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Unexpected Reactivities of T Cells Selected by a Single MHC-Peptide Ligand

Nagendra Singh and Luc Van Kaer

In H2-DM mutant mice, most MHC class II molecules are bound by a single peptide, CLIP, derived from the class II-associated invariant chain. Previous studies showed that H2-DM− cells are defective in presenting synthetic peptides to class II-restricted T cells. In sharp contrast, however, the same peptides elicited strong CD4+ T cell responses in H2-DM+ animals. We now provide an explanation for this apparent discrepancy. Peptide-specific CD4+ T cells from wild-type mice were efficiently stimulated by H2-DM−, but not by H2-DM− cells pulsed with the cognate peptide. In sharp contrast, CD4+ T cells from mutant animals specific for the same MHC-peptide combination recognized peptide-pulsed H2-DM− and H2-DM+ cells equally well. In addition, unlike Ag-specific T cells from wild-type animals, the reactivities of peptide-specific T cells from mutant animals could not be efficiently blocked by Abs specific for the cognate MHC class II-peptide combination. We further demonstrated that the distinct reactivities of CD4+ T cells from H2-DM+ and H2-DM− mice are due to differences in thymic selection. Collectively, these findings indicate that the CD4+ T cell repertoires of H2-DM+ and H2-DM− mice are remarkably different. The Journal of Immunology, 1999, 163: 3583–3591.

The mature T cell repertoire is shaped by cellular selection processes in the thymus. These processes are guided by the interaction of the TCRs expressed on immature T cells with complexes formed between self peptides and MHC molecules expressed on selecting cells (reviewed in Refs. 1–3). Thymocytes with very high affinity for self MHC/peptide complexes are eliminated by programmed cell death through the process of negative selection. In contrast, thymocytes with weak affinity for self MHC/peptide complexes are allowed to mature and populate the peripheral lymphoid organs in a process called positive selection. Finally, thymocytes with no or extremely low affinity for the selecting ligands die by neglect. Together these cellular selection events ensure that autoreactive and useless clones are removed from the repertoire, allowing only useful T cells, i.e., T cells specific for self MHC plus foreign Ag, to mature.

While the role of self peptides in negative selection is well established, much debate has surrounded the issue as to whether self peptides influence the specificity of T cell-positive selection (reviewed in Refs. 4–10). Recently, this issue was addressed by studying genetically engineered mice that express a single peptide on their MHC class II molecules. Two different methods were employed to generate such mice. In one approach, transgenic mice were made that express MHC class II Aβ molecules covalently attached to a peptide from the class II β2m-chain (11, 12). Similarly, we and others generated mice in which nearly all class II Aβ molecules are occupied by a peptide, CLIP, derived from the class II-associated invariant chain (13–15). This was accomplished by introducing a null mutation in the H2-DM peptide exchange factor. H2-DM mutant animals expressed surface levels of the Aβ-CLIP complex that were indistinguishable from the MHC class II molecules expressed by wild-type cells. Because numbers of mature CD4+ T lymphocytes in H2-DM mutant mice were reduced 2- to 3-fold, it was concluded that this MHC-peptide complex is an efficient ligand for positive selection. However, 60–80% of the CD4+ T cells selected in these mice were unusual in the sense that they reacted with class II molecules expressed by syngeneic wild-type cells. This finding was interpreted to suggest that a single MHC-peptide complex cannot induce intrathymic deletion of T cells reactive with the wide array of self peptides normally bound to the MHC class II molecules of wild-type cells. It was further shown that the CD4+ T cells from H2-DM mutant animals express a diverse set of TCRs and that these mice can generate strong CD4+ T cell responses when immunized with synthetic class II-binding peptides (16; unpublished results). Although these studies suggested that H2-DM mutant mice select a broad repertoire of CD4+ T cells, they did not preclude the possibility that significant restraints were imposed on the types of cells selected. Two lines of evidence indicated that this is indeed the case. First, five different TCR transgenics that were positively selected in wild-type mice were not selected in H2-DM mutants (16–18). Second, introduction of a TCRβ-chain transgene in the H2-DM-deficient background led to significant restraints on the selection of TCRβ-chains (19). Together these studies indicated that H2-DM mutant animals select a semidiverse CD4+ T cell repertoire.

