The Surprising Kinetics of the T Cell Response to Live Antigenic Cells

Aaron J. Tyznik and Michael J. Bevan

*J Immunol* 2007; 179:4988-4995; doi: 10.4049/jimmunol.179.8.4988

http://www.jimmunol.org/content/179/8/4988

---

**References**

This article cites 53 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/179/8/4988.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
The Surprising Kinetics of the T Cell Response to Live Antigenic Cells

Aaron J. Tyznik and Michael J. Bevan

Cooperation between CD4+ and CD8+ T cells is required for the proper development of primary effector and memory CD8+ T cells following immunization with noninflammatory immunogens. In this study, we characterized murine CD4+ and CD8+ T cell responses to male-specific minor histocompatibility (HY) Ags following injection of live male cells into females of the same strain. Male cells are rejected 10–12 days after transfer, coinciding with the expansion and effector function of CD8+ CTLs to two H-2Dk-restricted epitopes. Although anti-HY CD4+ T cell responses are readily detectable day 5 posttransfer, CD8+ responses are undetectable until day 10. The early CD4+ response is not dependent on direct presentation of Ag by donor male cells, but depends on presentation of the male cells by recipient APC. The CD4+ T cell response is required for the priming of CD8+ T cell effector responses and rejection of HY-incompatible cells. Unexpectedly, HY-specific CD4+ T cells are also capable of efficiently lysing target cells in vivo. In the delay in the CD8+ T cell response can be largely abrogated by depleting T cells from the male inoculum, and donor male CD8+ T cells in particular suppress host anti-HY CD8+ responses. These data demonstrate dramatic differences in host T cell responses to noninflammatory Ags compared with responses to pathogens. We explain the delayed CD8+ response by proposing that there is a balance between cross-presentation of Ag by helper cell-licensed dendritic cells, on the one hand, and veto suppression by live male lymphocytes on the other. The Journal of Immunology, 2007, 179: 4988–4995.

Studies of endogenous T cell responses to foreign Ag have focused on the rapid and extensive response of Ag-specific CD8+ T cells during acute bacterial and viral infections. Following encounter with these pathogens, naive CD8+ T cells become activated and rapidly undergo 15 or more divisions to establish an effector CTL population 7–8 days postinfection. This extensive proliferation is followed by contraction over the next 7–14 days during which 95% of Ag-specific cells undergo apoptosis, leaving a long-lived memory population (1, 2). Similar to CD8+ T cells, Ag-specific CD4+ T cells undergo a coordinate response of activation, expansion, contraction, and establishment of Ag-specific memory. The main differences observed were that CD4+ T cells had a roughly 20-fold lower expansion, peaked a day later than CD8+ T cells, had a protracted contraction phase, and memory cell numbers declined over time (3, 4).

Following immunization with most pathogens, primary CD8+ T cell responses are independent of CD4+ T cell help (5–7). Despite CD4+ T cell help being dispensable for primary CD8+ T cell responses, it is necessary for the maintenance and functionality of memory T cells (8–11). This is in contrast to the priming of most CD8+ T cell responses to cell-associated Ags where CD4+ T cells are necessary for the induction of optimal primary T cell responses (12–14). It has been demonstrated that the requirement for CD4+ T cell help is due to the requirement for APC conditioning and activation before priming CD8+ T cells. These differences appear to be related to the inflammatory nature of the immunogen such that antigenic tumor cells, minor H-disparate cells, and osmotically loaded cells are relatively noninflammatory and require help from activated CD4+ T cells. It is believed that activated CD4+ T cells reciprocally counter stimulate and license Ag-presenting dendritic cells (DC) through a CD40-CD40 ligand interaction, and the licensed APC can then properly stimulate naive CD8+ T cells (15–17). In contrast, inflammatory stimuli provided by viral and bacterial infections can overcome the CD4+ T cell requirement by directly licensing APC through TLR ligands or other intracellular innate receptors (18).

