Differential Requirement for CD4 Help in the Development of an Antigen-Specific CD8+ T Cell Response Depending on the Route of Immunization

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*J Immunol* 1998; 160:5522-5529; ;
http://www.jimmunol.org/content/160/11/5522
Differential Requirement for CD4 Help in the Development of an Antigen-Specific CD8\(^{+}\) T Cell Response Depending on the Route of Immunization\(^{1}\)

Hélène Bour,* Clotilde Horvath,* Christophe Lurquin,† Jean-Charles Cerottini,* and H. Robson MacDonald\(^{2*}\)

Previous studies in our laboratory have shown that DBA/2 mice injected i.p. with syngeneic P815 tumor cells transfected with the HLA-CW3 gene (P815-CW3) showed a dramatic expansion of activated CD8\(^{+}\)CD62L\(^{-}\) T cells expressing exclusively the $\gamma\delta$10 segment. We have used this model to study the regulatory mechanisms involved in the development of the CW3-specific CD8\(^{+}\) response, with respect to different routes of immunization. Whereas both intradermal (i.d.) and i.p. immunization of DBA/2 mice with P815-CW3 cells led to a strong expansion of CD8\(^{+}\)CD62L\(^{-}\), $\gamma\delta$10 cells, only the i.d. route allowed this expansion after immunization with P815 cells transfected with a minigene coding for the antigenic epitope CW3 170–179 (P815 miniCW3). Furthermore, depletion of CD4\(^{+}\) T cells in vivo completely abolished the specific response of CD8\(^{+}\)CD62L\(^{-}\), $\gamma\delta$10 cells and prevented the rejection of P815-CW3 tumor cells injected i.p., whereas it did not affect CD8\(^{+}\)CD62L\(^{-}\), $\gamma\delta$10 cell expansion after i.d. immunization with either P815-CW3 or P815 miniCW3. Finally, the CW3-specific CD8\(^{+}\) memory response was identical whether or not CD4\(^{+}\) T cells were depleted during the primary response. Collectively, these results suggest that the CD8\(^{+}\) T cell response to P815-CW3 tumor cells injected i.p. is strictly dependent upon recognition of a helper epitope by CD4\(^{+}\) T cells, whereas no such requirement is observed for i.d. injection. The Journal of Immunology, 1998, 160: 5522–5529.

D8\(^{+}\)CTL specific for tumor-associated Ags play a central role in antitumor immune responses leading to tumor rejection (1, 2). In many systems, differentiation of CTL precursors into functional CD8\(^{+}\)CTL is strictly dependent on CD4\(^{+}\) T cell help (3–5). However, in other models of Ag-specific immune responses, activation, differentiation, and expansion of CD8\(^{+}\) CTL are optimal even in the absence of CD4\(^{+}\) T cells (6, 7). Conflicting results have also been reported on the requirement for CD4 help in the development of CD8\(^{+}\) CTL against tumor cells (8–12). Most of these studies relied on in vivo depletion of CD4\(^{+}\) T cells, followed by either monitoring of tumor growth in vivo, or in vitro cytotoxic assays. However, no direct quantitative in vivo analysis of the Ag-specific CD8\(^{+}\) response was performed.

We previously established a murine tumor model in which the Ag-specific CD8\(^{+}\) T cell response in vivo can be longitudinally monitored in blood by flow cytometry (13–15). DBA/2 mice injected i.p. with syngeneic P815 tumor cells (H-2\(^{b}\)) transfected with the HLA-CW3 gene (P815-CW3) showed a dramatic expansion of CD8\(^{+}\) T cells expressing exclusively the $\beta$10 segment, and phenotypically defined as CD62L\(^{-}\)CD45RB\(^{-}\)CD44\(^{+}\) activated cells (14). The specific cytotoxic activity against the immunodominant peptide CW3 170–179, presented by the H-2K\(^{d}\) molecule, was found exclusively in the CD8\(^{+}\) $\gamma\delta$10 population (13).

In the present study, we have used this unique model system to address the requirement for CD4\(^{+}\) T cell help during the development of the CW3-specific CD8\(^{+}\) response, with respect to different routes of immunization. In particular, the CW3-specific CD8\(^{+}\) T cell response was analyzed after i.p. or intradermal (i.d.)\(^{3}\) immunization with tumor cells, in DBA/2 mice depleted or not of CD4\(^{+}\) T cells. Furthermore, immunizations were conducted either with P815 cells transfected with the complete HLA-CW3 gene, or with P815 cells transfected with a minigene coding only for the CD8 epitope CW3 170–179 (P815 miniCW3). Our results show that the CW3-specific CD8\(^{+}\) T cell response to tumor cells injected i.d. can develop without CD4\(^{+}\) T cell help, whereas help is required for the i.p. route of immunization.

Materials and Methods

Cells

Transfection of H-2\(^{b}\) mouse mastocytoma P815 with the HLA-CW3 gene has already been described (16). A 47-bp minigene coding for the CD8 epitope 170–179 of the CW3 molecule was constructed by oligonucleotide annealing and cloning at the unique EcoRI site of expression vector pCD-SR\(_{a}\) (17). A modification of the calcium phosphate DNA precipitation method of Graham and Van der Eb (18, 19) was used to transfect P815-HTR cells with the CW3 minigene cloned into pCD-SR\(_{a}\). Briefly, a calcium phosphate DNA precipitate, containing DNA clone pCD-SR\(_{a}-C W 3\) minigene and selective plasmid pVik-neo\(_{\gamma}\) (20) at a ratio of 10:1, was allowed to form for 30 to 45 min at room temperature. A quantity amounting to 5.10\(^{6}\) P815 cells was centrifuged for 10 min at 300 × g, and the cell pellet was resuspended directly in the calcium phosphate DNA precipitate containing 20 to 25 μg total DNA in 2.5 ml. The mixture was incubated for 30 min at 37°C and then added to an 80-cm\(^{2}\) flask (Nunc, Roskilde, Denmark) containing 22.5 ml DMEM

