{−/−}-transfected L cells (44/14.B5) (2) were used as APC. Alternatively, 1×10^5 hybridoma cells were stimulated with plate-bound purified LACK/I-A^K Fc protein at 0.5–200 ng/ml in flat-bottom 96-well plates. Tissue culture supernatant was removed 24 h after stimulation and IL-2 content was determined by sandwich ELISA using anti-mouse IL-2 Abs (BD PharMingen).

**TCR sequence analysis**

Total RNA was isolated from individual hybridomas and converted into cDNA using SuperScript II reverse transcriptase and random hexamers (Life Technologies). PCR amplification was performed on hybridoma cDNA using a Vβ4 primer 5′-GCC TCA AGT GGC TTC CAA CCT C3′ and a Cβ2 primer 5′-ATT GTC CTC GTC TTA GCC GTC CTG AC3′. PCR products were gel purified and sequenced using a nested Vβ4 primer, 5′-AGA ACG TCA GAT CAC AC3′. To obtain TCRs sequences, multiple PCR amplifications were performed on each hybridoma cDNA sample using a Cα primer NJ1105, 5′-GGC CCC ATT GTC CTT GGC ATC 3′; and one of the Vα-specific primers; Vα1, 5′-CAG CAG AGC CCA GAA TAC CTC 3′; Vα2, 5′-ACC TCA AAT AAA AAG GAG AAA GAC 3′; Vα3, 5′-CTG TAC TAT TCC GTG GCA CAC GTT GTC 3′; Vα4, 5′-AGG AGC AGG AGT TTA GGA AAT TCA 3′; Vα5, 5′-AAG ATT TTC TTA AGT GAG TCA GAT 3′; Vα6, 5′-AGT ATG GTC CTG ATT GGC TTC GAC 3′; Vα7, 5′-CGA ACA AGC TCT TCT ACT GCA AAA GAG 3′; Vα8, 5′-ACA AGG AAC AAG AGC GAG CAC CAA GG 3′; Vα9, 5′-CA AAG GCA AGC TTC GAC 3′; Vα10, 5′-CTG ACA TCC ACC ACA GTC ACT AAG GAA CGT 3′; Vα11, 5′-AAT GGG AGG TTA AAG TCA ACA TTC AAT 3′; Vα12, 5′-GTG GCA TCT CTG TTT ATC GCT GAC CCG 3′; Vα13, 5′-CGT ACA ATT AAT AGA GAA AAG CAC ACC AAG 3′; and Vα14, 5′-CGT GTG GAC CAC AAA GAC AAG ACC AAT 3′. PCR products were further amplified with the same Vα primer and a Cα primer, NJ109, 5′-AA TCA GAT TGG GGA GTC 3′. PCR products were purified from the second round of amplification were gel purified and sequenced with a third Vα primer NJ110, 5′-CAG GCA GAG GGT GCT GTC C3′.

**Conjugation formation assay**

LACK-specific T cell hybridomas were labeled with 667 nM CFSE (Molecular Probes) 16 h before analysis (46). At the same time, 2PK-3 cells were labeled with PKH26 according to the manufacturer’s (Sigma) instructions. The 2PK-3 cells were then loaded overnight with various concentrations of LACK peptide in complete DMEM supplemented with 25 mM HEPES. Twenty microliters of the LACK-specific hybridoma cells (10^5 cells/ml) was mixed with 20 μL of loaded 2PK-3 cells (10^5 cells/ml) and then incubated at 37°C for varying amounts of time. At the end of the incubation period, the cell mixture was vortexed for 10 s to disrupt nonspecific conjugation, transferred to 0.5 ml of FACS buffer, and analyzed on a BD Biosciences FACSscan flow cytometer using CellQuest software.