Although it is clear that the nature of the immunogen can dramatically affect the requirements for APC conditioning and CD4+ T cell help, the exact kinetics and functionality of CD4+ and CD8+ T cell populations following immunization with noninflammatory Ags have not been analyzed. To evaluate T cell responses to noninflammatory Ags, we used the well-characterized minor H Ag HY. This system allows us to track the survival of Ag-bearing cells and analyze both CD4+ and CD8+ Ag-specific T cell responses to three immunodominant epitopes with minimal introduction of inflammatory stimuli. Using this system, we describe the previously unappreciated differential kinetics of CD8+ and CD4+ T cell responses to HY Ags.

Materials and Methods

Mice

B6.129-H2-Ab1tm1Gtm N12 (MHC class II-deficient) and B6.SJL (129)-Ptprc-/BoyAllTac B2m2/2Iae N9 + N1 (β2m−/− SJL) mice were purchased from Taconic Farms. C57BL/6 (B6), B6(C)-H2-Ab1tm1/KhEgI (H2tm1), B6.SJL-Ptprc-/BoyI (B6.SJL), and C57BL/6-Tg(UbC-GFP)30Scha/J (UbC-GFP) mice were purchased from The Jackson Laboratory and housed in specific pathogen-free conditions in the animal facilities at the University of Washington.

Received for publication May 3, 2007. Accepted for publication August 3, 2007.

1 This work was supported by the Howard Hughes Medical Institute and National Institute of Health Grant AI91935 (to M.J.B.) and by Ruth L. Kirschstein National Research Service Award CA009537 (to A.J.T.).
2 Address correspondence and reprint requests to Dr. Michael J. Bevan, Howard Hughes Medical Institute, Department of Immunology, Box 357370, University of Washington, 1959 NE Pacific Street, Seattle, WA 98195. E-mail address: mbevan@washington.edu
A20), anti-CD62L-PE-Cy7 (clone MEL-14) anti-TNF-α (clone 53-6.7), and anti-CD8-PerCP (clone 53-6.7). Anti-MHC class II-CD44-FITC (clone IM7), anti-CD4-FITC (clone RM4-4), anti-CD4-PerCP (clone 53-6.7), and CD8-PerCP (clone 53-6.7). Anti-MHC class II-allophycocyanin (clone M5/114,15,2), anti-CD45.1-PE-Cy7 (clone A20), anti-CD62L-PE-Cy7 (clone MEL-14) anti-TNF-α-FITC (clone MP6-XT22), and anti-IL-2-PE (clone JES6-5H4) were purchased from BD Biosciences: anti-IFN-γ-phycoerythrin (clone MP6-XT22), and anti-IL-2-PE (clone JES6-5H4) were purchased from BD Biosciences. Anti-CD8-allophycocyanin-Alexa Fluor 750 (clone 5H10) was purchased from Caltag Laboratories. Synthetic peptides representing the defined HY epitopes (I-Ab-restricted Dby608–622 (NAGFNSNRANSR) and the H-2Db-restricted Smcy738–746 (KCSRNRQYL) and Uty246–254 (WMHHNMDLI)) were purchased from Chemicon International. Irrelevant synthetic H-2Db-restricted GP33–41 and I-Ab-restricted listeriolysin O (LLO)190–201 peptides were purchased from Invitrogen Life Technologies.

**Intracellular IFN-γ staining**

HY-specific CD8+ and CD4+ T cells were detected by measuring IFN-γ secretion in response to ex vivo stimulation by MHC class I or II peptides using the Cytofix/Cytoperm Kit Plus (with GolgiPlug; BD Biosciences) according to the manufacturer’s instructions. For T cell stimulation, 2 × 10^6 lymphocytes were resuspended in complete DMEM supplemented with 10% FCS and incubated with or without synthetic peptides in a 96-well plate in a volume of 200 µl. Cells were stimulated with the MHC class I H-2Db-restricted peptides (1 µM Smcy or Uty) or the MHC class II I-Ab-restricted peptide (1 µM Dby) for 4–5 h in the presence of 1 µg/ml GolgiPlug (BD Biosciences) at 37°C in 5% CO2. Cells were washed, resuspended in FACS staining buffer (PBS containing 1% FCS and 0.1% sodium azide) and typically stained on ice for 20 min with anti-C8D8-allophycocyanin-Alexa Fluor 750, anti-CD4-PerCP, and anti-CD45.1-PE-Cy7. Following surface staining, cells were washed, resuspended in permeabilization-fixation buffer, washed, and stained for intracellular cytokine expression with anti-IFN-γ-allophycocyanin and anti-IL-2-PE for 30 min at 4°C. Cells were washed in PermWash solution, resuspended in PBS plus 1% paraformaldehyde, and performed on a FACSCanto using CellQuest software (BD Biosciences).