In this work, we describe some unusual properties of the CD4+ T cell repertoire of H2-DM mutant mice. These studies were prompted by the observation that H2-DM mutant cells poorly present synthetic peptides to class II-restricted CD4+ T cells (13–15). However, despite this defect, H2-DM mutant mice generated strong peptide-specific CD4+ T cell responses when immunized with synthetic peptides (16; see below). We now show that H2-DM− cells efficiently present peptides to class II-restricted T cells from H2-DM+ mice, but not to similar cells from H2-DM− mice. Evidence is provided that these T cells recognize distinct configurations of the same class II-peptide complex on H2-DM+ and H2-DM− cells. We further show that the differential reactivities of
CD4+ T cells from H2-DM+ and H2-DM− animals reflect differences in thymic selection. These findings indicate that the reactivities of the CD4+ T cell repertoires of H2-DM+ and H2-DM− mice are remarkably different.

Materials and Methods

Mice

H2-DM-deficient mice have been described (14). Control C57BL/6 mice and allogeneic BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained and bred under specific pathogen-free conditions in the animal facility at Vanderbilt University School of Medicine (Nashville, TN).

Peptides

The Eot(52–68) (ASFEAQQALANIVDKA) and RNase(90–105) (SKYPNK CAYKTTPQANK) peptides and truncation variants of these peptides were synthesized by the biozolym facility of the Howard Hughes Medical Institute at the University of Texas Southwestern Medical Center (Dallas, TX). Purity of peptides was higher than 90%. Synthetic peptides were biotinylated as described (20). Briefly, 12 μl of d-biotin-N-hydroxy succinimide ester (10 mg/ml) (Boehringer Mannheim, Indianapolis, IN) in dimethylformamide was added to 1 ml of peptide solution (1 mg/ml) in 0.1 M methyl-bisucinate buffer and incubated overnight at 8°C. Because free biotin is hydrolyzed after prolonged incubation, further purification was not necessary. Residual-free biotin was neutralized by addition of 1% glycine to the solution.

Abs and flow-cytometric analyses

The H2-Aα-specific mAb Y-3P and the H2-Aα plus CLIP-specific mAb 30-2 (21) were obtained from Dr. Alexander Y. Rudensky (University of Washington, Seattle, WA); the H2-Aα plus Eot(52–68)-specific mAb Y-Ae (22, 23) was obtained from Donal B. Murphy (Wadsworth Center, Albany, NY); the H2-Aα-specific hybridoma BP107 was purchased from the American Type Culture Collection (Manassas, VA); the CD8-specific hybridoma 2.43, the CD4-specific hybridoma GK1.5, and a Thy-1.2-specific hybridoma were obtained from Dr. Barney Graham (Vanderbilt University); cytochrome- or FITC-labeled anti-TCR (clone H57-597), goat anti-mouse IgG (Fc)-FITC, goat anti-mouse IgG, and mouse anti-rat IgG (Fc) were obtained from Jackson ImmunoResearch (West Grove, PA).

Cell suspensions from spleens and lymph nodes were prepared according to standard procedures. For staining, cells or peptide-pulsed cells were incubated with mAbs for 45 min in PBS containing 2% FCS and 0.1% sodium azide. Cells were then washed and, where appropriate, incubated with secondary Abs or streptavidin-PE (Vector Laboratories, Burlingame, CA). After the final washes, cells were analyzed at the HHHI Flow Cytometry Core (Vanderbilt University) using a FACS Calibur flow system and CellQuest v. 3.1 software (Becton Dickinson, San Jose, CA).

