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Asynchronous Differentiation of CD8 T Cells That Recognize Dominant and Cryptic Antigens

Chantal Baron,†‡ Marie-Christine Meunier,*,† Étienne Caron,*,† Caroline Côté,*† Mark J. Cameron,‡ David J. Kelvin,‡ Richard LeBlanc,§ Vincent Rineau,*† and Claude Perreault‡*†

Restriction of T cell responses to a few epitopes (immunodominance) is a central feature of immune responses. We analyzed the entire transcriptome of effector CD8 T cells specific for a dominant (H7*) and a cryptic (HY) mouse Ag and performed a longitudinal analysis of selected T cell differentiation markers. We found that Ag specificity had a relatively modest influence on the repertoire of genes that are transcriptionally modulated by the CD8 T cell differentiation program. Although the differentiation programs of anti-H7* and anti-HY T cells were similar, they did not progress simultaneously. The expansion peak of anti-H7* T cells was reached on day 10 while that of anti-HY T cells was attained on days 15–20. Between days 10 and 20, anti-H7* T cells were in the contraction phase and anti-HY T cells in the expansion phase. Furthermore, expansion and development of effector function were well-synchronized in anti-H7* T cells but were disconnected in anti-HY T cells. We propose that, by leading to selective expansion of the fittest CD8 T cells, immunodominance may be beneficial to the host. Inhibition of the T cell response to cryptic Ag would ensure that host resources (APC, cytokines) for which T cells compete are devoted to T cells with the best effector potential. One implication is that favoring expansion of the fittest effector T cells in general may be more important than increasing the diversity of the T cell repertoire. *The Journal of Immunology, 2006, 177: 8466–8475.

Under experimental conditions, it is relatively easy to elicit CD8 T cell responses against an epitope presented alone on professional APCs, provided Ag-reactive T cells are present in the T cell repertoire. However, this situation has little to do with real-life conditions. Indeed, during the course of infection, T cells are confronted with APCs that present a multitude of nonself epitopes. Strikingly, under the latter circumstances, CD8 T cells respond to only a few immunodominant epitopes and neglect other potentially immunogenic peptides (cryptic epitopes). Restriction of CD8 T cell responses to a few selected epitopes has been termed immunodominance, a central and robust feature of immune responses (1–8). At face value, immunodominance is counterintuitive. Focusing on one or a few targets allows pathogens to escape by selectively mutating the relevant epitope(s) (9). The risk is particularly severe in the case of pathogens with a high mutation rate such as HIV, influenza virus, and hepatitis C virus (10–12). Thus, immunodominance presents a major hurdle to developing vaccines that generate effective CD8 T cell responses (7). In fact, the sole hypothetical justification for immunodominance is that limiting the diversity of the immune response might reduce the potential for cross-reactive autoimmune recognition (13).

The immunodominance hierarchy is largely dictated by the immunodomination process whereby dominant epitopes abrogate response to nondominant epitopes (14). Immunodomination results from cross-competition between T cells specific for different epitopes (15). The mechanisms of immunodomination remain ill-defined. In most models, the elusive key event occurs at the T cell/APC interface because immunodominination disappears when competing epitopes are presented on different APCs or when APCs are present in large excess (3, 16–21). Several nonmutually exclusive explanations have been proposed for this. Dominating T cells may kill or exhaust APCs, monopolize APC resources, or directly send inhibitory signals to nondominant T cells (7, 15, 19, 22, 23). Two studies reported that injection of very large numbers of memory CD8 T cells specific for immunodominated Ag did not enable these T cells to compete more successfully against T cells that recognized dominant epitopes (14, 24). Thus, except perhaps in some extreme situations, dominating T cells do not win the competition at the T cell/APC interface because they are more abundant than other T cell clonotypes in preimmune animals (23). Dominating T cells win the competition because they possess some elusive functional advantage over other T cells (14, 19, 23).

To decipher the mechanisms and the ultimate role of immunodominance, we sought to compare the differentiation program of T cells specific for dominant and cryptic Ags (cryptic Ags elicit T cell responses when they are presented alone, but not when presented with other Ags on the same APC). By ultimate role, we mean, as proposed by Casanova and Abel (25), the biologically relevant and evolutionarily selected function of immunodominance in host defense. Therefore, we analyzed global patterns of gene expression in effector CD8 T cells specific for two model
minor histocompatibility Ags (MiHAs)\(^3\) presented by H2D\(^b\)-HY and H7\(^a\) (formerly called B6\(^{dom1}\))—that are encoded by the Uty and Simp/Stt3b genes, respectively (26–28). We rationalized that selecting these Ags would maximize the sensitivity of our studies because H7\(^a\) and HY lie at opposite ends of the immunodominance scale (that is, in their ability to exert and resist immunodominance). Thus, while cell surface expression of H7\(^a\) prevents priming against many MiHAs present on C57BL/6 cells, H7\(^a\)-specific CD8 T cells consistently resist immunodominance by T cells specific for a myriad of other MiHAs (16, 19, 22). In contrast, HY MiHA elicits CD8 T cell responses only when presented alone. H7\(^a\) totally abrogates responses to HY when both Ags are presented on the same APC (16, 19). We found that the differentiation programs of anti-H7\(^a\) and anti-HY T cells were similar, but did not progress simultaneously. In addition, expansion and development of effector function were well-synchronized in anti-H7\(^a\) T cells but were disconnected in anti-HY T cells.