**Tetramer staining**

H-2Db tetramers bound to the Smcy-derived peptide KCSRRNYQYL were generated as described elsewhere (19). Single-cell suspensions were prepared from spleen and lymph nodes by mechanical dissociation and lysis of RBCs in hypotonic buffer. Cells were washed and resuspended in FACS staining buffer and stained on ice for 1 h with H-2D^b^ Te ramers and the following surface Abs: CD44-FITC, CD8-PerCP, CD4-allophycocyanin, and CD62L-PE-Cy7. Flow cytometry was performed on a FACSCanto and analyzed using FlowJo software (Tree Star).

**In vivo cytotoxicity assay**

The analysis of in vivo cytotoxicity was performed similarly to previously described protocols (20, 21). Naïve male or female B6.SJL splenocytes were differentially labeled with either 5.0 or 0.5 µM CFSE (Molecular Probes). For peptide-pulsed target cell killing, naïve B6.SJL CFSE<sub>high</sub> cells were pulsed with 1 µM Uty or Smcy peptides, whereas female CFSE<sub>low</sub> cells were pulsed with an irrelevant peptide, GP33–41. Cells were washed and transferred i.v. (1 × 10^5 cells of each population) into naïve or day 15 immune mice. For killing of target cells expressing endogenous levels of HY Ag, B6.SJL male CFSE<sub>high</sub> and female CFSE<sub>low</sub> cells were transferred i.v. into naïve or day 15 immune mice. Lymph nodes were harvested from donor mice at the indicated time points and analyzed by FACS for target cell clearance. For MHC class II-restricted killing, splenocytes from B6.SJL female or β2m<sup>−/−</sup> SJL male or female mice were differentially labeled with either 5.0 or 0.5 µM CFSE. CFSE<sub>high</sub> B6.SJL cells were pulsed with 1 µM Dby peptides, whereas CFSE<sub>low</sub> cells were pulsed with an irrelevant peptide, LLO<sub>190–201</sub>. Subsequently, naïve and day 9 immune mice were coinjected i.v. with 1 × 10^5 cells of each population. For endogenous MHC class II-restricted killing, naïve or day 9 immune mice received 1 × 10^7 male CFSE<sub>high</sub> β2m<sup>−/−</sup> SJL and 1 × 10^7 female B6.SJL male or female Ubc-GFP-transgenic cells in the spleen of host mice.
CFSElow /H9252 H11002 /H11002 SJL cells. Host mice were depleted of NK cells with 100 g of purified PK136-depleting Ab at day 5 postimmunization. At the indicated time points, lymph nodes were harvested and analyzed by flow cytometry for target cell clearance. For gating on CD45.1 target cells for MHC class I-restricted killing or CD45.1 I-Ab target cells for MHC class II-restricted killing, the percentage of killing was calculated as follows: 100% \[
\frac{\text{percent peptide pulsed in immune}}{\text{percent unpulsed in immune}} \times \frac{\text{percent peptide pulsed in naive}}{\text{percent unpulsed in naive}} \times 100.
\]
In vivo depletion of CD4 and CD8 donor cells
Male mice were injected i.p. with 200 g (day –3) and 50 g (day –1) of purified anti-mouse CD4 (clone GK1.5) or CD8 (clone 2.43) mAb before

**FIGURE 2.** In vivo cytolytic activity 15 days after primary immunization with male splenocytes. A. Equal numbers of Ly5.1 CFSEhigh HY peptide-pulsed female splenocytes and CFSElow control peptide-pulsed female splenocytes were injected i.v. into Ly5.2 C57BL/6 female mice (naive or day 15 postimmunization). In vivo cytotoxicity was assessed 8 h later by flow cytometry. Histograms are gated on Ly5.1 splenocytes in recipient mice. Numbers at the top of each plot represent the percentage of CFSElow or CFSEhigh cells of the total Ly5.1 donor cells recovered. Representative plots of Uty-pulsed (top panels) or Smcy-pulsed target cells (bottom panels).

