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References

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Self-Restricted Dual Receptor Memory T Cells

William T. Lee, Vaibhavi Shiledar-Baxi, Gary M. Winslow, Denise Mix, and Donal B. Murphy

Enhanced immune responses during secondary exposure to Ag result from the development of memory cells. In the present report we show that stimulation through one receptor on dual receptor CD4 cells can promote the generation of T cells capable of giving a memory response through the second receptor, even though the cells had not been previously exposed to the Ag recognized by the second receptor. Cloned cells generated from dual receptor memory T cells proliferated and secreted the same lymphokines after stimulation with either Ag, independent of both Ags by distinct TCRs was shown by production of variants that had lost either Ag specificity along with the corresponding TCR. Recognition of both Ags is MHC restricted, since the cells recognize Ag presented by self, but not non-self, MHC class II molecules. These results raise the possibility that one potential mechanism of maintaining specific memory to a given Ag is through stimulation by an unrelated Ag via the second TCR. The Journal of Immunology, 1998, 161: 4513–4519.

Current models of Ag-stimulated T cell differentiation suggest that naive (virgin) T cells can be stimulated to become short-lived effector cells or long-lived memory cells (reviewed in Refs. 1–4). Comparative studies of virgin and memory T cells have been facilitated by the use of markers, such as CD45 isoforms, which are believed to distinguish between the two cell types. In the mouse, high expression of the CD45RB isoform on resting CD4+ T cells is characteristic of naive cells, whereas resting memory cells are CD45RBlow (5). Using this marker to separate virgin and memory T cells, functional differences with respect to activation, lymphokine secretion, B cell helper ability, and homing have been observed (5–10).

Difficulties in studying T cell differentiation under Ag-specific conditions have been circumvented through the use of TCR transgenic mice. We have previously reported that DO11.10 TCR-αβ-transgenic mice (11) have clonotype-positive, OVA-specific memory T cells in the absence of prior exposure to OVA (12). Based upon further analyses showing that many of the T cells in these mice express two distinct TCR α-chains and that DO11.10 × RAG−/− mice lacked such memory cells, we postulated that these cells had probably arisen in response to antigenic stimulation of a secondary TCR, comprising of the transgenic β-chain paired with a nonallelically excluded, endogenous TCR α-chain. However, because the specific Ag(s) that had promoted memory development was unknown and because of recent demonstrations that memory cells can nonspecifically develop in response to inflammatory cytokines (13–15), we wished to show that dual receptor expression can act to increase the breadth of memory responses.

In the present report we show that immunization with a known Ag, keyhole limpet hemocyanin (KLH), can lead to memory responses to an unrelated Ag (OVA) via a second TCR. T cell clones were generated after isolating memory cells from KLH-immune DO11.10 mice. The clones expressed two TCRs, as evidenced by comparable TCR β-chain expression but reduced clonotype expression compared with single TCR-bearing DO11.10 cells. The specificities of both TCRs were defined, as the clones responded to both KLH and OVA presented by self-MHC class II molecules. Finally, we have confirmed that the recognition of the two Ags is mediated by distinct TCRs through the analysis of hybridoma variants, derived from the dual reactive clones, that had lost one or the other TCR.

The presence of peripheral T cells that express two distinct TCR specificities is not limited to transgenic mice. Studies have shown that humans and nontransgenic mice may have significant numbers of dual receptor cells (16, 17). Thus, TCR transgenic mice can be used as a model for this general phenomenon. Further, it has been proposed that dual receptor cells might contribute to autoimmune disease if one of the TCRs can recognize self Ag (16). Because memory cells have different avidities, activation requirements, and recirculation routes than virgin cells, we propose that the generation of dual reactive memory cells, where one of the TCRs is autoactive, may be a potential means for bypassing normal tolerance mechanisms of naive T cells that prevent autoimmunity. In addition, we speculate that stimulation by an unrelated Ag via the second TCR may be one mechanism for maintaining memory to an Ag recognized by the first receptor.

Materials and Methods

Animals

BALB/c ByJ (H2b), BALB.B/LiMrp (H2o), BALB.K/LiMrp (H2o), BALB.S/Mrp (H2o), and DO11.10 (11) mice were bred and maintained at the Wadsworth Center Animal Core Facility (Albany, NY). The DO11.10 mice were originally obtained from Dr. D. Loh (Washington University, St. Louis, MO). Unless otherwise indicated, the experiments were performed using 6- to 8-wk-old mice.

