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Quantitative Analysis of the Immune Response to Mouse Non-MHC Transplantation Antigens In Vivo: The H60 Histocompatibility Antigen Dominates Over All Others

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Minor histocompatibility Ags (minor H Ags) are substantial impediments to MHC-matched solid tissue and bone marrow transplantation. From an antigenic standpoint, transplantation between MHC-matched individuals has the potential to be remarkably complex. To determine the extent to which the immune response is simplified by the phenomenon of immunodominance, we used peptide/MHC tetramers based on recently discovered minor H Ags (H60, H13, and HY) and monitored in vivo CD8 T cell responses of female C57BL/6 mice primed with MHC-matched, but background-disparate, male BALB.B cells. CD8 T cells against H60 overwhelmed responses to the H13 and HY throughout primary and secondary challenge. H60 immunodominance was an inherent quality, overcoming a lower memory precursor frequency compared with that of H13 and evoking a T cell response with diverse TCRVβ usage. IFN-γ staining examining congenically defined minor H Ags extended H60 dominance over additional minor H Ags, H28, H4, and H7. These four minor H Ags accounted for up to 85% of the CD8 T cell response, but H60 stood out as the major contributor. These findings show that immunodominance applies to antigenically complex transplantation settings in vivo and that the responses to the H60 minor H Ag dominates in this model. We suggest that immunodominant minor H Ags are those that result from the absence of a self analog. The Journal of Immunology, 2001, 166: 4370–4379.
NK2D stimulatory receptors: however, it is not known whether this property impacts its immunodominance (21, 22).

The rules that govern immunodominance of minor H Ags remain to be established (8, 17). A major part of the uncertainty comes from the fact that the analysis of minor H Ag responses has been based, to a large extent, on extrapolations from in vitro analyses: conventional $^{51}$Cr release assays of CTLs after MLC and limiting dilution assays, both of which require an in vitro culture period. This is confounded by the fact that most previous data are based on single time point analyses, because the number of lymphoid cells typically used for in vitro culture precludes serial sampling. Methods that allow tracking of the T cell response to specific minor H Ags longitudinally in vivo would, therefore, be an important asset to understand the dynamic process by which immunodominance is achieved.

The goal of this study was to elucidate the dynamics of the minor H Ag-specific CD8 T cell responses in vivo following immunization. We show that CD8 T cells directed against the newly identified minor H Ag, H60, overwhelm responses to H13 and HY at all stages of the immune response in both spleen cell immunization and skin graft settings because of an inherent property of H60 to induce the disproportionate clonal expansion of specific CD8 T cells. Remarkably, H60-specific T cells dominate over all other minor H Ag-specific T cells in the B6 anti-BALB.B response.

Materials and Methods

Mice

C57BL/6J (B6) female mice were used as responders or recipients and C.B10-H2b/LiMcDr (BALB.B) male mice were used as donors of spleen cells or skin grafts. Congenic mouse strains, B6.C-H2b/b by female (H2 b congenic), B10.C-H2 b (3N)/Sn female (H13 congenic), B10.C-H2 b (4N7)/Sn female (H7 congenic), and B10.129-H46bH47b(21M)/Sn female (H4 congenic) have been described (7, 16, 23). H60 congenic B6.C-H60b/DCR mice were obtained from an outcross of BALB/cBy to B6, followed by 10 backcross generations to B6. Molecular typing indicated that the H60 congenic segment is <8 cM and is flanked by the D10Mit51 and D10Mit106 markers. All the mouse stains used in this study were maintained at The Jackson Laboratory (Bar Harbor, ME).