**FIGURE 3.** Primary CD4 T cell responses to HY are rapid compared with the CD8 T cell response. Female mice were injected with 2 x 10^7 male splenocytes and host CD4 T cell responses were followed. At the indicated time points, splenocytes were harvested and stimulated in vitro for 4 h with Dby or no peptide and stained for intracellular IFN-γ and IL-2 accumulation. A, Intracellular IFN-γ staining in the CD4 T cell population following in vitro peptide stimulation at the indicated times postimmunization. Numbers indicate the percentage of CD4 T cells producing IFN-γ. Results are representative of six separate experiments of two to three mice per time point. B, Percentage of Dby-specific CD4 T cells producing IFN-γ and IL-2 at the indicated time points. Numbers in parentheses indicate the percentage of IFN-γ, CD4 T cells producing IL-2. C, Kinetics of the Dby-specific CD4 T cell response. Values represent percentage of IFN-γ cell in the CD4 T cell population. Results are representative of six separate experiments of two to three mice per time point. Error bars, SEM.

CFSElow β2m-/- SJL cells. Host mice were depleted of NK cells with 100 µg of purified PK136-depleting Ab at day 5 postimmunization. At the indicated time points, lymph nodes were harvested and analyzed by flow cytometry for target cell clearance. For gating on CD45.1 target cells for MHC class I-restricted killing or CD45.1 I-Ab+ target cells for MHC class II-restricted killing, the percentage of killing was calculated as follows: 100% – [(percent peptide pulsed in immune/percent unpulsed in immune)/(percent peptide pulsed in naive/percent unpulsed in naive)] x 100.

**In vivo depletion of CD4 and CD8 donor cells**
Male mice were injected i.p. with 200 µg (day –3) and 50 µg (day –1) of purified anti-mouse CD4 (clone GK1.5) or CD8 (clone 2.43) mAb before
Efficient lysis of peptide-pulsed target splenocytes has been shown in many systems, but little is known about the lysis of target
postimmunization and splenocytes were assessed for intracellular IFN-γ responses were followed. Host mice were sacrificed at days 9, 12, and 15. The CD8+ T cell responses to HY Ag was cross-presented exclusively by host APC (Fig. 4 A). Recent studies have demonstrated that CD8+ T cell responses to HY in the B6 strain are primed by uptake of donor male cells by host APC and cross-presented to host CD8+ T cells (30). It is possible that the disparate CD8+ and CD4+ T cell kinetics we observed could be the result of a delay in cross-presentation of MHC class I-restricted epitopes by host APC while B cells or DC in the donor male inoculum directly present MHC class II-restricted Ag to host CD4+ T cells. To rule out direct presentation to CD4+ T cells, female mice were immunized with MHC class II-deficient male cells. The kinetics of the host CD4+ and CD8+ T cell responses to HY were unaffected when MHC class II-restricted Ag was cross-presented exclusively by host APC (Fig. 4A).