Reagents and Abs

Chicken OVA Asys 139 was synthesized and supplied by the Wadsworth Center Peptide Synthesis Core Facility. Polyclonal rabbit anti-mouse Ig was prepared by affinity purification on mouse Ig-Sepharose columns. mAbs GK1.5 (18) and 2B6 (19) (anti-CD4), 3.155 (anti-Lyt-2 (CD8)) (20),
Preparation of cells

Memory T cells from DO11.10 mice were prepared as previously described (12). Briefly, mice were immunized 6 wk before cell isolation by i.p. injection of 100 μg of KLH emulsified in CFA. Splenocytes from DO11.10 Tg mice that had been primed 6 wk previously with KLH in CFA were isolated. After enrichment for CD4+ T cells by depletion of rabbit anti-mouse Ig-coated B cells using goat anti-rabbit Ig-coated magnetic beads (Advanced Magnetics, Cambridge, MA), followed by complement-mediated lysis of residual B cells, macrophages, and CD8+ T cells with, respectively, anti-J11d, anti-MAC-1 plus MAR18.5, and anti-CD8. Baby rabbit serum (Wadsworth Center Animal Core Facility) was used as a source of complement. The CD4+ T cells were incubated with mAb 23G2 supernatant, and memory T cells were prepared by collecting the CD45RBlow cells after magnetic bead separation using the miniMACs system (Miltenyi Biotec, Sunnyvale, CA) and magnetic goat anti-rabbit Ig beads. APCs were prepared by T cell depletion of splenocytes using anti-Thy1.2 and complement followed by anti-CD4 (mAb 2B6) and anti-CD8 plus complement. Except for cell cloning, APCs were treated with mitomycin C (25 μg/ml) for 20 min at 37°C. For cell cloning, APCs were irradiated (3000 rad).

Generation of KLH-specific T cell clones and hybridomas

CD4+ memory cells from KLH-immune DO11.10 mice were stimulated with KLH (75 μg/ml) in the presence of exogenous rIL-2 (300 U/ml) and irradiated APCs. After 14 days, the cells were restimulated with APCs, OVA233-339 (0.1 μg/ml), and rIL-2. After two further restimulations with KLH, the cells were cloned by limiting dilution at 0.3 cells/well in the presence of APCs, KLH, and IL-2. In two separate cloning experiments (288 wells/experiment), the frequencies of wells that showed positive cell growth were 0.11 and 0.21, respectively. Multiple clones were randomly picked and expanded. T cell clones were maintained in tissue culture medium (RPMI 1640 medium supplemented with 10% FBS (Life Technologies, Grand Island, NY), 50 μM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, and 50 μg/ml gentamicin) and were stimulated every 2 to 3 wk with KLH. To generate T cell hybridomas that had lost specific TCR α-chains, selected T cell clones were fused to the BW-5417 thymoma cell line (28) using standard procedures. Because the construct used to generate DO11.10 mice contained the KJ1-26 TCR α- and β-chain genes, we wished to ensure that the loss of the KJ1-26 TCR α-chain was not accompanied by the loss of the KJ1-26 TCR β-chain. Hence, we first transfected the BW-5417 thymoma parent with the plasmid, pBDWMCβ2, which encodes the DO11.10 TCR β-chain (29) (a gift from Dr. A. Pullen, University of Washington, Seattle, WA). After fusion, hybridoma cells were cloned by limiting dilution and were maintained in the tissue culture medium described above, except containing 15% FBS.

Cell culture, proliferation, and lymphokine secretion

To test for functional responses, the T cell clones (nonhybridoma) were first rested in rIL-2-containing medium for 14 days, then washed and rested in medium without IL-2 for 2 days. Viable cells were collected by density gradient centrifugation over Ficoll. T cell clones or hybrids (1 × 10^6/well) were cultured in 96-well U-bottomed clusters (Corning Costar, Corning, NY) in 0.2 ml of tissue culture medium that contained exogenous APCs (2 × 10^5/well) and KLH, SEB, OVA, or OVA233-339 as indicated. For proliferation assays, the cells were cultured in duplicate wells for 3 days followed by a 12-h pulse with [3H]ThdR (1 μCi/well). Cells were harvested using a 96-well automated harvester, and radioactivity was measured using a BetaPlate (Wallac, Gaithersburg, MD). For measurement of secreted lymphokines, supernatants were collected after either 48 h (T cell hybrids) or 24 h (T cell clones) and analyzed by ELISA (IL-4) (30) or bioassay with HT-2 cells (IL-2) (31).