Cell lines and mixed leukocyte cultures

Cells lines were maintained in DMEM (Life Technologies, Grand Island, NY) supplemented with 5% FBS (HyClone, Logan, UT). TAP-deficient human T2 cells expressing H-2b, H-2d, or H-2k (H-2k) were provided by P. Cresswell (Yale University, New Haven, CT). The H13-specific H-2b,D-restricted CTL line B10.BR-3 and the HY-specific H-2D-restricted CTL clone CTL-10 (24) have been described previously. The H60-specific CTL line B6.1/H60 was generated by immunizing C57BL/6 mice with cells from H60 congenic B6.C-H60b/DCR mice and was maintained by weekly restimulation with BALB.B cells and 50 ml of rIL-2 following established protocols (25). Additional CTL lines were established from anti-H2Ab splenocytes from B6 female mice primed with 2 x 10^5 spleen cells from BALB.B male mice (lines SP/H60, SP/H13, and SP/HY) or MLCs from B6 female mice grafted with tail skin of BALB.B (H7 congenic) and then washed with PBS before coculture with responders. After 2-h restimulation, Brefeldin A (Sigma) was added to final concentration of 10 mg/ml, and cells were incubated for an additional 4 h to allow IFN-gamma to accumulate and in the endoplasmic reticulum of activated cells. Cells were then incubated with ethidium monoazide bromide (final concentration, 5 mg/ml; Molecular Probes, Eugene, OR) to label live cells. After washing, cells were surface-stained with FITC-conjugated anti-CD8 mAb, washed, fixed with 1% paraffin/methyldehyde in PBS at room temperature for 20 min, and then incubated with PE-conjugated anti-IFN-gamma mAb (PharMingen) diluted in PBS containing 0.1% saponin (Sigma). Stained cells were analyzed by flow cytometry with live cell gating.

Results

Longitudinal analysis of the B6 female anti-BALB.B male responses after spleen cell immunization

To monitor minor H Ag-specific T cells, soluble tetrameric peptide-MHC class I complexes for H60, H13, and HY minor H Ags were developed. The specificities of H60/H2-DK, H13/H2-Dd, and HY/H2-Dd tetramers are illustrated by their abilities to stain es-

Tetramers

Soluble MHC-peptide tetramers for H60, H13, and HY minor H Ags were prepared using a method similar to that described previously (27, 28). Recombinant MHC class I heavy chains and mouse $\beta_2$-microglobulin protein were produced in Escherichia coli. H2-Kb or H2-Dd complexes were folded in vitro with $\beta_2$-microglobulin and LTFNYRLN for H60, SSVIGVWYL for H13, and WMHHNSMILC1 HY-UTY, respectively. The MHC-peptide complexes were purified by gel permeation chromatography and enzymati-

Cell staining and flow cytometry

Cells (10^6) from fresh PBL, splenocytes, or cells derived from MLC were incubated at 4°C for 40 min in PBS with 0.1% BSA and 0.1% sodium azide containing PE-labeled tetrameric complexes and saturating amounts of anti-CD8 mAb conjugated to FITC (53-6.72, The Jackson Laboratory). For phenotypic analysis, cells from MLC were incubated with PE-labeled tetramer, one of a panel of anti-TCR mAbs directly conjugated to FITC (PharMingen, San Diego, CA), and an anti-CD8 mAb conjugated to Quan-

Intracellular IFN-gamma staining analysis

RBC-depleted spleen cells from immunized B6 mice were subjected to MLC with RBC-depleted and irradiated BALB.B male spleen cells for 11 days, during which IL-2 was added on day 7 at concentration of 10 U/ml. Cells (1 x 10^6) from MLC were used as responders and cultured with stimulators in 96-well round-bottom plates (Costar, Cambridge, MA) in DMEM (0.2 ml/well) for 2 h. The stimulators were RBC-depleted spleno-