**Generation of LACK-specific TCR-transgenic mice**

LACK-specific TCR cDNAs were cloned from the WT15, KO15, and KO23 hybridomas using a PCR strategy. The TCRα chain cDNAs were amplified with a Vβ5 primer, 5′-CCG GGA TAC GCC GGC ATG ACG AC3′ and a Cα primer, 5′-CGG GGA TAC GCC GGC ATG ACG AAC AAG 3′. The TCRβ cDNAs were amplified with a Vβ4 primer, 5′-CCG GGA TAC GCC GGC ATG ACG AAC AAG 3′ and a Cβ2 primer, 5′-CCG GGA TAC GCC GGC ATG ACG AAC AAG 3′. PCR products were then ligated into a construct (i) 41). The resulting hybridomas were then generated by pronuclear coinjection of CD2-TCRα and CD4-TCRβ constructs into B6/DBA/F1 eggs. WT15β, KO15αβ, and KO23αβ-transgenic founders were identified by Southern blot and FACS.
The cells were washed twice and stained with FITC-anti-TCR for 45 min on ice with intermittent mixing. The cells were subsequently washed twice and then stained with FITC-anti-TCR for 45 min on ice in the dark. After additional washes, the cells were resuspended in PBS/0.3% BSA/0.01% NaN₃ containing 1 μg/ml propidium iodide and then analyzed using a BD Biosciences FACScan flow cytometer and CellQuest software.

For the TCR-ligand dissociation analysis, 4.8 μg of LACK/I-A^d Fc protein was mixed with 0.84 μl of 0.5 mg/ml protein A-Alexa 488 (Molecular Probes) on ice for 30 min before adjusting the volume to 50 μl by addition of 2.5 μg of mouse IgG2a (Sigma) in PBS/0.3% BSA/0.01% NaN₃. Briefly, 0.3 × 10⁸ popliteal lymph node cells were then mixed with 20 μl of various dilutions of this staining reagent for 1 h on ice with intermittent mixing. The cells were washed twice and stained with FITC-anti-TCR, TC-anti-CD8, TC-anti-CD4, TC-anti-B220, TC-anti-Mac-1, and TC-anti-TCR-γδ for 20 min on ice in the dark. After additional washes, the cells were resuspended in PBS/0.3% BSA/0.01% NaN₃ containing 1 μg/ml propidium iodide and then analyzed immediately using a BD Biosciences FACScan flow cytometer and CellQuest software.

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Results

TCR repertoire diversity in CD4 lineage T cells selected without CD4

Helper T cell function in Cd4^-/- mice can be attributed to a small population of peripheral MHC class II-restricted lymph node cells that are CD3^- and CD4^-CD8^+. These cells can be assigned to the CD4 lineage on the basis of their functional properties and their expression of reporter transgenes or targeted insertions controlled by Cd4 regulatory elements (49, 50). To survey the TCR repertoire expressed by CD4 lineage cells selected in the absence of CD4, we initially used a panel of mAbs specific for individual TCR Vc and Vβ domains. CD4 lineage T cells were identified by their lack of CD8 and their expression of either Thy-1 or a transgenic CD4 lineage reporter (the chicken receptor for avian leukosis virus under the control of the murine Cd4 promoter, enhancer, and silencer elements; Refs. 41 and 51).

As shown in Fig. 1, regardless of CD4 expression, a diverse TCR repertoire was expressed by CD4 lineage T cells selected on either H-2^d or H-2^b backgrounds. Nevertheless, despite the apparent diversity of the TCR repertoire, there were some notable differences between the usage of certain Vc and Vβ elements by cells selected in the presence or absence of CD4. Specifically, the populations differed in their relative usage of Vc3.2, Vβ5, and Vβ12 in H-2^d mice and the usage of Vc3.2, Vβ3, Vβ5, and Vβ10 in H-2^b mice. The observed differences in the TCR repertoire were specific to the CD4 lineage because they were not observed in CD8^- T cells from Cd4^-/- and Cd4^+/+ mice (Fig. 1, B and D).
junctions. These residues are likely to participate in a direct interaction with histidine at position 164 in the LACK peptide and are therefore crucial for the Ag specificity of the TCRs (53). Hybridomas from CD4-deficient mice showed a similarly high frequency (20 of 21) of acidic residues at the V-D junctions of their TCRs, whereas those from CD4+/− mice did not express CD4 (KO, dashed lines). B. LACK/I-A^d Fc concentrations required for half-maximal IL-2 production by hybridomas that had been sorted for comparable CD4 and TCR expression. The left and right panels show the responses made by cells that did or did not express CD4, respectively. Variant hybridomas were derived from WT hybridomas that had lost CD4 expression spontaneously (WT-4) or from CD4−/− hybridomas (KO) that had gained it by retroviral transduction (KO+4).