\(^3\) Abbreviations used in this paper: MiHA, minor histocompatibility Ag; qrt-PCR, quantitative real-time PCR; MFI, mean fluorescence intensity; Tet, tetramer.

**Materials and Methods**

**Mice**

C57BL/10Snj (B10), and B10.C-H7\(^a\)(47N)/SN (B10.H7\(^a\)) mice were obtained from The Jackson Laboratory. Mice were bred in the Guy-Bernier Research Center and maintained in specific pathogen-free conditions according to the standards of the Canadian Council on Animal Care.

**Immunization and cell preparation**

Six- to 10-wk-old B10.H7\(^a\) female mice were immunized by i.p. coinjection of 2 × 10\(^7\) B10 and 2 × 10\(^7\) B10.H7\(^a\) male spleen cells. Single-cell suspensions were prepared from the spleen of immunized mice on the indicated days after Ag priming. Erythrocytes were lysed and CD8\(^+\) cells were enriched by magnetic depletion of B220\(^-\) and CD4\(^-\) cells with microbeads (Dynal).

**Microarray experiments**

**Cell sorting.** Enriched CD8 cells were stained with FITC-labeled anti-CD8a Ab (BD Pharmingen), H7\(^a\) (KAPDNRETL)/H2D\(^b\)-PE tetramers (Tet), and HY (WMHHNNMDLI)/H2D\(^b\)-allophycocyanin Tet (CANVAC). CD8\(^+\)H7\(^a\)Tet\(^+\), CD8\(^+\)HYTet\(^+\), and CD8\(^+\)H7\(^a\)HYTet\(^-\) (CD8\(^+\)Tet\(^-\))
cells were sorted using a FACS Vantage SE with Diva option (BD Biosciences). Purity of sorted cell populations was always ≥98%. Tet+ populations containing between 8,000 and 300,000 cells and Tet− populations containing 2–3 million cells were obtained for each mouse and were processed separately.

**Oligonucleotide microarrays and data analysis.** Total RNA was isolated from sorted populations using TRIzol (Invitrogen Life Technologies). Due to the small numbers of cells involved, 100–1000 ng of total RNA was linearly amplified using MessageAMP aRNA kits (Ambion) as per the manufacturer’s instructions. Two Tet+ samples (one HY+ and one HY−) provided insufficient amounts of cRNA and were not processed further. The remaining samples were prepared for oligonucleotide microarray analysis using Affymetrix two-cycle cDNA synthesis and IVT kits according to the manufacturer’s instructions. Two Tet+ samples (one HY+ and one HY−) were used in the analysis. Hybridization of cRNA (15 μg) to Affymetrix Mouse Genome 430 2.0 oligonucleotide arrays, and scanning on an Affymetrix GeneChip scanner 3000 were performed according to standard Affymetrix protocols (www. affymetrix.com) at the Centre for Applied Genomics at the Hospital for Sick Children (Toronto, Ontario, Canada). The numbers of independent biological replicates were as follows: CD8+ H7aTet+ (four samples), CD8+ HYTet+ (four samples), and CD8+ Tet− (five samples). After quantile normalization, Affymetrix probe set summaries were obtained from raw intensities using the Robust MultiArray Analysis subroutine implemented in R and obtained from the Bioconductor program repository website (http://bioconductor.org/). A numerical filter was used to select genes with median expression levels above 50, allowing for poorly measured genes and genes not appreciably expressed in a sample to be excluded. Significant genes were then selected through pairwise comparisons of the cell types using the Student t test. Transformed data were displayed using the Gene Linker Platinum software (Biosystexm). Microarray data are Minimum information about a microarray experiment (MIAME) compliant and have been submitted to the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo; accession no. GSE2924).

**Quantitative real-time PCR (qrt-PCR) analysis**

qrt-PCR was performed on cDNA obtained from Tet+ CD8 T cells using an ABI Prism Sequence Detection System 7000 (Applied Biosystems) as previously described (29). Triplicate wells were averaged and the target gene values were normalized for Hprt content. We used specific primers and probes (TaqMan gene expression assays) from Applied Biosystems.