Measurement of immune responses

Mice were immunized with 20 μg of peptide emulsified in CFA (Becton Dickinson). Seven days later, 5 × 10^6 draining lymph node cells were cultured with graded doses of immunizing peptide in flat-bottom 96-well tissue culture plates in 200 μl of RPMI 1640 medium containing 10% FBS (Life Technologies, Gaithersburg, MD), 50 μM 2-ME, 2 mM glutamine, penicillin and streptomycin, and 10 mM HEPES. For the experiments shown in Figs. 1B, 6, and 7D, lymph node cells from responder mice were cultured with peptide-loaded spleen stimulator cells that were depleted of CD4+ T cells by panning with anti-CD4 Abs on mouse anti-rat IgG (Fc)-coated plates. After 72 h, cells were pulsed with 1 μCi of [3H]thymidine (NEC Life Science Products, Boston, MA) per well and cultured for another 12–16 h, harvested with a cell harvester (Tomtec, Orange, CT), and counted with a betaplate reader (EG&G Wallac, Gaithersburg, MD).

For priming with peptide-pulsed APC, spleen cell suspensions were enriched for dendritic cells, as described (24). Briefly, single cell suspensions from spleen tissue were prepared by collagenase D (Boehringer) digestion of the tissue that was then filtered through 35% BSA (Sigma, St. Louis, MO) density gradients. After washing, low buoyant density cells were cultured overnight with 50 μg/ml of peptide. Nonadherent cells were washed and injected i.v. into mice (5 × 10^6 cells/mouse).

For mixed lymphocyte reactions, responder spleen cells were first depleted of CD8+ and MHC class II+ cells by panning with anti-CD8 and Y-3P Abs, respectively, on goat anti-mouse Ig-coated plates. Purity of cells was higher than 90%. Responder cells (2 × 10^6) were then cultured with varying numbers of irradiated spleen stimulator cells from BALB/c mice for 3 days at 37°C. Proliferation of responder cells was measured as above.

Production and assay of T cell hybridomas

H2-DM+ and H2-DM− mice were immunized with 20 μg of peptide emulsified in CFA. Seven days later, 12 × 10^6 lymph node cells from these immunized mice were restimulated in vitro for 3 days in the presence of the relevant peptide (20 μg/ml). Cells were counted, fused with BW5147 α-β cells (obtained from Dr. Willi Born, National Jewish Center, Denver, CO) using polyethylene glycol 1500 (Boehringer Mannheim), and plated in HAT selection medium (Boehringer Mannheim) under limiting dilution conditions.

Reactivities of T cell hybridomas were measured by coculture of a fixed number (5 × 10^4) of hybridoma cells with irradiated stimulator cells (4 × 10^5) and titrated doses of peptide. In most experiments, varying doses of synthetic peptides were also added to the cultures. For the experiment shown in Fig. 5B, APC were fixed with paraformaldehyde (0.04%) before addition of peptides and hybridoma cells. For Ab-blocking experiments, graded doses of mAbs were also added to the cultures. After 24 h of culture, supernatants were collected and assayed for IL-2 contents using the IL-2-dependent cell line HT-2. Fifty microliters of supernatant were mixed with 10^3 HT-2 cells and incubated at 37°C for 20 h. IL-2-dependent proliferation of HT-2 cells was measured by pulsing the cells with 1 μCi of [3H]thymidine and further culture for 16 h. [3H]Thymidine incorporation was measured as described above.

Generation of radiation bone marrow chimeras

Bone marrow cells from H2-DM+ or H2-DM− mice depleted of T cells by panning with a mixture of anti-Thy-1.2, anti-CD4, and anti-CD8 Abs on goat anti-mouse Ig-coated plates were injected i.v. into irradiated (920 rad) recipient mice. Four weeks after bone marrow transfer, T cell subsets in the lymph nodes and/or blood of chimeric animals were analyzed. Eight weeks after transfer, mice were immunized with peptides as above. Successful reconstitution was confirmed by staining of blood lymphocytes with the mAbs Y-3P, BP107, and 30-2.