Immunofluorescence staining and analysis

Fluorescence staining was performed at 4°C in 100 μl containing 1 × 10^6 cells and a predetermined optimal amount of FITC-conjugated mAb in balanced salt solution containing 2% FBS, 20 mM HEPES, and 0.1% NaN3. Flow cytometric analyses of stained cells were performed using a BD-FACScan (Becton Dickinson, Mountain View, CA). As a model Ag, we chose KLH because we reasoned that its large size would increase the probability that a processed peptide would be recognized by the random pairing of the DO11.10 TCR β-chain with an endogenous TCR α-chain and because CD4+ cells from unprimed DO11.10 mice do not respond to KLH in primary cultures. Thus, DO11.10 mice were immunized with KLH in CFA. After 6 wk, splenic CD4+ cells were prepared, and the CD45RBlow (memory) cells were isolated (Fig. 1). As we have previously shown, this population contains a high frequency of KLH-specific T cells (12). The isolated memory cells were then restimulated with KLH in the presence of exogenous IL-2. Additionally, we included a single round of stimulation with OVA233-339 so that we would facilitate the growth of KJ1-26+ cells. Consequently, a T cell line developed that grew in culture in response to KLH. Similar experiments performed with

![FIGURE 1. Isolation of CD45RB^low memory cells from KLH-primed DO11.10 mice. Splenocytes from DO11.10 Tg mice that had been primed 6 wk previously with KLH in CFA were isolated. After enrichment for CD4+ T cells, the cells were stained for CD45RB expression before (A) and after (B) isolation of memory cells by magnetic-activated cell sorting. The memory cells were stimulated with KLH to generate the DO-KLH line. Staining with a negative control Ab is also shown (dashed lines).](http://www.jimmunol.org/)

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restricted by self-MHC class II molecules. APCs, prepared from the spleens of BALB/c (H2b), BALB.B (H2d), BALB.K (H2k), and BALB.S (H2l) mice were cultured with DO-KLH cells. Varying doses of intact Ag (KLH, OVA), peptide (OVA323–339), or superantigen (SEB) were added to the cultures, and the responses of the DO-KLH cells were measured (Fig. 3). As indicated in Figure 3A, the responses to KLH were restricted by H2d, as only APCs from BALB/c mice could productively present the Ag. APCs from the H2 recombinant strain B10.6D, which expresses class II Aδ but no E molecules, also presented KLH, and anti-I-Aδ mAb (MK-D6) blocked the response (data not shown). As previously reported (26, 32), the DO11.10 TCR recognizes OVA323–339 when presented in the context of Aδ class II molecules (Fig. 3B). Consistent with these earlier studies, we also observe a slight response to both OVA323–339 and OVA (Fig. 3C) when they are presented in the context of H2o. To ensure that the congenic APC populations were effective to Ag presentation, we measured the response of the DO-KLH cells cultured with the various APC types and SEB. Recognition of superantigens is not an MHC-restricted event (33, 34); accordingly, all the APC types stimulate DO-KLH cells (Fig. 3D). In agreement with previous studies showing preferential usage of class II E molecules over A molecules in presentation of SEB (35), the E-negative strains (BALB.B and BALB.S) were less efficient at stimulating a response to SEB. Hence, these data show that KLH-responsive T cells can be isolated from the memory population of DO11.10 mice after exposure to KLH. Within this population are memory cells that respond not only to KLH but also to OVA. The responses to both Ags are restricted by self-MHC class II molecules.