**Flow cytometry analysis**

Enriched CD8+ cells were labeled with anti-CD8-allophycocyanin or -PE, anti-CD62L-FITC, anti-IL-7R (CD127)-PE, anti-CD43-FITC (B111), anti-KLRG1-FITC, and H7a/H2Dd or HY/H2Dd Tet (PE or allophycocyanin conjugated). Stained cells were analyzed by flow cytometry using a FACSCalibur and the CellQuest program (BD Biosciences). We obtained all Ab from BD Biosciences, except for anti-IL-7R that we purchased from eBioscience.

**Results**

**Immunization and design of microarray experiments**

Our first specific objective was to compare the transcriptome of anti-H7+ and anti-HY CD8 effector T cells using the integrative potential of genome-scale microarrays (30). Because we were searching for intrinsic differences between these two T cell populations, we made every effort to eliminate extraneous confounding variables. Thus, we studied CD8 T cell populations that a priori differed only by their Ag specificity (H7+ or HY). Our experimental protocol led to expansion of anti-HY and anti-H7+ CD8 T cells that were primed concomitantly in the same host and received similar CD4 help (Fig. 1). Thus, B10.H7b+ female mice were primed by i.p. injection of a cell mixture containing B10 and B10.H7b+ male splenocytes. Because of the immunodominance phenomenon, H7b abrogates recognition of HY presented on the same APC (B10 male splenocytes), but not of HY presented on separate APCs (B10.H7b+ male splenocytes) (3). Thus, with this immunization scheme, each population of APC triggered CD8 T cells specific for a single MHC class I-associated epitope. Recognition of B10.H7b+ male splenocytes led to expansion of CD8 T cells specific for the H2Dd-restricted HY Ag, whereas B10 male APC entailed selective expansion of CD8 T cells specific for the H2Dd-restricted H7b Ag (16, 19, 22, 26, 27). Of note, both populations of Ag-specific CD8 T cells received CD4 help solely from CD4 T cells specific for the MHC class II-restricted HY Ag (31). Mice were sacrificed on day 14 after priming, and splenocytes from each mouse were processed separately (biological replicates). After depletion of B220+ and CD4+ cells, splenocytes were stained with anti-CD8 Ab as well as H7aTet and HYTet (labeled with different fluorochromes). Then, three populations of CD8+ splenocytes were purified using FACS cell sorting: HYTet+, H7aTet−, and Tet−. RNA
A differentiation program can modify transcription of hundreds of experiments on mouse CD8 T cells have shown that this culminates with cell proliferation, cytokine secretion, acquisition of memory cells. This process involves a complex differentiation program that is initiated by CD8 T cell activation (34–36).

Table I. Partial list of genes differentially expressed in H7aTet+ vs Tet− CD8 T cells

<table>
<thead>
<tr>
<th>Effector function</th>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>Fold Difference (H7aTet+ vs Tet−)</th>
</tr>
</thead>
</table>
| GenBank Accession No. | Expression profile of HYTet+ T cells was remarkably similar to that of H7aTet+ T cells (Fig. 2, A and B). Strikingly, taking a >1.5-fold difference and a p value <0.05 as selection criteria, we found that only 15 of 308 genes were differentially expressed in H7a− vs HY− specific T cells (Table II). This means that CD8 T cells specific for a dominant and a cryptic MiHA had very similar gene expression profiles. Seven genes were expressed at higher levels in H7a-specific T cells relative to Tet− CD8 control cells (Fig. 2, A and B). Differentially expressed genes are involved in multiple cellular processes such as transcription, signal transduction, protein synthesis, cell proliferation, and transport (Fig. 2C); the complete 308-gene data set is shown in supplementary Table I. * A partial list of the differentially expressed genes is provided in Table I to illustrate that the gene expression profile of anti-H7a CD8 effector T cells was similar to that of effector CD8 T cells specific for viral or OVA epitopes (34–36).