Cell-mediated lysis assay

The standard $^{51}$Cr release assay has been described previously (26). For peptide-loaded targets, $^{51}$Cr-labeled T2-D and T2-K cells were incubated with 1-mM concentration of synthetic peptides for 30 min at 37°C, washed twice with PBS to remove unbound peptide, and then coincubated at 37°C with effector cells in 1:5 ratio of effector to target cells. Lysis of target cells was measured as specific cytolyis, based on the level of $^{51}$Cr released into the supernatant relative to spontaneous and maximal $^{51}$Cr, and is shown as the mean of triplicate wells.
or preceding days (data not shown). However, on day 5, H60 tetramer⁺ CD8 T cells were distinguished as a grouped population that increased to 3–4% of splenic CD8 T cells on day 7. Even though the early kinetics of expansion of H60 tetramer⁺ CD8 T cells in PBL paralleled those of splenocytes (Fig. 2A), the frequencies in the spleen were always equivalent to or lower than those observed in the PBL, implying an immediate release of activated T cells from the peripheral lymphoid organs into the bloodstream (29). However, H13 and HY tetramer⁺ CD8 T cells were undetectable in both splenocyte and PBL samples (data not shown).

To characterize the activation state of the H60 tetramer⁺ CD8 T cells, both naïve and immunized splenocytes and PBL from a separate experimental set were examined for the expression of CD11a and CD62L on tetramer-positive cells via flow cytometry on day 7 postimmunization. All H60 tetramer⁺ CD8 T cells from immunized mice were CD11a[high] and CD62L[low] (Fig. 2B), indicative of prior TCR activation. As a consequence of alloimmune stimulation by multiple minor H Ags following BALB.B spleen cell immunization, 20–30% of the total CD8 T cells were in an activated state (CD11a[high] CD62L[low]), reflecting a lower level of activation compared with immune responses against viral infection (11, 30, 31). In this experiment approximately 15–21% of CD11a[high] cells and 7–15% of CD62L[low] cells bound the H60 tetramer.

Serial PBL samples pooled from five immunized mice were used for a longitudinal analysis of CD8 T cell responses to H60, H13, and HY. H60 tetramer⁺ CD8 T cells were undetectable on day 0, but, after primary immunization with BALB.B male splenocytes, increased to 0.34% of CD8 T cells on day 4 and to about 5.7% on day 7. The frequency of H60 tetramer⁺ CD8 T cells peaked at approximately 6.3% of CD8 T cells on day 10, with the most rapid increase between days 4 and 7. The frequency of H60 tetramer staining CD8 T cells then declined to a stable level of about 0.7% of CD8 T cells after 30 days and persisted at that level for 150 days (Fig. 2C). The slowing of clonal expansion after day 7 correlates with clearance of donor APCs by effectors cells (32), followed by a dramatic decline of responding T cells, presumably by apoptotic cell death. After secondary immunization with BALB.B male spleen cells, H60 tetramer⁺ CD8 T cells expanded to 3.3% of CD8 T cells on day 4 after secondary challenge, increasing to 7.1% on day 7. Then it stabilized at 4% of CD8 T cells, an almost 4-fold higher frequency than that observed after the primary response, for at least 150 days. However, neither H13 nor HY tetramer⁺ CD8 T cells were detected at any time point during the primary or secondary responses (Fig. 2C). These results strongly suggest that CD8 T cell responses against H60 overwhelm responses against H13 and HY, resulting in a higher frequency of memory cells. These memory cells can persist over a considerable period of time and undergo a classical anamnestic response after a second challenge. Thus, H60 dominance persists at all points in the primary and secondary responses and continually represses immune responses to H13 and HY.

**FIGURE 1.** Specificity of tetramers for minor H Ag-reactive T cells.

H60- (B6.1/H60), H13- (B/NX-3), and HY- (CTL-10) specific CTL lines were stained with PE-conjugated minor H Ag/MHC class I tetramers and FITC-conjugated anti-CD8 mAb and analyzed by flow cytometry.  