Results

H2-DM− cells efficiently present peptides to CD4+ T cells from H2-DM+ but not H2-DM− mice

Previously, we and others showed that APC from H2-DM-deficient animals are unable to present intact protein Ags to peptide-specific H2-Aα-restricted CD4+ T cell hybridomas (14, 15). Surprisingly, mutant cells also demonstrated strongly reduced capacity to present exogenous peptides to these hybridomas (e.g., see Fig. 1A) (14, 15). This is also true when bulk CD4+ T cell cultures from immunized mice are used as responder cells. Fig. 1B shows that CD4+ T cells from wild-type mice previously immunized with synthetic peptides are poorly stimulated by peptide-pulsed H2-DM+ cells, but strongly react with peptide-pulsed H2-DM− cells. These findings may be interpreted to suggest that, in H2-DM mutant cells, few empty or otherwise peptide-receptive MHC class II molecules reach the cell surface. This conclusion appeared inconsistent with other experiments that indicated that H2-DM mutant animals generate strong CD4+ T cell responses when immunized with synthetic peptides (16). Fig. 1C shows such an experiment with the Eot(52–68) and RNase(90–105) peptides. Consistent with results obtained by Tourne et al. (16), immunized H2-DM− mice generated strong CD4+ T cell proliferative responses when stimulated with H2-DM− APC in the presence of the immunizing peptide. In fact, mutant mice consistently generated stronger responses than wild-type mice against these two peptides. Thus, at least in these experiments, H2-DM-deficient APC were perfectly capable of presenting synthetic peptides to Ag-specific T cells.

To provide an explanation for these surprising results, we decided to investigate whether peptide-specific CD4+ T cells from H2-DM− and H2-DM+ mice differed in their reactivities. We first generated panels of peptide-specific T cell hybridomas from these mice. H2-DM+ and H2-DM− mice were immunized with the Eot or RNase peptides, lymph node cells of these mice were then cultured in vitro for 3 days in the presence of the immunizing peptide,
The reactivities of hybrids from H2-DM+ mice poorly present peptides to class II-restricted T cells, yet these mice generate strong responses when immunized with synthetic peptides. A. Presentation of Eα(52–68) peptides by H2-DM+ and H2-DM- APC to the Eα-specific T cell hybridoma W13. Irradiated splenic APC from H2-DM+ and H2-DM- mice were cultured with the W13 T cell hybridoma in the presence of graded doses of Eα peptide. IL-2 produced in the supernatant was measured by incorporation of [3H]thymidine into the DNA of IL-2-dependent HT-2 cells. B. Presentation of Eα(52–68) peptides by H2-DM+ and H2-DM- APC to CD4+ T cells from Eα-immunized H2-DM+ mice. Purified CD4+ T cells from the lymph nodes of H2-DM+ mice immunized 7 days earlier with the Eα peptide were cultured with peptide-pulsed irradiated splenic APC from H2-DM+ or H2-DM- mice. Proliferation of responder cells was measured by uptake of [3H]thymidine. Results are expressed as mean ± SD of triplicate measurements. The SD for the data points with 0 µg/ml of peptide and those for peptide-loaded H2-DM+ cells were too low for visualization in these plots. C. Peptide-specific immune responses in H2-DM+ and H2-DM- mice. Lymph node cells from H2-DM+ and H2-DM- mice immunized with the indicated peptides were cultured with graded doses of the immunizing peptides. Proliferation of responder cells was measured by uptake of [3H]thymidine. For each peptide, results for two wild-type and two mutant mice are shown.

All hybrids from wild-type mice were efficiently stimulated by peptide-loaded cells from wild-type mice, but were poorly stimulated by similarly treated cells from mutant mice. Interpretation of the reactivities of hybrids from H2-DM+ mice is complicated by the fact that approximately one-half of these reacted with spleen cells from wild-type mice in the absence of peptide. This was perhaps not surprising, because 60–80% of all CD4+ T cells from H2-DM+ mutant mice can react with H2-DM+ cells (16, 18). Presumably, these hybrids cross-react with some endogenous peptides bound to the class II molecules of wild-type mice. These reactivities probably reflect the redundancy that is inherent to all cell recognition (reviewed in Ref. 25). Among the remaining hybrids from H2-DM- animals, one RNase-specific hybrid (hybrid MR2) showed reactivities similar to the hybrids isolated from wild-type mice. All other hybrids from mutant animals recognized peptide-pulsed H2-DM+ and H2-DM- APC equally well (see Fig. 2 and the reactivities of hybrids M5, M11, M37, and M38 in Fig. 3A).