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold Difference (H7aTet+ vs Tet−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granzyme K</td>
<td>Gzmk</td>
</tr>
<tr>
<td>TNF (ligand) superfamily, member 6</td>
<td>Tnfsf6</td>
</tr>
<tr>
<td>Granzyme A</td>
<td>Gzma</td>
</tr>
<tr>
<td>S100 calcium-binding protein A6 (calcyclin)</td>
<td>S100a6</td>
</tr>
<tr>
<td>Chemokine (CC motif) ligand 4</td>
<td>Ccl4</td>
</tr>
<tr>
<td>Interferon γ</td>
<td>Ifny</td>
</tr>
<tr>
<td>Neutral sphingomyelinase activation-associated factor</td>
<td>Nsmaf</td>
</tr>
<tr>
<td>Annexin A2</td>
<td>Anxa2</td>
</tr>
<tr>
<td>Fyn proto-oncogene</td>
<td>Fyn</td>
</tr>
<tr>
<td>S100 calcium-binding protein A10 (calpactin)</td>
<td>S100a10</td>
</tr>
<tr>
<td>Guanine nucleotide-binding protein (G protein), γ 10</td>
<td>Gng10</td>
</tr>
<tr>
<td>Interleukin 7 receptor</td>
<td>Il7r</td>
</tr>
<tr>
<td>Basic helix-loop-helix domain containing, class B2</td>
<td>Bhlhb2</td>
</tr>
<tr>
<td>Lectin, galactose binding, soluble 1</td>
<td>Lgals1</td>
</tr>
<tr>
<td>Epithelial membrane protein 3</td>
<td>Emgp3</td>
</tr>
<tr>
<td>S100 calcium-binding protein A4</td>
<td>S100a4a</td>
</tr>
<tr>
<td>Heterogeneous nuclear ribonucleoprotein R</td>
<td>Nrp</td>
</tr>
<tr>
<td>Chemokine (CXC motif) receptor 3</td>
<td>Cxcr3</td>
</tr>
<tr>
<td>Chemokine (CC) receptor 2</td>
<td>Ccr2</td>
</tr>
<tr>
<td>Prostaglandin E receptor 4 (subtype EP4)</td>
<td>Prger4</td>
</tr>
<tr>
<td>Killer cell lectin-like receptor subfamily G, member 1</td>
<td>Klrg1</td>
</tr>
<tr>
<td>Killer cell lectin-like receptor, subfamily D, member 1</td>
<td>Klrd1</td>
</tr>
<tr>
<td>Selectin, lymphocyte</td>
<td>Sell</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>Ctsd</td>
</tr>
<tr>
<td>Serine (or cysteine) proteinase inhibitor, clade B, member 6b</td>
<td>Serpinb6b</td>
</tr>
<tr>
<td>Solute carrier family 4, sodium bicarbonate cotransporter, member 7</td>
<td>Slc4a7</td>
</tr>
<tr>
<td>Hemoglobin, β adult major chain</td>
<td>Hbb-b1</td>
</tr>
<tr>
<td>Latexin</td>
<td>Lxn</td>
</tr>
<tr>
<td>Thioreductin 1</td>
<td>Txnl</td>
</tr>
</tbody>
</table>

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Differential gene expression in anti-H7a vs anti-HY CD8 effector T cells

We next asked whether the 308 transcripts that characterize T cells primed against H7a were differentially expressed among H7a− and HY-specific T cells. Cluster analysis revealed that the gene expression profile of HYTet+ T cells was remarkably similar to that of H7aTet+ T cells (Fig. 2, A and B). Strikingly, taking a >1.5-fold difference and a p value <0.05 as selection criteria, we found that only 15 of 308 genes were differentially expressed in H7a− vs HY− specific T cells (Table II). This means that CD8 T cells specific for a dominant and a cryptic MiHA had very similar gene expression profiles. Seven genes were expressed at higher levels in H7a− specific than HY-specific T cells. Il7r codes for IL-7R (CD127) whose expression provides survival signals that allow Ag-primed T cells to become memory cells (37, 38). Granzyme A (Gzma), a serine protease in the cytotoxic granules of CTL, induces caspase-independent cell death when introduced with perforin into target cells (39, 40). Vamp5 is a member of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor family that is responsible for the last stage of docking and subsequent fusion in diverse intracellular or intercellular processes (41); the vesicle fusion mediated by members of that receptor family is involved in TCR targeting to the immunological synapse (42). S100a4 regulates cell cycle progression and differentiation, whereas Csf1r (Gene Expression Omnibus data sets 433 and 755), Ftil 43), and Edg8 (44) are up-regulated on activated relative to naive T cells, but definition of their precise roles awaits further studies.
Eight genes were overexpressed in HY-specific relative to H7a-specific T cells. Sell codes for CD62L; down-regulation of CD62L decreases T cell re-entry in the lymph nodes and thereby favoring migration of effector T cells to nonlymphoid tissues (45). Up-regulation of Klrg1 is associated with replicative senescence and may functionally participate in down-regulation of CD8 T cell responses (37, 46–49). In CD4 T cells, Fus up-regulation is associated with Th2 as opposed to Th1 polarization (50). Up-regulation of Eomes may promote terminal differentiation and impair the survival potential of CD8 T cells (51). Mhas, Pchp2, Rps8, and Ung are housekeeping genes whose precise roles in T lymphocytes have yet to be evaluated.

qrt-PCR and flow cytometry analyses

To validate the Affymetrix array profiles and to provide further quantitative data, we assessed by qrt-PCR or flow cytometry the expression of four genes that were differentially expressed in HY- vs H7a-specific CD8 T cells and were deemed to be of particular relevance: Il7r, Klrg1, Sell, and Gzma. qrt-PCR confirmed the relative changes in expression of Il7r, Klrg1, and Gzma while Ab staining confirmed the differential expression of CD62L (Fig. 3).