**Requirement for CD4+ T cells**

To confirm the requirement of CD4+ T cell help for primary CD8+ T cell responses in our model, we transferred male cells into female mice deficient in CD4+ T cells. HY-specific CD8+ T cell responses were not detectable in these mice and donor male cells were not rejected up to 45 days after transfer (Fig. 4B and our unpublished observations). However, it was possible that Ag-non-specific CD4+ T cells could provide the necessary help for the primary CD8+ T cell response. To address this, we analyzed HY responses in H2bkn12 mice. These mice have an H2 I-Ak mutation, to Smcy peptide in the spleen following immunization with total, T-depleted, CD8-depleted, or CD4 depleted male splenocytes. Numbers indicate the percentage of IFN-γ+ cells in the CD8+ population. B, Total HY-specific CD8+ T cell responses to intact and T subset-depleted splenocytes as indicated. Values represent the combined response to Uty and Smcy peptides in each mouse at the indicated time points. Values plotted are the mean of two to three mice per time point from a minimum of four independent experiments. Statistically significant differences using the equal variable Student’s t test are indicated with * (p ≤ 0.0005) or ** (p ≤ 0.03). C, CD8+ T cell response to HY Ags was suppressed with increasing numbers of male donor cells. Female mice received 1 × 10^7 (■), 2 × 10^7 (□), or 4 × 10^7 (▲) male UbC-GFP splenocytes and host T cell responses were measured at days 9, 12, and 15. Numbers represent the combined CD8+ T cell responses to Uty246-254 and Smcy738-746 measured by intracellular IFN-γ production. Error bars, SEM (n = 4 mice for each group) and are representative of two independent experiments. Statistically significant differences using Student’s t test are indicated with * (p ≤ 0.02).
but possess an intact CD4+ T cell compartment. It has been suggested that these mice are unable to present antigenic peptides derived from male cells to CD4+ helper T cells (31). Similar to the result in the CD4- T cell-depleted mice, female H2<sup>bmi12</sup> mice were unable to mount an effector CTL response or reject male cells despite the presence of CD8+ T cells (our unpublished observations).

MHC class II-restricted killing has been observed following immunization with pathogens (32, 33). To determine whether CD4+ T cell lytic activity played any role in the HY cell transfer model, we examined the killing of Dby peptide-pulsed and control LLO peptide-pulsed female target cells in vivo. By 48 h after transfer, 65% specific lysis was seen in the lymph nodes (Fig. 5). We also observed efficient MHC class II-restricted lysis of unpulsed male target cells presenting endogenous levels of HY peptide. To demonstrate this, β<sub>m</sub>-deficient male and female spleen cells were used as target cells to avoid class I killing. They were injected into naïve or day 9 immunized, NK-depleted mice and showed a specific loss of male cells only in the HY-primed recipients (Fig. 5).

**Delayed kinetics due to direct presentation**

Previous studies have suggested that live antigenic donor T cells may alter the ability of the recipient to mount an effective immune response against the donor Ag (34). To initially test this, we immunized female mice with male splenocytes depleted of CD4+ and CD8+ cells and followed the kinetics of host T cell responses by ICS. Unexpectedly, HY-specific CD8+ T cell responses were detectable and peaked 3 days earlier following immunization with T-depleted vs intact splenocytes (Fig. 6A). In the same animals, host CD4+ T cell responses remained unaffected regardless of the treatment of the male inoculum (our unpublished observations). We further defined the subset of cells responsible for delaying the host CD8+ T cell response. Donor male cells were depleted of either CD8+ or CD4+ T cells and used as immunogen. Donor CD8+ cells appeared to be more efficient at delaying host CD8+ T cell responses than donor CD4+ cells (Fig. 6, A and B).

Despite evidence that cross-presentation primes CD8+ effector T cell responses to live HY-bearing cells in the B6 strain, there was a possibility that some direct presentation of HY Ag was occurring by donor DC, and our immunization strategy with T-depleted splenocytes introduced a higher proportion of donor APC to more efficiently directly prime the CTL response. It is also possible that the male-specific genes are differentially expressed in different cell populations within the spleen, altering the amount of Ag introduced in our system. To address this, we immunized female mice with varying doses of unfractionated male splenocytes. This allowed us to immunize mice with the same proportion of each cell population, but allowed us to add varying numbers of donor male T cells. Female mice were immunized with increasing doses of male cells and the kinetics of the CD8+ T cell response was measured by ICS. When female mice were immunized with a low dose of male cells, effector host CD8+ T cell responses were rapid and easily detected 9 days after cell transfer. At this time, 1.0% of all host CD8+ T cells were Uty or Smcy specific compared with 0.2 and 0.0% in mice immunized with 2 × 10<sup>7</sup> and 4 × 10<sup>7</sup> splenocytes, respectively. By 12 days postimmunization, HY-specific CD8+ T cell responses were comparable in mice immunized with 1 × 10<sup>7</sup> and 2 × 10<sup>7</sup> splenocytes with 2.8 and 2.2% of CD8+ T cells producing IFN-γ, respectively, but these responses were significantly greater than the HY-specific response in mice immunized with 4 × 10<sup>7</sup> splenocytes. By 15 days after cell transfers, responses to all three doses of male cells were equivalent (Fig. 6C). Therefore, higher doses of antigenic male cells delayed the host CTL response.