T cells can form conjugates in vitro with Ag-presenting B cells in a fashion that is both Ag dependent and can also be sensitive to the involvement of cell adhesion/signaling proteins such as CD2 (54, 55). We therefore sought to determine whether the enhanced sensitivity demonstrated by KO LACK-reactive TCRs might affect their capacity to promote the Ag-dependent formation of T cell/B cell conjugates. LACK-specific hybridomas that had been sorted for comparable surface TCR and CD4 expression were labeled with the green fluorescent dye CFSE. As APC, we used the H-2^d B cell lymphoma 2PK-3 labeled with the red fluorescent dye PKH26 and loaded with the LACK peptide. The labeled hybridomas and 2PK-3 cells were mixed at 37°C and the formation of conjugates was followed over time by FACS analysis (Fig. 3A). Conjugate formation between LACK-specific hybridomas and LACK-presenting 2PK-3 cells was peptide specific and dose and time dependent (data not shown). Conjugation was also potentiated by the presence of CD4, as CD4-expressing hybridomas formed conjugates more rapidly than their CD4−/− counterparts when they were matched for CD4 expression (compare WT-4 vs KO, p = 0.003, or WT vs KO+4, p = 0.044, in Fig. 2B).
effective than WT TCRs at driving conjugation between a T cell and its target. Thus, together with the Ag response data in Fig. 2, the conjugation data revealed a modest selection for enhanced reactivity among Ag-specific TCRs selected in the absence of CD4. Nevertheless, both datasets show overlap between the properties observed for TCRs selected in the presence vs the absence of CD4.

Analysis of TCRs selected in Cd4−/− mice expressing a transgenic LACK-reactive TCRβ chain

In search of a way to enhance the statistical power of the above studies and also to examine additional differences between Cd4+/+ and Cd4−/− TCRs, we used the PCR to clone cDNAs encoding LACK-reactive TCR chains from the T cell hybridomas. We then used these TCR cDNAs to generate lines of transgenic mice expressing either αβ TCR heterodimers from the KO15 and KO23 hybridomas or a TCRβ chain from the WT15 hybridoma (without its partner TCRα chain). αβ TCR transgenic mice were used to address the specific question of whether TCRs selected in the absence of CD4 could also be selected in its presence (see below). In contrast, the TCRβ-transgenic mice were generated to increase the frequency of LACK-reactive precursor T cells in mice and thereby to facilitate immunization experiments (56). WT15β mice expressed the LACK-reactive Vβ4 transgene in all αβ lineage T cells, but the majority of such cells were not LACK-reactive because they did not express LACK-reactive TCRα chains. WT15β transgenic animals were backcrossed to H-2d CD4-deficient mice to produce cohorts of transgenic offspring that differed in whether they did or did not express CD4.

Immunization of the TCRβ (WT15β)-transgenic mice led to rapid and substantial clonal expansion of LACK-reactive T cells in the lymph nodes draining the sites of immunization. These LACK-reactive cells could be readily detected by flow cytometry using a fluorescent I-A<sup>d</sup>/LACK reagent (44), which is structurally distinct but functionally similar to a MHC/peptide tetramer (Fig. 4A). LACK-reactive T cells from immunized WT15β-transgenic Cd4−/− mice showed an I-A<sup>d</sup>/LACK staining distribution that overlapped with the distribution observed for T cells from immunized CD4-expressing control mice. Despite this overlap, however, the mean fluorescence intensity of the positively stained population was reproducibly higher in the absence of CD4 than in its presence (Fig. 4B). These data suggested that the absence of CD4 selected against T cells that fell at the low end of the positively stained distribution. Because staining intensity with multimeric MHC/peptide reagents such as LACK/I-Ad is determined by TCR-ligand interaction kinetics, the data implied that the absence of CD4 produced a constriction of the Ag-specific TCR repertoire at the expense of TCRs that bound their ligands with low affinity and/or fast dissociation rates.