Of note, different mice were used for the microarray experiments (n = 5) and the validation studies (n = 3), further underpinning the robustness of the differences that we found between H7a- and HY-specific CD8 T cells.

Time-sequential evaluation of anti-H7a and anti-HY T cell differentiation

Time is an essential dimension of complex systems (52). Therefore, we asked whether differences in gene expression profile of anti-H7a and anti-HY T cells were peculiar to day 14 or would be present during all phases of the immune response. To this end, we assessed from days 7 to 100 the expansion of anti-H7a and anti-HY T cells and their expression of selected genes. Based on our qrt-PCR data (Fig. 3), we analyzed the expression of the following cell surface proteins: CD62L, IL-7R (CD127), Klrg1, and the activation-induced glycoform of CD43 (recognized by the 1B11 Ab). Because no Ab are available against mouse granzyme A, we used 1B11 staining as a surrogate marker for the granule exocytosis pathway (53, 54).

At all time points, the frequencies of H7aTet+ T cells were greater than those of HYTet+ T cells (Fig. 4A). Because the frequencies of anti-H7a and anti-HY T cell precursors in the preimmune repertoire are similar (23), anti-H7a T cells underwent more extensive expansion than anti-HY T cells. The salient finding though was the lack of synchronicity between expansion of the two T cell populations. Anti-H7a T cells reached maximal expansion

![FIGURE 3. Validation by qrt-PCR and flow cytometry analysis of genes differentially expressed by anti-HY and anti-H7a T cells. Fold changes of transcript levels in H7aTet+ vs HYTet+ cells obtained in microarray experiments (A) and by qrt-PCR (B). C, Percentage of H7aTet+ and HYTet+ cells that were CD62L− (CD62L is the product of the Sell gene). The histogram shows one representative experiment and the boxed values represent the mean ± SD for three mice. Note that data in B and C were obtained from different mice (n = 3) from those in A (n = 5).](http://www.jimmunol.org/)

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**Table II. Genes differentially expressed in H7aTet+ relative to HYTet+ CD8 T cells**

<table>
<thead>
<tr>
<th>GenBank Accession No.</th>
<th>Gene Name</th>
<th>Fold Difference (H7aTet+ / HYTet+)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_007779NMNM</td>
<td>Colony stimulating factor 1 receptor</td>
<td>4.3</td>
<td>0.009</td>
</tr>
<tr>
<td>NM_010240</td>
<td>Ferritin L chain 1</td>
<td>2.8</td>
<td>0.036</td>
</tr>
<tr>
<td>NM_053190</td>
<td>Endothelial differentiation, sphingolipid G-protein-coupled receptor</td>
<td>2.1</td>
<td>0.025</td>
</tr>
<tr>
<td>NM_008372</td>
<td>IL-7 receptor</td>
<td>2.0</td>
<td>0.006</td>
</tr>
<tr>
<td>NM_011311</td>
<td>S100 calcium binding protein A4</td>
<td>1.8</td>
<td>0.028</td>
</tr>
<tr>
<td>NM_016872</td>
<td>Vesicle-associated membrane protein 5</td>
<td>1.8</td>
<td>0.014</td>
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<tr>
<td>NM_010370</td>
<td>Granzyme A</td>
<td>1.6</td>
<td>0.044</td>
</tr>
<tr>
<td>XM_356068</td>
<td>Malignant fibrous histiocytoma amplified sequence 1</td>
<td>1.6</td>
<td>0.033</td>
</tr>
<tr>
<td>NM_009098</td>
<td>Ribosomal protein S8</td>
<td>1.6</td>
<td>0.028</td>
</tr>
<tr>
<td>NM_016970</td>
<td>Killer cell lectin-like receptor subfamily G, member 1</td>
<td>1.7</td>
<td>0.025</td>
</tr>
<tr>
<td>NM_010136</td>
<td>Eomesoderm homolog (Xenopus laevis)</td>
<td>1.7</td>
<td>0.024</td>
</tr>
<tr>
<td>NM_011042</td>
<td>Pol(rC)-binding protein 2</td>
<td>1.7</td>
<td>0.006</td>
</tr>
<tr>
<td>NM_011677</td>
<td>Kruppel-like factor 2 (lung)</td>
<td>1.7</td>
<td>0.019</td>
</tr>
<tr>
<td>NM_011346</td>
<td>Selectin, lymphocyte</td>
<td>2.0</td>
<td>0.021</td>
</tr>
<tr>
<td>NM_139149</td>
<td>Fusion, derived from t(12;16) malignant liposarcoma (human)</td>
<td>2.1</td>
<td>0.030</td>
</tr>
</tbody>
</table>

* Fifteen genes were differentially expressed in CD8+ H7aTet+ compared with CD8+ HYTet+ cells (p < 0.05, fold change > 1.5).