One way to interpret the differences in reactivities between hybrids from wild-type and mutant mice would be that these hybrids recognize distinct processed forms of the peptides. To exclude this possibility, APC were fixed with paraformaldehyde before addition of the synthetic Eα peptide. Again, H2-DM+ hybrids were preferentially stimulated by H2-DM+ APC (see results for hybrids W4, W6, and W13 in Fig. 3B), whereas H2-DM- hybrids were stimulated equally well by H2-DM+ and H2-DM- APC (see results for hybrids M5, M11, and M37 in Fig. 3B). These findings were confirmed by using truncation variants of Eα(52–68) to stimulate the hybridomas. Results for one representative wild-type hybrid (W13) and one representative mutant hybrid (M5) are shown in Fig. 3C. Because similar differences in reactivities were found as with the full-length peptide, we concluded that it is unlikely that differences in reactivities between wild-type and mutant hybrids are caused by the loading of differentially processed peptides or by the differential recognition of these MHC-peptide complexes by T cells.

Taken together, these hybridoma data indicate that Ag-specific CD4+ T cells from H2-DM+ and H2-DM- mice are differentially stimulated by peptide-pulsed H2-DM+ and H2-DM- cells.

Reactivities of H2-DM+ and H2-DM- hybrids are differentially blocked by peptide-specific mAbs

We wanted to determine the molecular basis for the differential reactivities of H2-DM+ and H2-DM- hybrids. All evidence indicated that these hybrids recognized the same MHC class II-peptide complex. To analyze the specificity of these hybrids further, we performed Ab-blocking experiments with the Y-Ae Ab, which is specific for the complex between H2-Aζ and Eα(52–68) (22, 23). Fig. 4 shows that responses by H2-DM+ hybrids (W4, W6, W9,
and W13) against peptide-pulsed wild-type APC were efficiently blocked by Y-Ae. In sharp contrast, Y-Ae only partially blocked the responses of H2-DM$^2$ hybrids (M5, M11, M37, and M38) against peptide-pulsed H2-DM$^1$ and H2-DM$^2$ APC. However, responses of all hybrids were efficiently blocked by the Y-3P Ab, which recognizes H2-A$^b$ irrespective of the bound peptide.

One way to interpret these data is that hybrids from wild-type and mutant animals recognize distinct forms of the A$^b$-Ea complex. The major form of these complexes expressed by wild-type APC may be recognized by the Y-3P Ab, which recognizes H2-A$^b$ irrespective of the bound peptide.

FIGURE 2. Peptide-specific T cell hybridomas from H2-DM$^1$ and H2-DM$^2$ mice have different reactivities. Hybridomas were generated from H2-DM$^1$ and H2-DM$^2$ mice immunized with the Eo(52–68) peptide (A) or the RNase(95–105) peptide (B). Reactivities of these hybrids against 8 x 10$^5$ splenic APC in the absence of peptide or against 2 x 10$^5$ APC in the presence of peptide (5 $\mu$g/ml) were measured. Results are shown for 10 randomly selected hybrids from each experimental group. None of these hybrids reacted with splenic APC from MHC class II-deficient mice (data not shown).