**Dominance of H60 is maintained in vitro, but is not absolute**

The great majority of information concerning minor H Ag responses is based on analyses of primed T cells after restimulation in vitro following several days of tissue culture. To understand how in vitro culture influences the frequency of dominant and subordinate minor H Ag-specific T cells, we compared the frequencies of tetramer-staining CD8 T cells directly ex vivo on day 7 to those observed after restimulation in vitro. Spleen cells from three BALB.B-immunized B6 female mice were harvested, pooled, and restimulated in MLC with irradiated BALB.B male splenocytes. Additionally, to expand minor H Ag-specific CD8 T cells with a potentially low frequency, parallel MLCs were set up using the same primed responder spleen cells and stimulated selectively with irradiated splenocytes from congenic (H60 or H13), B6 male (HY) or female (syngeneic control) mice. H60 tetramer⁺ CD8 T cells expanded from approximately 6% ex vivo to 30 and 29.7% of CD8 T cells after a 5-day MLC with BALB.B and H60 congenic stimulators, respectively (Fig. 2A). Thus, CD8 T cells directed against H60 were activated to the maximal possible extent under conditions in which many minor H Ags were presented during BALB.B restimulation in vitro. In contrast, both H13 and HY tetramer⁺ CD8 T cells remained below the limit of significance after in vitro restimulation with BALB.B cells. However, selective restimulation in vitro with H13-congenic cells led to, at best, a minimal enrichment in H13 tetramer⁺ cells (0.86% of CD8 T cells). In contrast, HY-responding cells were barely detected even after selective restimulation with B6 male cells (<0.2% of CD8 T cells).

To determine whether the frequencies observed by tetramer staining and specific cytotoxic activity correlated, standard 4-h ⁵¹Cr release assays were conducted using effector cells from the MLCs described above. Results from CML assays were concordant with the tetramer data (Fig. 3B). Thus, strong cytotoxic activity against H60 peptide-loaded T2-K[γ] cells was evident in MLCs with both H60 congenic (79% lysis) and BALB.B male (87% lysis) stimulators (Fig. 3B, upper panel). In contrast, while H13-specific cytotoxicity was negligible after MLC with BALB.B, in vitro culture with H13 congenic stimulators resulted in H13-specific cytotoxicity, which increased minimally to 10%, thus coinciding with increased H13 tetramer staining (Fig. 3, A and B, middle panel). No HY-specific cytotoxicity was detected after MLC with BALB.B or with male stimulators (Fig. 3B, lower panel). The combined results from tetramer staining and CML assays indicate that the H60 response dominates H13- and HY-specific responses in terms of both minor H Ag-specific cell number and cytotoxicity. Immunodominance, therefore, is established as a consequence of immunization in vivo and is not biased by short term in vitro culture with stimulator cells expressing the same set of minor H Ags. However, repeated minor H Ag-specific restimulation in vitro did indeed encourage the clonal expansion of CTLs specific not only for H60 (line SP/H60), but also for H13 (line SP/H13) and HY (line SP/HY; Fig. 3C). These results indicate that mice generate CTLs, albeit at very low numbers, directed against subordinate minor H Ags even when confronted by an overwhelmingly dominant minor H Ag-specific response.
Immunization by skin graft relaxes, but does not abrogate, the immunodominance of H60

The type of APC is thought to influence the outcome of immune responses (33–35). To test whether the dominance hierarchy induced by immunization with spleen cells is the same when the source of alloantigen is a skin graft, we conducted parallel experiments as described above, except that the B6 female mice were immunized by tail skin grafts from BALB.B male mice. In comparison with spleen cell immunization, the frequency of splenic H60 tetramer-positive CD8 T cells was lower, comprising 1.5% of the CD8⁺ splenic T cells on day 10 postgraft. The frequency increased to 7.4% after in vitro culture with BALB.B cells and to 15.7% after in vitro culture with H60 congenic cells (Fig. 4A, upper panel). The lowered frequency compared with spleen cell immunization might reflect increased competition among CD8 T cells responding to diverse minor H Ags. This possibility is reinforced by finding a higher frequency of H13 tetramer⁺ cells following skin graft rather than spleen cell immunization (2.6 and 3.2% of CD8 T cells after stimulation with BALB.B and H13 congenic cells, respectively; Fig. 4A, middle panel). A concordant increase in CTL activity of MLC cells to H13 peptide-loaded T2-Db cells was observed (35 and 55.8% lysis after MLC with BALB.B and H13 congenic cells, respectively; Fig. 4B, middle panel). However, HY-specific CD8 T cells were still undetectable in both cases of tetramer staining and cytotoxicity (Fig. 4, A and B, lower panel). We were able to establish specific CTL lines for H60 (SK/H60), H13 (SK/H13), and HY (SK/HY) after repeated minor H Ag-specific restimulation in vitro (Fig. 4C). These results suggest that immunization via a skin graft diversify somewhat the immune response, attenuating the dominance of H60 and enabling detection of
CD8 T cells responding to other minor H Ags, including H13. However, the overall hierarchy of immunodominance, H60>H13>HY, was still retained.