* Ratios preceded by a minus sign (−) indicate that transcripts were expressed at higher levels in HY-specific T cells; remaining ratios refer to genes expressed at higher levels in H7a-specific T cells.
(100%) on day 10 when anti-HY T cells had only reached 20% of their peak response (Fig. 4B). Expansion of anti-HY T cells reached its maximum between days 15 and 20, when anti-H7a T cells were in their contraction phase.

IL-7R is down-regulated following Ag stimulation and is re-expressed on memory T cells (37). The proportion of IL-7R+ T cells decreased to attain a nadir of 12% in both anti-H7a and anti-HY T cell populations, and thereafter increased in memory T cells (Fig. 5A). Notably, during the first 20 –30 days after immunization, the profile of IL-7R expression correlated with the kinetics of expansion and contraction: IL-7R modulation lingered 5 days behind in anti-HY relative to anti-H7a T cells. The lowest proportions of IL-7R+ T cells (Fig. 5A) were observed at about the same time as the expansion peaks (Fig. 4). The picture was different with CD62L (Fig. 5B). The overall evolution of CD62L as a function of time was similar for the two T cell populations, but during the effector phase (day 7–30) the proportion of CD62L+ elements was consistently lower in anti-H7a than anti-HY T cells (Fig. 5B). In the memory T cell pool (day 100), the proportions of IL-7R+ and CD62L+ elements were similar for anti-HY and anti-H7a T cells (Fig. 5).

From days 10 to 20, the proportion of 1B11+ cells as well as the mean fluorescence intensity (MFI) of 1B11+ cells were slightly though significantly lower for HY-specific relative to H7a-specific T cells (Fig. 6A). Here, the notable point is that for both T cell populations, expression of 1B11 epitopes reached a peak between days 7 and 10 and decreased rapidly thereafter. On day 10, numbers of anti-H7a T cells were at their zenith whereas anti-HY T cells had reached only 20% of their maximal expansion (Fig. 4). Because expression of 1B11 epitopes (activation-induced CD43 glycoform) on CD8 T cells correlates with cytotoxic effector function (53, 55), our data suggest that expansion and acquisition of effector function dovetailed well in H7a-specific, but were ill-synchronized in HY-specific, T cells.
KLRG1 is an inhibitory receptor. Its ligation by cadherins hampers CD8 T cell proliferation and cytotoxic activity (48, 49). We found that accumulation of KLRG1⁺/H11001 cells in the two T cell populations proceeded at a similar pace for the first 10 days after immunization, but diverged by day 15 (Fig. 6B). From days 15 to 100, the proportion of KLRG1⁺/H11001 cells was consistently higher for HY-specific relative to H7a-specific T cells.

Anti-HY CD8 T cell response following priming against HY without H7a

As mentioned in the Introduction, H7a totally abrogates responses to HY when both Ags are presented on the same APC but not when they are presented on separate APCs (16, 19). We can infer from the H7a/HY model as well as other models (20, 56) that presentation of the dominant and cryptic epitope on separate APCs mitigates immunodomination. However, this does not mean that presentation of the two epitopes on separate APCs totally eliminates immunodomination. Therefore, we asked whether H7a-specific T cells might interfere with the expansion and differentiation of HY-specific T cells even when they are primed by distinct APCs. To this end, we compared the expansion and differentiation of HY-specific CD8 T cells in two groups of mice: mice primed against H7a and HY (as depicted in Fig. 1) and a cohort of B10.H7b female mice primed only against HY (by injection of B10.H7b male splenocytes). We evaluated in both groups the expansion of HY-specific T cells and their expression of four differentiation markers (IL-7R, CD62L, activation-induced CD43 glycoforms, and KLRG1) (Fig. 7). Two key points can be made from analyses of anti-HY CD8 T cells obtained after priming with HY alone vs HY + H7a. First, the peak of expansion was reached on day 15 (Fig. 7, A and B), 5 days later than for anti-H7a T cells (Fig. 4). Second, modulation of expression of the four differentiation markers as a function of time was identical in both groups (Fig. 7, C–F). Thus, absence of competing T cells does not modify the cardinal features of anti-HY CD8 T cells, that is, their slow expansion rate and their differentiation program. Nevertheless, lack of competing H7a T cells slightly increased the expansion rate of HY-specific T cells and significantly augmented the magnitude of their expansion. This suggests that even when competing T cells are not in close proximity (their cognate Ag is not presented by the same APC), the H7a-specific CD8 T cells may hamper expansion of HY-specific CD8 T cells, presumably by competing for cytokines.