**Discussion**

In this report, we provide a detailed analysis of the surprising in vivo kinetics of CD8+ and CD4+ T cell responses of female B6 mice immunized with live male splenocytes. Although the primary effector CD8+ T cell response expands and contracts in a manner observed in other immunization strategies, we find that this response is considerably delayed following immunization with HY. The lack of detectable HY-specific CD8+ T cells capable of IFN-γ production before day 10 postimmunization was due to the absence of Ag-specific effector T cells as confirmed by tetramer staining. This is in contrast to primary effector responses peaking 7–8 days postimmunization with irradiated cells, peptide-pulsed DC, or acute infections (14, 35–37). Despite numerous studies analyzing responses to HY, the delay in the CD8+ T cell response has not been appreciated. In addition to the slow CD8+ effector response, the time required to clear the immunogen from the host was also delayed and correlates with the expansion of effector CD8 cells. Despite this delay, HY-specific CD8+ T cells were similar to pathogen-induced CTLs as measured by effector cell surface molecule expression, cytokine production, and CTL activity in vivo (Fig. 1 and data not shown).

Recently, many studies have analyzed the role of CD4+ T cells in the generation of functional primary, memory, and recall CD8+ T cell responses to noninflammatory, cell-associated Ag require CD4+ cell help. In ours and similar models of immunization, generation of a primary CD8+ T cell response requires an active CD4+ T cell response to HY (Fig. 4B and our unpublished observations) (38). Because a CD4+ helper response is required, we examined whether the delay in the CD8+ T cell response could be attributed to a slow CD4+ T cell response. On the contrary, we found that the kinetics of the CD4+ T cell response to HY was similar to that seen postimmunization with other pathogens and peaks many days before the detection of a primary CD8+ T cell response (Fig. 3). The CD4+ T cell response is characterized by a high percentage of IL-2 and IFN-γ double-producer HY-specific cells detectable as early as 5 days postimmunization. The fraction of cells making IL-2 declines rapidly however.

We investigated the possibility that HY-specific CD4+ T cells had effector lytic activity in vivo. Similar to previous reports following immunization with pathogen, HY-specific CD4+ T cells efficiently killed peptide-pulsed target cells in an Ag- and MHC class II-dependent manner in vivo (Fig. 5). Additionally, to determine the ability of CD4+ T cells to lyse targets with endogenous levels of HY Ag, we performed an in vivo killing assay with β<sub>m</sub>-deficient male and female target cells in mice acutely depleted of NK cells. MHC class II-restricted CD4+ T cells lysed male target cells in a HY-specific manner, suggesting that rejection of the male cells between days 8 and 12 after transfer may be partially attributed to MHC class II-restricted killing of donor B cells. However, CD4+ T cells alone are unable to mediate complete rejection of male splenocytes (38). It remains possible that in vivo killing of male cells by lytic CD4+ T cells contributes to the available Ag load necessary to cross-prime effector CD8+ T cells. In support of this idea, CD4+ and CD8+ T cell responses to irradiated male cells were weak relative to immunization with live male cells. This may be due to the rapid clearance of the male cells and the absence of prolonged Ag exposure necessary to prime efficient CD4+ and CD8+ T cell responses.