Responses to KLH and OVA are mediated by two distinct receptors

The observation that DO-KLH cells responded to KLH and OVA suggested that the Ag response was mediated by two distinct TCRs. This hypothesis is supported by flow cytometric analysis of DO-KLH and DO11.10 T cells. Both cell types were stained for the expression of either the KJ1-26 clonotype or the TCR Vβ8 chain. As shown in Figure 4A, Vβ8 expression is similar on DO-KLH and DO11.10 cells, indicating that the overall TCR numbers are similar. In contrast, the staining pattern for KJ1-26 differs between the two cell types. As shown previously (12) and in Figure 4B, not all Vβ8+ CD4+ splenic T cells in DO11.10 mice express the KJ1-26 TCR. Further, even on the clonotype-bearing cells, KJ1-26 expression is heterogeneous, suggesting multiple populations of cells. Most cells express high levels of the clonotypic TCR; however, some of the cells have an intermediate level of KJ1-26 expression. Both KJ1-26− and KJ1-26intermediate populations are missing when DO11.10 mice are crossed to RAG−/− mice (12), making it likely that the KJ1-26+ cells possess two distinct TCRs due to incomplete allelic exclusion (16, 17). In contrast to the pattern exhibited by DO11.10 CD4+ T cells, examination of the DO-KLH T cell lines shows that the clonotype is expressed in a homogeneous fashion. Moreover, the overall KJ1-26 TCR levels are reduced and are similar to those in DO11.10 KJ1-26−/− cells (Fig. 4B). Because of the similarity in total TCR expression levels, it is likely that the lower clonotype levels on DO-KLH cells reflect the presence of an endogenous TCR α-chain that is paired with the DO11.10 TCR β-chain to form an alternate receptor and that the second TCR accounts for the KLH reactivity. To formally prove that the KLH and OVA activities resulted from the expression of distinct TCRs, we derived variants that had lost either KLH or OVA specificity and, accordingly, the corresponding TCR. To accomplish this goal, a subclone of the DO-KLH cell line, G1-G4, was fused to the TCR− thymoma, BW-5417 (28), and the T hybridoma fusion products were

FIGURE 2. DO-KLH cells are activated by both OVA and KLH. A, DO-KLH cells were cultured with either KLH (triangles) or OVA (squares) and APCs. After 3 days, proliferation was determined by [3H]Tdr incorporation. In the absence of T cells, background responses were <1000 cpm/well. B, DO-KLH cells were stimulated with either KLH (triangles) or OVA (squares) as described above. After 2 days, supernatants were collected and were analyzed for the secretion of IL-4 (dark symbols) or IFN-γ (open symbols) by ELISAs. DO-KLH secreted no detectable IL-2; freshly isolated DO11.10 cells secreted IL-2 but neither IL-4 nor IFN-γ, and only in response to OVA323–339 (data not shown).

DO-KLH cells respond to both OVA and KLH in an MHC-restricted fashion

We first determined whether DO-KLH T cells could be activated by the immunizing Ag, KLH, as well as by the Ag (OVA) that is recognized by the KJ1-26 TCR (Fig. 2). DO-KLH T cells were rested in the absence of IL-2 for 2 days (16 days after the last stimulation) and then were cultured with exogenous APCs and KLH or OVA. When presented with Ag, DO-KLH cells proliferated in response to both KLH and OVA (Fig. 2A). Further examination of the DO-KLH T cells showed that they were of the Th2 type, as both OVA and KLH stimulated the secretion of IL-4, but not IFN-γ (Fig. 2B) or IL-2 (data not shown). In addition, all the subclones derived from the DO-KLH cells exhibited the same lymphokine secretion pattern (data not shown). Similar results were obtained with either intact OVA or the specific peptide (OVA323–339) recognized by KJ1-26 TCR.