Quantitative differences in clonal expansion do not contribute to the dominance of H60 over H13

Given the considerable extent to which H60 dominates H13 after spleen cell immunization, we tested whether the dominance hierarchy is affected by increasing the frequency of H13-specific CD8 T cells. To do so, we performed in vivo competition analysis. B6 female mice were injected with H60 congenic spleen cells from male mice. (The use of male mice results in the induction of CD4 T cell-derived helper factors in response to HY minor H Ags.) This resulted in a small, but persistent, population of H60-specific CD8 memory T cells (Fig. 5). To generate H13-specific CD8 T cell responses and to fix the frequency of H13-specific memory cells at a higher level than H60-specific memory cells, the mice were then immunized twice with spleen cells from H13 congenic mice. (This congenic strain includes the H3b minor H Ag that is known to induce CD4 T cell-derived helper factors.) Two immunizations with H13 congenic cells increased the frequency of H13-specific CD8 T cells to a maximum of 1.43% of CD8 T cells (10-fold higher than H60-specific CD8 T cells), which decreased to 0.63% of CD8 T cells 14 days later (5-fold higher than H60-specific CD8 T cells). Despite the fact that the frequency of H60-specific T cells was 5-fold lower than that of H13-specific CD8 T cells at that time, challenge with H13- and H60-bearing BALB.B male spleen cells expanded the H60-specific CD8 T cell population 60-fold to 7% of CD8 T cells, while H13-specific CD8 T cells increased to only 0.9% of the total CD8 T cells. These results suggest that H60 dominance in vivo is an inherent qualitative property of T cells responding to this Ag.

Heterogenous TCRβ usage in responses to H60

The rapid appearance of H60 tetramer+ cells in the blood and spleen after immunization (Fig. 4) could be facilitated by a greater diversity of CD8 T cell clonotypes responding to H60 compared with subdominant minor H Ags. To address this possibility, TCR

FIGURE 3. Tetramer and CML analyses of responder cells from B6 mice after priming with BALB.B spleen cells. A, Seven days after immunization, splenocytes from responder mice were stained with PE-conjugated tetramers for H60, H13, and HY and FITC-conjugated CD8 Ab. Responder splenocytes were also stimulated in 5-day MLCs with irradiated splenocytes from BALB.B, congenic (H60, H13), or B6 male (HY) mice and, then, stained as ex vivo cells were. B, Effector cells from the above 5-day MLCs with BALB.B male or Ag-specific stimulators (H60-Con, H13-Con, and B6 male) were analyzed for specific cytotoxicity against the indicated peptide-loaded T2 target cells. MLC with syngeneic B6 female stimulators included a negative control and failed to demonstrate any specific CTL activity. C, Minor H Ag-specific CTL lines (SP/H60, SP/H13, and SP/HY) were established from the above MLCs with Ag-specific stimulator after six rounds of restimulation.
phenotyping for Vβ usage was performed with cells from short term MLCs using flow cytometry. Independent MLCs were set from two B6 female mice (mice A and B) 7 days after immunization with BALB.B spleen cells. On day 5 post-MLC, cells were subjected to tricolor flow cytometric analysis using anti-Vβs mAbs-FITC, anti-CD8 mAb-QR, and H60 tetramer-PE. Not surprisingly, Vβ usage of the total CD8 T cell set responding to BALB.B stimulation was heterogeneous with all 14 Vβ TCRs represented (Fig. 6, upper plot). Vβ usage by H60-specific T cells was only slightly less diverse, with representation in 11 of 14 Vβs (Fig. 6, middle plot). Vβ4, -5, -8, and -11 were Vβs commonly used in both animals with high frequency and comprised up to 94.5% of H60-specific T cells in the mouse A. In mouse B the common families accounted for 69.1% of H60-specific CD8 T cells, with percentages of 16.3% (Vβ4), 13.5% (Vβ5), 2.6% (Vβ8), and 13% (Vβ11). Since H13-specific CD8 T cells were not easily detectable during primary MLC, the CTL line for H13 (SP/H13), along with the CTL line for H60 (SP/H60) from Fig. 3C were analyzed for TCR Vβ usage. Broad TCR usage was maintained by the SP/H60 CTL line cells again; 8 of 14 Vβs (Fig. 6, lower plot), but only two Vβs (Vβ7 and 17), were used by the H13-specific CTL line, SP/H13, with the overwhelming usage of Vβ7 (97%). These results are consistent with a diverse TCR repertoire of H60-responding CD8 T cells, but a much more limited repertoire responding to H13. Clonal diversity may thus enable H60-responding T cells to overwhelm T cells responding to H13.