A concern with our immunization strategy is that the injected, live male cells are capable of directly presenting HY Ag to host T cells. A recent study has determined that the primary CD8+ T cell
response to immunization with live male cells is differentially presented directly by donor cells or cross-presented by host APC depending on the epitope and strain of mouse analyzed (30). A possible explanation for the rapid CD4+ and delayed CD8+ response was that host CD4+ T cells are directly primed by donor male B cells or DC, while the CD8+ T cell responses are slowly cross-presented by host APC. Following immunization with class II-deficient male cells incapable of directly priming host CD4 cells, the kinetics of the primary CD4+ and CD8+ T cell responses remained unchanged (Fig. 4A). This would suggest that donor male cells are taken up by host APC and presumably both MHC class I- and MHC class II-restricted peptides are presented by host DC as early as 5 days postimmunization. Despite cross-presentation of HY by female APC, CD8+ T cell responses were not detectable for another 5 days.

Another explanation for the delayed CD8 response is the action of suppressor cells in the immunizing donor cell population. FoxP3+ CD4+ T regulatory cells are thought to have a self-reactive TCR repertoire that is necessary to suppress autoreactive T cells that escaped negative selection (39). Our immunization strategy with live male cells introduces a significant number of FoxP3+ CD4+ T regulatory cells that may be capable of suppressing host cells that recognize HY as foreign. To investigate this, female mice were immunized with donor male cells depleted of total T cells. Following T cell depletion of the male inoculum, the CD8+ T cell response was accelerated by 3 days while the CD4+ T cell response remained unaffected. We further defined the T cell subset responsible for delaying the host CD8+ T cell response by depleting either CD4+ or CD8+ subsets from the donor inoculum. Although depletion of CD4+ cells from the male cell immunogen accelerated the CD8+ T cell response to HY, the effect was not as great as depleting donor splenocytes of CD8+ cells. This indicates that the CD8+ donor cell population was predominantly responsible for delaying the host CD8+ T cell response to HY (Fig. 6). To determine whether the delay in the CD8+ T cell response was dependent on the number of CD8+ cells transferred during the immunization, female mice were immunized with varying doses of male cells. Despite receiving a lower dose of Ag, female mice mounted a more rapid CD8+ T cell response compared with mice receiving a higher dose of male cells (Fig. 6C). These data demonstrate that despite an increased Ag load, the host CD8+ T cell response to male cells is delayed in a dose-dependent manner. This observation has been described previously as a “veto” cell phenomenon observed following immunization with live allografts (34, 40, 41). The veto cell is characterized by the capacity of certain subsets of cells to specifically suppress CTL precursors directed against Ag presented by the veto cells themselves (40–43). It has been shown that veto cells are ubiquitously distributed and include cells of T cell origin (34, 44–49). The TCR specificity of the veto cell is inconsequential, but instead the suppression is likely dependent on the specificity of the precursor CTL recognizing Ag on the veto cell itself (50–52). As a result, there is recognition of the veto cell by the precursor CTL but not vice versa. According to this phenomenon, when naive responder CD8+ T cells meet Ag on other T cells, their response is delayed.

It has been suggested that veto cell activity is mediated by apoptosis (53). We investigated the possibility that CD8+ veto cells kill naive HY-specific CD8+ T cells and the delay in the host CD8+ response requires de novo generation of HY-specific precursor cells. However, the host T cell kinetics was unaffected when wild-type mice were immunized with perforin- or granzyme B-deficient male splenocytes or when thymectomized female mice were vaccinated with wild-type male cells (our unpublished observations). Instead, our data would suggest that the veto phenomenon is due to sequestration of naive HY-specific CD8+ T cells around male donor T cells that have migrated into the T cell zones of secondary lymph nodes presenting self-HY Ag on MHC class I molecules. This is supported by the absence of a veto phenomenon when male CD8 cells lacking β2m isolated from mixed radiation bone marrow chimeras are used as the immunogen (our unpublished observations). This interaction does not appear to be permanently tolerizing because the host ultimately generates an HY-specific CD8+ T cell response presumably primed from cross-presented Ag on host APC. This explanation accounts for the fact that donor T cells veto a primary CD8+ T cell response more effectively than B cells (because of the different homing to T or B areas). However, it does not explain why CD8+ T cells are more effective than CD4+ T cells, since both subsets commingle in the T areas.

Acknowledgment

We acknowledge B. Deree for technical assistance in the generation of MHC tetramers.

Disclosures

The authors have no financial conflict of interest.

References