Discussion

H7a and HY lie at opposite ends of the immunodominance scale in H2b mice. To evaluate and compare their differentiation program, we first analyzed the entire transcriptome of day 14 H7a- and HY-specific effector CD8 T cells, and then performed a time-sequential study of selected differentiation markers. Few microarray experiments have been performed on purified mouse T cell subsets (34–36), and to the best of our knowledge, none has compared the transcriptome of T cells with different Ag specificity. Ag encounter triggers an instructive developmental program that unfolds over weeks or months following initial Ag priming (33, 57, 58). Our initial finding was that on day 14, T cells specific for the dominant (H7a) and the cryptic Ag (HY) had very similar gene expression profiles. Of the 308 genes that were most differentially expressed by Tet⁺ CD8 T cells relative to naive CD8 T cells, 293 were
expressed at similar levels in anti-H7α and anti-HY T cells (Fig. 2). Thus, about the time of maximal T cell expansion (day 14), Ag specificity has a relatively modest influence on the repertoire of genes that are transcriptionally modulated by the CD8 T cell differentiation program. The gene expression profiles of anti-H7α and anti-HY T cells may display more dramatic differences during the earliest stages in the responses, perhaps principally vis-a-vis production of IFN-γ and TNF-α (59–61). However, it will be necessary to devise new methodological approaches to address this important issue. Indeed, the amount of RNA (100 ng) required to study the entire transcriptome and the very low numbers of HY-specific T cells detectable before days 10–14 (Figs. 4 and 7) precluded us from performing Affymetrix GeneChip experiments in the earliest stage of the response. Further studies are also mandatory to evaluate whether posttranslational modifications such as phosphorylation and ubiquitination are differentially regulated in T cells specific for dominant and cryptic Ag. Nevertheless, we found on day 14 some significant differences in the expression of a small subset of genes that can impinge on T cell fitness.

In complex systems, timing is everything. Perhaps the most salient finding of the present study is that although the differentiation programs of anti-H7α and anti-HY T cells were similar, they did not progress simultaneously. That expansion of anti-H7α and anti-HY T cells would have different magnitudes (Fig. 4A) is consistent with their position in the immunodominance hierarchy: H7α is dominant and HY cryptic. What was not expected, however, was that their expansion and contraction would not be synchronized. The expansion peak of anti-H7α T cells was reached on day 10 while that of anti-HY T cells was attained on days 15–20 (Fig. 4B). Between days 10 and 20, anti-H7α T cells were in the contraction phase and anti-HY T cells were in the expansion phase. Both on anti-H7α and anti-HY T cells, the proportion of IL-7Rα+ cells (Fig. 5A) was negatively correlated with T cell pool sizes (Fig. 4); IL-7Rα expression reached its nadir at the time of maximal expansion and increased afterward. At face value, our findings appear to be at odds with studies showing that CD8 T cells with different Ag specificities expand and contract synchronously in response to lymphocytic choriomeningitis virus or Listeria monocytogenes infection (57, 58, 62). We propose that this discrepancy can be explained by the nature of the Ags. Studies on immune response to pathogens have focused on the strongest epitopes, those at the high end of the immunodominance scale. Immune response to cryptic epitopes (such as HY) cannot be studied in classical infection models because, under these conditions, multiple epitopes are presented on the same APC and the immune system therefore neglects cryptic determinants (2). In our model, the dominant and cryptic epitopes were presented by different APC. Accordingly, we have found no other in-depth analysis of the differentiation program of CD8 T cells specific for cryptic determinants (determinants that are immunogenic only when presented alone). Further studies of T cell responses to cryptic determinants will be required to evaluate the generality of our observations on HY. However, one current difficulty with such studies is the lack of cryptic MHC class I-restricted epitopes whose molecular structure is well-defined (a prerequisite for generation of MHC–peptide Tet).