By Scatchard analysis, we found that the LACK-reactive TCRs expressed by T cells selected in the absence of CD4 were characterized by higher average apparent K<sub>D</sub> values than the TCRs that...
were selected in the presence of CD4 (Fig. 4C). Following procedures similar to those used previously by Savage et al. (57), we also examined the rate at which the LACK/I-A^d reagent dissociated from the surface of the CD4^+ or CD4^- LACK-reactive T cell populations (Fig. 4D). This analysis revealed a reproducible difference in the dissociation rates measured over the first 3 min of the assays, such that the CD4^+ population exhibited more rapid decay in mean fluorescence intensity than the CD4^- population during this time period (Fig. 4, D and E). At later time points, the decay curves were essentially parallel with little evident difference in the slopes from 3 to 30 min of the assays (Fig. 4E). These patterns of dissociation were consistent with the fact that the LACK/I-A^d staining profiles overlapped considerably at the high end of the staining distributions (Fig. 4B) wherein TCRs with the slowest dissociation rates should be found. By contrast, the LACK/I-A^d staining profile for CD4^- mice was noticeably enriched for weaker staining cells that would be expected to show rapid dissociation kinetics. It is the presence of these latter cells in the CD4^- population that we had cloned from the T cell hybridomas. Multimeric MHC/peptide reagents (58, 59), the LACK/I-A^d preparation was useful in informing selectively on the ligand-binding kinetics of Ag-specific TCRs and, as shown here, it revealed a reproducible constriction of the range of TCR-binding kinetics that was selected in the absence of CD4.

**Selection of CD4^- TCRs in CD4-expressing transgenic mice**

As an alternative approach for examining differences between TCRs selected in the presence and absence of CD4, we attempted to determine whether the properties of CD4^- TCRs might be sufficiently distinctive as to preclude their inclusion in a normal CD4-expressing TCR repertoire. Here, we were interested in exploring the possibility that the absence of CD4 might impose selection for a type of TCR that would normally be excluded from the TCR repertoire because of overly strong interactions with thymic peptide/MHC class II ligands. As mentioned above, we therefore generated transgenic mice expressing two of the KO TCR heterodimers that we had cloned from the T cell hybridomas. Multiple transgenic founders were obtained for both of the TCRs and several of these were bred to create TCR-transgenic H-2^d CD4^- and CD4^+ control mice.

In the presence of CD4, both of the transgenic TCRs allowed for efficient development of CD4 lineage cells (Fig. 5). The majority of mature CD4 lineage cells in the thymi and periphery of these
mice expressed both chains of the transgenic TCRs on their surfaces, as shown by staining with the LACK/I-A<sup>d</sup> reagent. Complementary analysis of TCR<sup>α<sup>+/−</sup></sup> TCR-transgenic mice showed that both TCR heterodimers could allow for positive selection of CD4<sup>+</sup> T cells without the involvement of endogenous TCR<sup>α<sup>+</sup></sup> chains (Fig. 5). T cells from both strains mounted robust IL-2 responses when they were stimulated with the LACK peptide in vitro, although the KO15 T cells were reproducibly more responsive than cells from KO23 mice (data not shown). Thus, despite their distinctive in vitro properties, these experiments demonstrated that the KO TCRs could be selected into the Th cell repertoire in the presence of CD4.

In the absence of CD4, we found that the KO15 TCR but not the KO23 TCR was inefficient at promoting selection of CD4 lineage T cells (Fig. 5). Thus, there were very few CD8<sup>+</sup> mature CD4 lineage cells in the thymi and periphery of transgenic mice expressing the KO15 TCR and most of their peripheral T cells were CD8<sup>+</sup>. By contrast, the KO23 TCR allowed for more efficient CD4-independent selection of CD4 lineage cells than KO15, with the frequency of thymic CD4 lineage cells approaching that observed in the presence of CD4. To confirm that the CD8<sup>+</sup> population of T cells in the KO23 TCR-transgenic mice included cells that had passed through the equivalent of the double-positive stage, we analyzed the methylation status of the <i>Cd8</i> gene. This analysis