T cell “accounting” shows that H60 dominates over all other minor H Ags in the B6 anti-BALB.B CD8 T cell response

Although H60 dominance over H13 and HY was established by flow cytometry using tetramers and by functional CML analysis, the level of participation of a larger array of minor H Ags that are considered to be immunodominant remained unresolved. To address this question, we employed a modified IFN-γ staining method to quantitate low frequency T cell responses. Two independent MLCs were made from B6 female mice immunized with
The immune response to minor H Ags is a serious clinical concern for both solid and bone marrow allogenic transplantation. However, it has been extremely difficult to understand how the responses proceed in vivo because of the lack of quantitative analytical tools. This work is the first to exploit tetramer technology to understand the in vivo dynamics of the minor H Ag response. We show that H60 dominates over H13 and HY at all phases of the immune response, continuing through secondary challenge, and persisting potentially lifelong. Intracellular IFN-γ analysis examining congenically defined minor H Ags allowed us to extend the H60 dominance to additional minor H Ags. As a result, the hierarchy in the immune responses of B6 female mice against BALB.B male spleen cells is H60>H28, H4 and H7>H13>HY. However, it is important to note that while CD8 T cells responding to H60 predominated, they did not completely prevent T cells from responding to subordinate H13 and HY minor H Ags. The fact that immunodominance is not absolute, even under the most competitive situations, has implication for understanding the immunogenetics of tissue transplantation. Moreover, the immunodominance of minor H Ags appears to be heavily dependent on the route of immunization and/or the type of APCs (7, 35–38). In our study immunization via skin graft, in which Langerhans cells play a role as APCs, relaxed immunodominance, thus broadening the antigenic diversity presented to the host immune system. However, the consequence of this broadening is not absoluted from the dominance hierarchy.

Comparison of the dynamics of immune responses against minor H Ag and viral Ags

The overall kinetics of the T cell response to H60 paralleled responses to dominant viral Ags: 1) rapid expansion of CD8 T cells after primary Ag encounter, 2) a substantial decline via activation-induced death, and 3) establishment of a small and persistent memory population (39, 40). However, the maximum frequency observed in the H60 response was 5- to 10-fold lower than that

Discussion

The immune response to minor H Ags is a serious clinical concern for both solid and bone marrow allogenic transplantation. However, it has been extremely difficult to understand how the responses proceed in vivo because of the lack of quantitative analytical tools. This work is the first to exploit tetramer technology to understand the in vivo dynamics of the minor H Ag response. We show that H60 dominates over H13 and HY at all phases of the
reported for dominant viral Ag epitopes (30, 31, 41). Presumably these differences in magnitude result from the fact that viruses can amplify and generate a more efficient T cell-activating signal than do the nonexpansive allogeneic minor H Ag stimuli.