In contrast to expansion and IL-7Rα expression, levels of CD62L and activation-induced CD43 glycoforms evolved concurrently in anti-H7α and anti-HY T cells (Figs. 5B and 6A). Nevertheless, during the effector phase of the immune response, down-regulation of CD62L and up-regulation of CD43 glycoforms were more pronounced on anti-H7α than anti-HY T cells. Considering the strong correlation between expression of activation-induced CD43 glycoforms and cytotoxic activity of CD8 T cells (53, 54), our data suggest that the differentiation program of anti-HY effector T cells is not well-adjusted. In anti-H7α cells, up-regulation of 1B11 epitope was greatest around the time of maximal expansion (days 7–10) (Figs. 4 and 6A). Up-regulation of 1B11 epitope on anti-HY T cells also reached a peak around days 7–10 when expansion of anti-HY T cells was minimal. However, when numbers of HY-specific T cells attained their zenith, levels of activation-induced CD43 glycoforms had declined considerably. In other words, expansion and development of effector function were well-synchronized in anti-H7α T cells but were disconnected in anti-HY T cells. Anti-HY T cells had another handicap: from day 15 onward, the proportion of KLRG1+ cells was greater among anti-HY relative to anti-H7α CD8 T cells. KLRG1 up-regulation has a negative impact on CD8 T cell function because KLRG1 interactions with cadherins on normal tissues inhibit CD8 T cell proliferation and cytotoxic activity (46–49). How KLRG1 expression is regulated is basically unknown. Nevertheless, evidence suggests that KLRG1 expression on CD8 T cells identifies cells that have undergone extensive cell division (46, 47, 63). How can we reconcile the latter concept with the fact that 1) H7α- and HY-specific T cell precursors have similar frequencies in naive mice (in in vitro limiting dilution assays using peptide coated APCs (23)) and that 2) HY-specific T cells accumulate to a lower level than H7α-specific T cells following Ag priming (Fig. 4)? One plausible explanation is that although H7α- and HY-specific T cell precursors have similar frequencies in naive mice, the proportion of T cell precursors that are effectively primed following in vivo immunization is different. Thus, the absolute number of precursor T cells that are recruited and participate to the immune response could be orders of magnitude greater for H7α relative to HY-specific T cells. Accordingly, because HY-specific T cells found after day 15 would derive from a very limited number of primed precursors, they would show evidence of replicative senescence even though they do not accumulate to high levels.

Collectively, our data demonstrate that the rapid expansion of H7α-specific T cells together with their well-synchronized modulation of Gzma, CD62L, and activation-induced CD43 glycoforms makes them better effectors than HY-specific T cells. We reported that H7α- and HY-specific T cell precursors have similar frequencies in naive mice, and that H7α-specific CD8 T cells harvested at the time of primary response show highly restricted TCR diversity (23). Thus, the immunodominant status of H7α is not a question of T cell precursor frequency or TCR diversity. Furthermore, H7α/Dα and H-Y/Dα complexes have similar half-lives (8 h) (22). However, two observations strongly argue that H7α must deliver stronger TCR signals than HY; H7α is expressed at much higher levels than HY at the cell surface (16, 22) and anti-H7α T cells exhibit a much more rapid TCR:epitope on-rate than anti-HY T cells (23). During the course of infection, a 24–48 h delay in generation of effector CD8 T cells can make the difference between life and death (64). From this perspective, the discrepancies between anti-H7α and anti-HY T cells are dramatic (Table II, Figs. 4–6). Moreover, the concept that immunodomination leads to selective expansion of the fittest CD8 effector T cells suggests that, contrary to the dominant paradigm, immunodomination may be beneficial to the host. Indeed, during the course of immune responses, T cells compete for Ag and other APC resources as well as for prosurvival cytokines (65–67). We propose that inhibition of T cell response to cryptic Ags ensures that host resources for which T cells compete are devoted to T cells that have the best effector potential. This might favor rapid clearance of pathogens not only at the time of primary but also upon the subsequent encounters, because immunodomination has a major (though not exclusive) role in shaping the repertoire of the memory T cell compartment (24, 68, 69). One implication is that, in general, favoring
expansion of the fittest effector T cells may have more importance than increasing the diversity of the T cell repertoire. Usually, immunodominant T cells recognize Ag-bearing APC with higher avidity than nondominant T cells (70, 71). Because high-avidity CD8 T cells are more effective at pathogen clearance than the low-avidity CD8 T cells (72), it might therefore make sense to "neglect" T cells with the lowest functional avidity, that is, those specific for cryptic epitopes. However, whether immunodominance of subdominant epitopes is advantageous to the host is not clear because, at least in some cases, T cell responses to subdominant epitopes may contribute to pathogen eradication (73, 74).

Finally, that effector CD8 T cells are generated more rapidly after priming with H7α than HY explains previous observations on in vivo clearance of APC: H7α, but not HY-specific CD8 T cells rapidly eliminate APC bearing their cognate Ag (75). Because cryptic epitopes are poor immunogens, it can be assumed that they require longer duration of Ag presentation than dominant epitopes (32, 76). Therefore, we postulate that by curtailling the duration of Ag presentation (through APC deletion or exhaustion (75, 77–81)), immunodominant CD8 effector T cells selectively impair cryptic epitopes. This model may explain why HY is immunogenic when presented alone (unlimited duration of Ag presentation), but silent when presented with H7α on the same APC (short duration of Ag presentation).

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Disclosures

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