We next confirmed that the response to both KLH and OVA is restricted by self-MHC class II molecules. APCs, prepared from memory cells from non-KLH-primed mice failed to result in a KLH-responsive T cell line (W. T. Lee, unpublished observations), suggesting that the KLH-specific memory cells developed as a consequence of immunization. The T cell line, referred to as DO-KLH, was cloned by limiting dilution. All the resulting subclones had response characteristics similar to those of the parental line, suggesting that the line developed from a single KLH-specific clone. Immunofluorescent staining with KJ1-26 shows that the KJ1-26 TCR. Further, even on the clonotype-bearing cells, KJ1-26 expression is heterogeneous, suggesting multiple populations of cells. Most cells express high levels of the clonotypic TCR; however, some of the cells have an intermediate level of KJ1-26 expression. Both KJ1-26− and KJ1-26intermediate populations are missing when DO11.10 mice are crossed to RAG−/− mice (12), making it likely that the KJ1-26+ cells possess two distinct TCRs due to incomplete allelic exclusion (16, 17). In contrast, the staining pattern for KJ1-26 differs between the two cell types. As shown previously (12) and in Figure 4B, not all Vβ8+ CD4+ splenic T cells in DO11.10 mice express the KJ1-26 TCR. Further, even on the clonotype-bearing cells, KJ1-26 expression is heterogeneous, suggesting multiple populations of cells. Most cells express high levels of the clonotypic TCR; however, some of the cells have an intermediate level of KJ1-26 expression. Both KJ1-26− and KJ1-26intermediate populations are missing when DO11.10 mice are crossed to RAG−/− mice (12), making it likely that the KJ1-26+ cells possess two distinct TCRs due to incomplete allelic exclusion (16, 17). In contrast to the pattern exhibited by DO11.10 CD4+ T cells, examination of the DO-KLH T cell lines shows that the clonotype is expressed in a homogeneous fashion. Moreover, the overall KJ1-26 TCR levels are reduced and are similar to those in DO11.10 KJ1-26−/− cells (Fig. 4B). Because of the similarity in total TCR expression levels, it is likely that the lower clonotype levels on DO-KLH cells reflect the presence of an endogenous TCR α-chain that is paired with the DO11.10 TCR β-chain to form an alternate receptor and that the second TCR accounts for the KLH reactivity. To formally prove that the KLH and OVA activities resulted from the expression of distinct TCRs, we derived variants that had lost either KLH or OVA specificity and, accordingly, the corresponding TCR. To accomplish this goal, a subclone of the DO-KLH cell line, G1-G4, was fused to the TCR− thymoma, BW-5417 (28), and the T hybridoma fusion products were

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cloned by limiting dilution. The resulting clones were then screened for the ability to secrete lymphokines (IL-2) in response to either KLH or OVA. The ability of the hybrids to secrete IL-2 is derived from the BW-5417 parent, since the original DO-KLH cell line does not secrete this cytokine. As shown in Table I, three distinct types of hybridoma subclones were generated. Most of the clones examined retained the ability to secrete large amounts of IL-2 when cultured with either OVA or KLH (type I). However, the responses to KLH and OVA were independent of each other, as subclones could be identified that responded to OVA and not to KLH (type II) or responded to KLH and not to OVA (type III). All the subclones could respond vigorously to the superantigen, SEB, which reacts with the TCR V\(_{\beta}8\) chain used by the KJ1-26 TCR. The ability to respond to OVA was directly related to the expression levels of the KJ1-26 TCR (Fig. 5). Hence, cells that responded to OVA but not to KLH had comparable KJ1-26 and V\(_{\beta}8\) staining, cells that responded to KLH but not to OVA had little detectable KJ1-26 staining, and cells that responded to both Ags had an intermediate level of KJ1-26 staining. Taken together, these data show that the responses to OVA and KLH are mediated by two distinct TCRs and that cells, including the original DO-KLH line, that respond to both Ags express both TCRs.

**Discussion**

Our previous work provided evidence that dual TCR expression could permit the acquisition of T cell memory to a specific Ag without prior priming (12). This conclusion was based upon the following findings: 1) the existence of KJ1-26\(^{+}\), OVA-specific memory T cells in nonimmune DO11.10 mice; 2) the presence of secondary TCR\(\alpha\)-chains on KJ1-26\(^{+}\) T cells, including memory cells; 3) the absence of both dual TCR cells and memory cells in DO11.10 \(\times\) RAG\(^{-/-}\) mice. Hence, the most likely explanation for the presence of the OVA-specific memory cells was the specific stimulation of alternate TCRs by environmental Ags on T cells bearing the KJ1-26 clonotype. However, the possibility existed that the paucity of B cells in the RAG\(^{-/-}\) mice could have contributed to the failure to find memory cells, as opposed to lack of stimulation by environmental Ags. Further, as suggested by others

**FIGURE 3.** DO-KLH cells respond to Ag in an MHC-restricted fashion. DO-KLH cells were cultured with APCs and KLH (A), OVA\(_{323-339}\) (B), OVA (C), or SEB (D). Ag concentrations are in micrograms per milliliter, except for OVA\(_{323-339}\) which is in micrograms per milliliter \(\times 10^{-2}\). APCs from the following congenic mouse strains were used in the cultures: BALB/c (H2\(^d\); squares), BALB.B (H2\(^b\); circles), BALB.K (H2\(^k\); triangles), and BALB.S (H2\(^s\); diamonds). After 3 days, proliferation was determined by \[^{3}H\]Tdr incorporation. In the absence of either T cells or APCs, the background responses were <1000 cpm/well.