Role of the thymus in the differential reactivities of CD4$^+$ T cells from H2-DM$^+$ and H2-DM$^-$ mice

We considered two possible explanations for the differential reactivities of CD4$^+$ T cells from H2-DM$^+$ and H2-DM$^-$ animals that are not necessarily mutually exclusive. One possibility is that the priming strategy employed may have selected CD4$^+$ T cells with these reactivities. In wild-type mice, T cells are primed with peptides presented by wild-type APC, whereas in mutant mice T cells are primed with peptides presented by mutant APC. This priming strategy may have biased the immune response. Another possibility is that differences in the CD4$^+$ T cell repertoire of H2-DM$^+$ and H2-DM$^-$ mice account for our observations.
To distinguish between these possibilities, we tested whether Eα peptide-pulsed dendritic cells from H2-DM$^+$ and H2-DM$^+$ mice can generate a peptide-specific response when used to immunize wild-type mice. Fig. 6B shows that this immunization protocol did not elicit an immune response in wild-type mice. However, two control experiments demonstrated that this immunization protocol was effective. First, wild-type mice generated strong responses when immunized with peptide-pulsed wild-type dendritic cells (Fig. 6A). Second, mutant mice immunized with peptide-pulsed mutant cells similarly generated a strong response (Fig. 6C).

To investigate the role of thymic selection in the differential reactivities of CD4$^+$ T cells from wild-type and mutant mice more directly, a set of reciprocal bone marrow chimeras was generated. T cell-depleted bone marrow cells from H2-DM$^+$ and H2-DM$^+$ mice were transferred into lethally irradiated H2-DM$^+$ or H2-DM$^+$ mice; 4 wk later, the level of chimerism in these mice was measured by staining PBL with the Abs 30-2 (specific for H2-A$^+$ + CLIP), BP107 (specific for H2-A$^+$ + non-CLIP peptides), and Y-3P (specific for H2-A$^+$ + any peptide) (data not shown); and after an additional 4 wk, T cell compartments in the lymph nodes of successfully reconstituted mice were analyzed, or alternatively, mice were immunized with Eα (52–68) or RNase(90–105) to measure immune responses. The CD4$^+$ T cell compartments in the lymph nodes of these reconstituted mice (Fig. 7A) were very similar to those observed in previous studies (16, 18). Numbers of CD4$^+$ T cells in H2-DM$^+$→H2-DM$^+$ chimeras and H2-DM$^+$→H2-DM$^+$ chimeras were very similar to the numbers of CD4$^+$ T cells in H2-DM$^+$ and H2-DM$^+$ mice, respectively. As expected, these two sets of chimeras generated strong immune responses when immunized with either the Eα or RNase peptide.
The responses of these chimeric animals were very similar to the responses of nonchimeric H2-DM1 and H2-DM2 mice (compare Figs. 1C and 7B).

In the H2-DM1→H2-DM2 chimeras, in which T cells are positively selected on H2-DM2 epithelium and negatively selected on H2-DM1 hemopoietic cells, few CD4+ T cells developed (3- to 4-fold fewer cells than H2-DM2 animals). This has been noted before (16, 18) and is probably due to deletion of the population of autoreactive CD4+ T cells. These chimeras generated very weak peptide-specific immune responses (presented by H2-DM1 hemopoietic cells). This may be because of the low numbers of CD4+ T cells in these mice. Alternatively, the unresponsiveness of these chimeras may be due to the memory phenotype of their CD4+ T cells. Indeed, Surh et al. (18) showed that most of the CD4+ T cells in H2-DM1→H2-DM2 chimeras have a memory phenotype (CD44+) and are largely unresponsive.

In the H2-DM1→H2-DM2 chimeras, thymocytes are positively selected on H2-DM2 thymic epithelium and negatively selected on H2-DM1 hemopoietic cells. Consistent with previous results (16, 18), the CD4+ T cell population in these chimeras was expanded about 1.7-fold. These chimeras allowed us to test whether CD4+ T cells positively selected on wild-type epithelium are able to generate peptide-specific immune responses when primed in vivo with peptides presented by H2-DM2 hemopoietic cells. Fig. 7B shows that these chimeras generated very weak immune responses. This was not due to general anergy of the CD4+ T cell population, because these cells strongly responded to allogeneic BALB/c cells (Fig. 7C). Furthermore, to demonstrate that

**FIGURE 4.** The Y-Ae Ab differentially blocks the reactivities of Ea(52–68)-specific hybridomas from H2-DM+ and H2-DM− mice. Graded amounts of the Y-Ae (specific for H2-Aα plus Ea) or Y-3P (specific for H2-Aα plus any peptide) Abs were added to cultures of peptide-pulsed (4 μg/ml) irradiated APC (1.5×105) and the indicated T cell hybridomas. W4, W6, W9, and W13 are Ea-specific hybrids isolated from wild-type mice, and M5, M11, M37, and M38 are Ea-specific hybrids from H2-DM− mice. The response in the absence of Ab was taken as 100%.