**FIGURE 5.** Positive selection of T cells expressing LACK-specific αβ TCR transgenes derived from <i>Cd4<sup>−/+</sup></i> mice. The FACS plots show the cell surface phenotype of mature (CD24<sup>−</sup>) thymocytes and lymph node T cells (B220<sup>−</sup>Mac-1<sup>−</sup>) from TCRα<sup>−/+</sup> (A) or TCRα<sup>−/−</sup> (B) KO15 and KO23 αβ TCR-transgenic mice. T cells were costained with PE-conjugated Abs specific for CD4 and CD8, anti-MAC-1, B220, and LACK/I-A<sup>d</sup>. T cells expressing CD4 can be distinguished from those expressing CD8 because the latter show brighter mean fluorescence intensity. CD4 lineage T cells in CD4<sup>−/+</sup> mice fall in the CD4<sup>−</sup>CD8<sup>−</sup> double-negative quadrant. Thymocytes were stained with anti-CD8, anti-CD24, and LACK/I-A<sup>d</sup>. Each plot is representative of at least two individual determinations on mice of the same genotype. C, Numbers of LACK/I-A<sup>d</sup>-labeled CD24<sup>−</sup> thymocytes and T cells of the indicated CD4/CD8 phenotypes in TCR-transgenic mice of the specified genotypes.
showed that the Cd8 gene was substantially demethylated in the CD4+/Cd8− T cells in Cd4+/Cd8− KO23 TCR-transgenic mice (data not shown). Such data indicate that the Cd8 gene was previously active in the DN cells and would be consistent with them passing through the equivalent of the CD4+/CD8− double-positive stage of thymocyte development (60) before completing their development into CD4+ lineage cells. Thus, the results suggest that the KO23 TCR could allow for positive selection of CD4 lineage T cells in the absence of CD4.

Discussion

In the few cases in which it has been measured, the affinity of CD4 for MHC class II molecules has appeared to be very low, with an apparent Kd for the interaction being on the order of 10−4 M for mouse CD4 (61, 62) and 10−6 M for human CD4 (1). This low affinity would predict a modest (largely p56lck-dependent) contribution of CD4 to Ag recognition, such as the 5- to 10-fold enhancing effect presented here (Fig. 2B) and elsewhere (63). It would also be consistent with the observation that a substantial fraction (20–25%) of precursor cells can successfully commit to the helper cell lineage in mice that do not express CD4 (34, 35). It is obvious, nonetheless, that the absence of CD4 impairs the ability of many cells to commit to the CD4 lineage, indicative of atypical TCR selection that can be visualized simply by comparing the relative usage of TCR variable domains in peripheral CD4 lineage T cells of WT and Cd4+/Cd8− mice (Fig. 1).

Despite abnormal selection of TCRs in Cd4+/Cd8− mice, we have found evidence for substantial overlap between the TCR repertoires that are selected in these and WT mice. This was initially apparent in the properties of hybridomas derived from Cd4+/Cd8− mice—these being distinctive collectively, but nonetheless still largely overlapping in their properties with those derived from WT mice. In other experiments, we also found that Cd4+/Cd8− αβ TCRs could be positively selected in the presence of CD4. This latter outcome would not be expected if the absence of CD4 selected only for TCRs that bound their thymic ligands with peculiarly high affinity, such that signaling from them would cause negative selection when enhanced by the involvement of CD4. In experiments using TCRαβ-transgenic mice, we found that the kinetic properties exhibited by Ag-specific Cd4+/Cd8− TCRs clearly overlapped with those of TCRs selected in CD4−expressing mice, with there being no obvious selection for abnormally high-affinity TCRs in the absence of CD4. Nevertheless, these last studies showed that the loss of CD4 resulted in a constriction of the Ag-specific TCR repertoire such that it was depleted of receptors that bound their ligand with low affinity. In aggregate, therefore, the data suggest that the function of CD4 is most important when TCRs demonstrate weak binding to their ligands.