Secondary challenge with viruses is typically associated with a remarkable and immediate proliferative burst, which then enables the secondary response to proceed with greater efficiency (41, 42). However, with the exception of a 3-day lag phase in the primary response, the induction kinetics and the peak frequency of H60-specific CD8 T cells in the PBL after second immunization barely differed (~7% of CD8) from those in the primary responses (~6%), despite substantial differences in the starting precursor frequencies. The lack of an appreciably expanded burst size in the H60-specific secondary response could be caused by more efficient elimination of APCs by CTLs in the secondary response compared with the primary response. However, we cannot exclude the possibility that, in the secondary response, covert clonal amplification occurs in peripheral sites with gradual release into the bloodstream. In this context, Lefrancois et al. recently observed that memory cells take up residence in nonlymphoid peripheral tissues (Ref. 43 and unpublished observations). Nevertheless, despite a similar burst size, the percentage of H60-specific T cells that converted to memory T cells was substantially increased after secondary challenge: approximately 4% of CD8 T cells compared with about 0.7% after primary challenge. The results represent an extreme example of selection for memory cells imprinting to resist programmed cell death, presumably as a consequence of up-regulation of anti-apoptotic molecules, such as Bcl-2 (44).

**What explains dominant minor H Ags?**

Immunodominance is a response pattern adopted by CD8 T cells when confronted with numerous peptide-MHC epitopes during antiviral, anti-tumor, and autoimmune responses (45, 46). Although its multifactorial basis has been explained in several ways (19, 47, 48), shaping of the naive T cell repertoire by thymic selection has gained increasing support as a potentially important mechanism underlying the generation of immunodominant responses (49–51). The presence/absence of self-minor H Ag analogs (H60) could influence thymic selection of minor H Ag-specific T cells quantitatively (the number of cognate T cells that survive negative selection) and qualitatively (the diversity of cognate TCRs that survive negative selection). However, the inability of H13-specific memory T cells to compete successfully with H60-specific memory cells (Fig. 5) does not support the quantitative model. Similar conclusions have been made from in vivo competition studies to viral Ags (47). Immunodominance of H60 over H13 is more consistent with a diversity model. It is notable that a remarkable diversity of Vβ families participated in the H60-specific CD8 T cell response, while the response against H13 was much more constricted. In this context, several common features are shared by the dominant minor H Ags but not by the subordinate minor H Ags. Both H60 and H28 are antigenic because B6 responder mice carry a null allele (19, 20). In contrast, both H13 (16) and another newly identified subordinate minor H Ag, H47, have closely matched allelic analogs that act as self-peptides in B6 mice.4 Self B6 peptides might, therefore, act as partial deleting ligands for TCRs with high affinity for allelically different H13 and H47 major H Ags, while H60 and H28 probably have no comparable self analogs. Thus, the complete lack of negative selection to dominant minor H Ags in the latter situation would provide a more diverse peripheral repertoire of precursor T cells that possess TCRs with high avidity for the minor H Ag/MHC complex with increased frequency. T cells with high avidity TCR for cognate minor H Ags would assume dominance by higher efficiency triggering, resulting in a competitive proliferative advantage over T cells with lower avidity TCRs. However, this selection-avidity model does not explain why

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HY is at the lowest level of the immunodominance hierarchy. The HY-encoding gene Uty has an X chromosome Utx homologe, but this homologe does not encode a peptide sequence that is sufficiently matched with the Uty-encoded HY-D6 epitope to bind D6 (15). Malarkannan et al. showed that Uty is minimally expressed by dendritic cells while H60 and H28 are well expressed (19). Thus, the extreme subordinance of HY minor H Ag may occur because of defective expression of the Ag by professional APCs.

The majority of minor H Ags arise as a consequence of naturally occurring polymorphisms, resulting in amino acid changes in expressed proteins, e.g., ND1 (52), CO1 (53), ATPase 6 (54), H3a (55), H13 (16), H47,4 and HA-1 (56), rather than the presence/pressed proteins, e.g., ND1 (52), CO1 (53), ATPase 6 (54), H3a occurring polymorphisms, resulting in amino acid changes in ex...