**FIGURE 5.** Recognition of the Aα-Ea complex on H2-DM+ and H2-DM− cells by the H2-Aα plus Ea(52–68)-specific Ab Y-Ae. Splenic H2-DM+ and H2-DM− cells were incubated with varying amounts of the biotinylated Ea peptide, and subsequently stained with Y-Ae, followed by streptavidin-PE or FITC-labeled goat anti-mouse IgG (Fc). FACS plots shown (A) represent data electronically gated on TCRβ cells. Results for the staining intensity with the biotinylated Ea peptide were also plotted as mean fluorescence intensity (B).
H2-DM−→H2-DM+ chimeras are immunologically competent, they were immunized with wild-type or mutant dendritic cells loaded with the Eα peptide. These chimeras generated strong responses when immunized with peptide-loaded wild-type but not mutant cells (Fig. 7D), indicating that Eα-specific T cell precursors are present, but that these cells are poorly activated with peptides presented by H2-DM+ APC.

Collectively, these data argue that intrathymic selection is responsible for the differential reactivities of CD4+ T cells from H2-DM+ and H2-DM− mice.

Discussion

In H2-DM-deficient mice, T cell differentiation proceeds on a limited array of MHC-peptide complexes, most of which consist of class II H2-Ak molecules bound by CLIP (13–17). Prior studies indicated that H2-DM-deficient animals select a semidiverse CD4+ T cell repertoire (13–19). Surprisingly, however, a large number (60–80%) of the CD4+ T cells selected in these mice were unusual because they reacted with the wide array of MHC-peptide complexes expressed by syngeneic wild-type mice (13–15). In this work, we have described another unusual aspect of the CD4+ T cell repertoire of H2-DM− mice. Our results demonstrate that Ag-specific CD4+ T cells from H2-DM− mice recognize their cognate peptide Ag equally well when presented by H2-DM+ and H2-DM− APC. This is in sharp contrast with the reactivities of Ag-specific CD4+ T cells from wild-type mice, which recognize their cognate Ag preferentially on H2-DM+ cells. These findings indicate that the CD4+ T cell repertoire of H2-DM− mice is quite different from the T cell repertoire of wild-type mice.

Previous studies with H2-DM− mice suggested that cells from these animals express very few empty or otherwise peptide-receptive class II molecules at their cell surface (13–15). Our peptide-binding experiments indeed indicate that H2-DM− cells require ~5–10 times more peptide than wild-type cells to stabilize the same number of class II molecules. In agreement with this defect in peptide loading, T cell hybrids from wild-type mice required at least 10-fold more peptide on H2-DM+ APC for the same level of stimulation (Fig. 3A). In sharp contrast, however, CD4+ T cells from mutant mice recognized peptide-pulsed H2-DM+ and H2-DM− APC equally well (Figs. 2 and 3A). Thus, we conclude that peptides can be loaded on APC from H2-DM− mice and that these MHC-peptide complexes are differentially recognized by peptide-specific T cells from H2-DM+ and H2-DM− mice.

Our results indicated that CD4+ T cells selected in H2-DM− mice are biased toward recognizing Ags presented by H2-DM+ cells, whereas CD4+ T cells selected in H2-DM− mice are biased toward recognizing Ags presented by H2-DM− cells. This was not caused by the immunization protocol used: H2-DM+ mice generated strong responses against peptide-loaded dendritic cells from wild-type mice, but very weak responses against similar cells from mutant mice (Fig. 6); furthermore, H2-DM− mice consistently generated strong responses against peptide-loaded H2-DM− APC. These findings suggested that the distinct reactivities of the T cell hybrids reflect differences in the T cell repertoires of these mice. The bone marrow transfer experiments further showed that this is caused by differences in thymic selection.