**FIGURE 4.** DO-KLH cells express the DO11.10 clonotype but at lower levels than DO11.10 cells. DO-KLH cloned T cells (solid lines) and freshly isolated, purified CD4\(^{+}\) T cells from DO11.10 mice (dotted lines) were stained for the expression of V\(_{\beta}8\) (A) or the DO11.10 clonotype (B). Fluorescence staining using a negative control Ab was limited to the first decile.
stimulation of T cells that do not express the KJ1-26 clonotype and that react with environmental Ags could have led to a nonspecific inflammatory condition with associated lymphokine release. This could have prompted the development of KJ1-26+ memory cells in a bystander-like fashion. In this case, elimination of the non-clonotype-bearing cells in the DO11.10 × RAG<sup>−/−</sup> mice might have also prevented the inflammation.

The present study provides clear evidence that immunization of DO11.10 mice with a known Ag, KLH, permits the development of dual receptor memory cells that recognize the immunizing Ag as well as the Ag recognized by the DO11.10 transgenic TCR, OVA. The cells proliferated and secreted lymphokines after stimulation with either Ag. Independent recognition of both Ags by distinct TCRs was shown by production of variants that had lost either specificity along with the corresponding TCR. Recognition of Ag by both TCRs is MHC restricted, since the cells recognize Ag presented by self, but not nonself, MHC class II molecules. These results are more compatible with direct activation of cells bearing the transgenic TCR through the second receptor rather than with bystander activation. Together with our previously published results, the data provide strong evidence that stimulation through one receptor on dual receptor T cells can lead to the generation of memory T cells capable of giving a memory response through the second receptor, even though the cells had not been previously exposed to the Ag recognized by the second receptor.

Studies by others have shown that dual receptor T cells can be identified at significant frequencies (16, 17, 36). Indeed, Padovan et al. have estimated that up to 30% of human peripheral blood T cells can express two different receptors due to a failure of TCR α/β allelic exclusion (16). Similar estimates have been made in the mouse (17, 36). Several studies have also shown that both receptors are functional, i.e., both receptors can transduce signals after ligation with anti-TCR Abs (16) or physiologic ligands, such as superantigens (37) or transplantation Ags (38). Our results are compatible with these studies and show further that the same lymphokines are produced following stimulation of either TCR.

Of particular importance in considering the significance of dual receptor T cells is their MHC specificity. If only one of the receptors is capable of recognizing Ag presented by self-MHC molecules, the cell will be functionally nonspecific, like single receptor T cells. The existence of such T cells has been reported by Hardardottir et al. (39). In the same study data were presented suggesting that strong positive selection through the self-restricted TCR precluded acquisition of a second TCR. This observation, coupled with accumulating evidence that positive selection down-regulates active recombination (40, 41) raised the possibility that on all T cells bearing two TCRs, only one is specific for peptides bound to self-MHC molecules. Under this view, dual receptor T cells would be expected to function as single receptor T cells in

### Table I. Analysis of IL-2 secretion by T cell hybridomas<sup>a</sup>

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<th>Stimulus&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup> Proliferation of HT-2 cells (cpm of incorporated [3H]TdR) after addition of supernatants from stimulated T cell hybridoma subclones (50% v/v).

<sup>b</sup> T cell hybridoma subclones were cultured with APCs and different concentrations of the indicated stimulus. Only a single dose point is shown; however, in all cases, the values are at peak or plateau values.

<sup>c</sup> Data shown are from representative subclones derived from three separate cloning experiments. Roman numerals denote patterns of hybridoma clones. The cpm from HT-2 cells stimulated without added supernatant in experiments 1 to 3 are 381, 116, and 548, respectively.

<sup>d</sup> Original parental T cell hybridoma from which subclones are derived.