A role for CD4 in potentiating the recognition of low-affinity TCR ligands has been suggested by several previous studies (40, 64–66). Nevertheless, such a role has not been obvious in all circumstances (e.g., Ref. 63), prompting the conclusion that the significance of CD4 in promoting Ag recognition depends largely on the nature of the TCR and the ligand being recognized (64). Thus, in some cases, it seems reasonable that the kinetics of the TCR-ligand interaction will presumably not allow adequate time for efficient recruitment of CD4 to the TCR-ligand complex and/or, even if CD4 is recruited, the complex may dissociate too quickly for a signal to be efficiently propagated. In other cases, the kinetics of the TCR-ligand association will be sufficiently favorable to allow time for CD4 to make a meaningful contribution to the signaling process. It is unclear, however, whether this contribution would come primarily by way of CD4-dependent recruitment of p56lck or whether CD4 is also important in stabilizing the extra-cellular engagement between the TCR and its ligand. Although microscopic data have not provided obvious support for this latter type of contribution (29), there are supportive data from studies using forms of CD4 that do not bind to p56lck and yet retain coreceptor function that is demonstrably significant for the recognition of low-affinity ligands (14, 40, 64). Whether stabilization of the TCR-ligand complex by CD4 is important throughout, or primarily at the onset of the TCR signaling process, remains to be determined and may not be easily addressed microscopically, particularly for low-affinity ligands that do not appear to induce large-scale reorganization of surface molecules at the T cell-APC interface.

Inefficient recognition of low-affinity ligands by TCRs in the absence of CD4 provides a potential explanation for the observation that the survival and homeostatic expansion of CD4 lineage cells is impaired in Cd4+/Cd8− mice (37, 67). Several studies have pointed to a necessary role for ligand-dependent TCR signaling in the proliferation of T cells that occurs when they are transferred into T lymphopenic environments (68–73). Although impaired proliferation is the most consistent observation that has been made, there are also several examples of naive T cells showing reduced survival when they are deprived of ligands for the TCRs they express or when signaling from their TCRs is inhibited. For instance, loss of p56lck impairs proliferation but not survival of T cells (74), whereas extrathymic absence of MHC class II expression in mice causes progressive Th cell depletion indicative of a survival defect (75, 76). Proliferation and survival in this general context are likely to be dependent on TCR recognition of low-affinity (MHC/self-peptide) ligands that do not normally induce overt T cell activation and immune responses. Given the nature of the repertoire constriction described here, the recognition of these low-affinity (MHC/self-peptide) ligands would be expected to be inefficient in the absence of CD4, leading to suboptimal delivery of TCR signals and consequently compromised T cell survival and “homeostatic proliferation.” Thus, by facilitating the recognition of self-peptides presented by MHC class II molecules, CD4 performs an essential role in regulating extrathymic selection of the TCR repertoire.

Previous work has shown that CD4-deficient Th cells manifest a defect in Th2 differentiation (38–40). Although this defect is not insurmountable (C. Peña Rossi and N. Killeen unpublished observations and Ref. 77), it is nonetheless profound and therefore suggestive of an important role for CD4 in allowing a form of TCR signaling that is permissive for the Th2 fate. Such a role would in turn be consistent with the results of several studies that have established correlations between TCR signaling and selection for a Th1 or Th2 fate (78, 79). The data presented in this paper offer some additional insight into a possible mechanism by which CD4 may potentiate Th2 differentiation, particularly when considered in the context of a recent study showing that Leishmania (LACK)-specific Th2 cells selected in TCRαβ-transgenic mice typically express TCRs that bind their ligands with lower affinity and faster dissociation kinetics than Th1 cells (44). By impairing clonal expansion of T cells bearing low-affinity TCRs, the absence of CD4 would incur a defect in T cells expressing the type of TCRs that might be more typically associated with the Th2 phenotype. In contrast, the retention of high-affinity TCRs in Cd4+/Cd8− mice presumably accounts for the fact that these mice retain the ability to make Th1 responses (34). It remains to be established, however, whether the defect in Th2 cells expressing low-affinity TCRs reflects a homeostatic or thymic depletion of precursor cells, or a failure primarily at the level of clonal expansion.

In conclusion, the results of this study reveal an important role for CD4 in allowing for breadth in the TCR repertoire. This role
involves the facilitation of the clonal expansion of T cells expressing TCRs that bind their ligands with low affinity and has the potential to explain a defect in T cell survival and homeostatic proliferation caused by loss of CD4. It also offers a clue to the mechanism by which the CD4 molecule enhances Th2 differentiation. Thus, the study makes clear the importance of CD4 in promoting immunological fitness and functionality beyond the simple augmentation of Th cell responsiveness.

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