What is the molecular basis for the differential reactivity of hybrids from H2-DM+ and H2-DM− mice? We considered a number of different possibilities. First, our experiments with fixed APC and truncated peptides (Fig. 3, B and C) excluded the possibility that these reactivities were caused by different processing of the peptide after uptake by the APC.

Second, we considered the possibility that wild-type and mutant hybrids preferentially recognize different configurations of the same MHC-peptide complex. The idea that peptides can bind with MHC class II molecules in different configurations has been made before (26–30). For example, Viner et al. (26, 27) studied the reactivities of a set of hybridomas isolated from mice immunized with synthetic peptides that correspond to naturally processed peptides of the immunodominant, H2-Ak-restricted epitope of the model Ag HEL. The majority of these hybridomas were unable to react with intact HEL after processing by various APC. Based on these studies, it was proposed that peptides can generate complexes that are antigenically dissimilar from those generated by natural processing. Thus, the reactivities of the hybrids investigated in this study may be related to the reactivities of the hybrids that were studied in the HEL system: most of the hybrids from H2-DM− animals may recognize a configuration that is the predominant form on wild-type cells, whereas most of the hybrids from H2-DM+ animals may recognize a distinct configuration that is the predominant form on mutant cells. This possibility is supported by our Ab-blocking experiments with the Aβ+ Eα-specific mAb Y-Ae (Fig. 4) and by our flow-cytometric analyses (Fig. 5), which suggest that Y-Ae efficiently binds with the Aβ-Eα complex expressed by wild-type cells, but poorly recognizes this complex on mutant cells.
A third possibility was suggested by the recent study of Lee et al. (31), which suggests that most T cells selected in H2-DM-deficient cells are low affinity cells. It is hard to imagine, however, how differences in the affinity of the TCRs would result in such profound differences in the recognition of peptides presented by wild-type vs H2-DM
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APC. In addition, the low affinity T cells studied by these investigators were part of the autoreactive pool, which was excluded from our analysis, and these investigators did not study Ag-specific T cell responses. Nevertheless, it remains possible that our observations are somehow related to those of Lee et al. (31). Thus, our findings may reflect differences in TCR affinity or, alternatively, the findings of Lee et al. (31) may be due to conformational differences in MHC-peptide complexes.

At present, it is unclear why thymic selection in H2-DM
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mice results in a T cell repertoire that is profoundly different from that of wild-type animals. In H2-DM
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mice, T cell selection predominantly proceeds on the A\textsuperscript{b}-CLIP complex. Previous studies have suggested that the conformation of the A\textsuperscript{b}-CLIP complex is structurally different from the majority of A\textsuperscript{b}-peptide complexes expressed by wild-type cells (13–15, 32; data not shown). Perhaps T cell selection on the unique conformation of the A\textsuperscript{b}-CLIP complex contributes to the unusual CD4
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T cell reactivities of H2-DM
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mice. However, it has been previously suggested that a substantial proportion of the CD4
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T cells of H2-DM
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animals is, in fact, selected by the small amount of A\textsuperscript{b}/non-CLIP complexes that are expressed in the thymus of these animals (17). An alternative explanation would therefore be that the unusual reactivities of T cells from H2-DM
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animals are caused by selection on MHC class II molecules at very low peptide density.

Regardless of the explanation that will be found for these observations, our results, and those of Lee et al. (31), indicate that thymic selection on the A\textsuperscript{b}-peptide complexes of H2-DM-deficient mice leads to the maturation of CD4
+ T cells with unusual reactivities. These findings indicate that the CD4
+ T cell repertoires of
H2-DM− and H2-DM+ mice are remarkably different. Our results may have important implications for Ag presentation, MHC-peptide/TCR interactions, and T cell repertoire selection.

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