### Figure 5.

Expression of KJ1-26 and Vβ8 on DO-KLH hybridoma variants. Representative hybridoma subclones from groups that respond to both OVA and KLH (A: type I; clone P6-B7), only OVA (B: type II; clone P6-B4), or only KLH (C: type I; clone P8-B4) were stained for the expression of Vβ8 (solid line) or the DO11.10 clonotype (dashed line). In all panels, staining of an irrelevant control mAb is shown (dotted line). Also shown are the counts per minute obtained from HT-2 cells that were cultured with supernatants from hybridomas previously stimulated with either OVA<sub>323–339</sub> or KLH.
response to peptides presented by self-MHC molecules. No response, except possibly an allogeneic response against a foreign MHC molecule or a response induced by a superantigen, would be mediated through the second receptor.

In contrast, if both receptors are capable of recognizing Ag presented by self-MHC molecules, the cell will be functionally bispecific. A study by Simpson et al. (38) and our study showing that both TCRs on a single cell recognize Ag presented by self, but not nonself, MHC molecules provides clear evidence for the existence of such bispecific cells. Also compatible with this alternative are results from our previous study with dual receptor T cells, where it appears that OVA-specific memory T cells can be generated through specific stimulation of the second TCR by an unknown environmental Ag presented by a self-MHC molecule (12). Although we do not know which of the two TCRs is used to mediate positive selection, these studies indicate that positive selection through one self-MHC-restricted TCR does not necessarily prevent the expression of another self-restricted TCR on the same cell.

The existence of self-restricted dual receptor memory T cells has important implications for the maintenance of memory and autoimmune disease. With regard to the former, it is generally accepted that an initial encounter with foreign Ag stimulates the development of long term immunologic memory. However, the mechanisms responsible for the long term preservation of specific memory are unclear (reviewed in Ref. 1; 42–45). With T cells, one hypothesis is that memory cells are long-lived cells that revert to a resting state until reexposed to the priming Ag. An alternative viewpoint is that long-lived memory is maintained because memory T cells are periodically restimulated (44–47). The agent responsible for restimulation is unclear. While some studies suggest that the priming Ag persists in vivo and is required to maintain memory, recent data strongly suggest that the priming Ag is not necessary (42, 48). However, these latter studies cannot rule out other means of stimulation, such as cross-reacting Ag or nonspecific cytokine effects (13, 46). Studies that have measured the life span of T cells demonstrated that although some memory T cells remain quiescent for extended periods (44, 49), most memory cells undergo rapid turnover in vivo even in the absence of the priming Ag (49). This strongly suggests that some mechanism other than Ag persistence contributes to the maintenance of memory. It is tempting to speculate that one potential mechanism is stimulation by an unrelated Ag via a second TCR. The unrelated Ag could be derived from a foreign pathogen, a ubiquitously expressed environmental molecule, or possibly a self-peptide.

In addition to contributing to memory maintenance, our data fit well with previous suggestions that dual reactive cells may play a role in autoimmune disease. It has been postulated that the expression of a second TCR might facilitate escape from negative selection due to lowered expression of a self-reactive TCR (16). We propose further that specific properties of memory cells are critical to autoimmunity mediated by dual TCR cells. Reduced TCR expression coupled with activation and costimulation requirements specific to naive cells (50) prevent activation of an autoreactive naive T cell. However, stimulation of the alternate TCR (due to infection or environmental Ags) leads to memory generation. This would permit the autoantigen to stimulate memory cells under conditions that would not normally stimulate naive cells. In addition, since homing and trafficking patterns differ between naive and memory T cells (51), an autoreactive memory T cell might encounter Ags that are not normally seen by naive cells. Stimulation of dual TCR memory cells in this fashion would be analogous to “molecular mimicry” (52). As in mimicry models, we would predict that an encounter with a pathogen stimulates an autoreactive T cell. However, stimulation would occur not through cross-reaction of the same TCR but by stimulation of a second TCR that is fortuitously expressed by the pathogen-specific memory T cell. In this model the stimulating Ag and the autoantigen need not be related.

In summary, our data show that stimulation through one receptor on dual receptor T cells can lead to the generation of memory T cells that are capable of giving a memory response through the second TCR, even though the cells had not been previously exposed to the Ag recognized by the second receptor. This raises the possibility that such a mechanism may also account at least in part for the maintenance of T cell memory. Our study provides evidence that both receptors on memory T cells recognize Ag presented by self-MHC molecules. Stimulation of bispecific naive T cells through one TCR by a pathogen or environmental Ag could lead to the generation of memory T cells that express a second receptor that is autoreactive. Unlike naive T cells, such memory T cells would be more likely to respond to the autoantigen and cause autoimmune disease.

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References