\(^{3}\) Abbreviations used in this paper: i.d., intradermal; CD62L, CD62 ligand; PEL, peritoneal exudate lymphocyte.
(Life Technologies, Paisley, U.K.) supplemented with 10% heat-inactivated FCS (Irvine Scientific, Santa Ana, CA). After 24-h incubation at 37°C, cells were centrifuged and resuspended in 40 ml fresh medium. Forty-eight hours after transfection, cells were washed and selection was conducted in DMEM supplemented with 10% heat-inactivated FCS and 1.5 mg/ml G418 (Genetix; Life Technologies). Transfected cells were subsequently cloned at 1 cell/well in the selection medium, and clones were screened in a cytolytic assay with the CTL clone 1.1 specific for the CW3 peptide 170–179 presented by H-2Kd (21).

### Mice and immunizations

Adult female DBA/2 mice (Harlan Olac, Bicester, U.K.) were injected i.p. or i.d. on the back with, respectively, 107 or 2.107 viable P815 tumor cells transfected with the complete HLA-CW3 gene (P815-CW3) or the CW3 minigene (P815 miniCW3). Both tumor cells were maintained as an ascitic tumor passaged weekly in nude mice. For some experiments, mice were boosted with 107 P815-CW3 cells injected i.p. At various times after primary immunization or challenge, mice were bled by the tail vein and PBL were isolated by Ficoll-Hypaque gradient centrifugation (Pharmacia Biotech, Uppsala, Sweden).

#### In vivo CD4 depletion

Mice were injected i.p. with anti-CD4 mAb (GK1.5 ascites) diluted at 1/20 in 0.2 ml PBS, on days −3, −2, −1, +3, +7, and +11, and immunized at day 0. Residual CD4+ T cells represented less than 1 to 2% of total T cells at the time of immunization, and this level remained constant throughout the primary response. Control mice were injected with PBS on the same schedule.

**Flow cytometry analysis**

Triple stainings were performed on PBL with FITC-conjugated anti-CD62L (Mel-14, prepared in our laboratory), phycoerythrin-conjugated anti-CD8 (53.6.7; Boehringer Mannheim, Mannheim, Germay), and biotinylated anti-Vβ10 (B21.5, prepared in our laboratory) revealed with streptavidin-Tricolor (Caltag Laboratories, San Francisco, CA). Samples were analyzed on an FACSscan equipped with LYSIS II software (Becton Dickinson, San Jose, CA).

For cell-sorting experiments, PEL and spleen cells were triple stained, as indicated above, and CD8+CD62L+Vβ10+ or Vβ10−cells were sorted on a FACStar (Becton Dickinson), collected in DMEM supplemented with 5% heat-inactivated FCS, and directly tested for ex vivo cytolytic activity.

**Cytolytic assay**

Mice were killed 2 wk after i.p. immunization with P815-CW3 cells. PEL were purified by nylon wool columns, and single cell suspensions of splenocytes were prepared by standard procedures. Activated CD8+ T cells were sorted on the basis of Vβ10 expression, as described above, and used as effector cells in the cytolytic assay.

P815-CW3 and control P815 cells were labeled with 100 μCi of 51Cr, as previously described (22), for 1 h at 37°C, and washed five times. A quantity amounting to 2.106 51Cr-labeled target cells was mixed with varying numbers of freshly sorted effector cells in V-bottom microplates, in DMEM supplemented with 5% heat-inactivated FCS. 51Cr release in supernatant was measured after 4-h incubation at 37°C. The percent specific lysis was calculated as described (22).

In some experiments, CTL clone 1.1 (21), specific for the CD8 epitope 170–179 of HLA-CW3, was used as effector cell.

**Results**

**CW3-specific CTL are contained exclusively within the Vβ10−CD62L− subset of CD8+ T cells in HLA-CW3 immune mice**

Previous reports from our laboratory have shown that the expanded CD8+Vβ10+ T lymphocyte population in HLA-CW3 immune DBA/2 mice showed significant ex vivo CW3-specific cytolytic activity, whereas CD8+Vβ10+ cells did not (13). To rule out the presence of CW3-specific CTL expressing another Vβ segment than Vβ10 in the activated CD8+CD62L+ population, CD8+CD62L+PEL or splenocytes (isolated from DBA/2 mice immunized i.p. with P815-CW3 cells) were sorted on the basis of Vβ10 expression and subsequently tested for CW3-specific cytolytic activity. As shown in Figure 1, both in PEL and spleen, CW3-specific cytolytic activity was only detected in the CD8+CD62L−Vβ10+ population, even at high E:T ratio. Thus, the phenotypically defined CD8+CD62L−Vβ10+ T cell population will hereafter be referred to as CW3-specific CTL.

**Intradermal and intraperitoneal immunization with P815-CW3 tumor cells leads to a strong and comparable Ag-specific primary CD8 response**

Intradermal immunization with P815-CW3 tumor cells led to a very strong CW3-specific CTL response in blood, similar to what was observed after i.p. immunization (Table I, Fig. 2): Vβ10+ cells represented 70% on average, and up to 80% in some experiments, of the activated CD8+CD62L− T cells in PBL, as compared with 7% in naive mice. The percentage of CD62L− cells in the CD8+ population also notably increased, whereas the proportion of CD8+ T cells only increased marginally as compared with control mice (Table I).

Taking these values into consideration, both i.p. and i.d. immunization with P815-CW3 cells led to a dramatic expansion of CW3-specific T cells, which accounted for 20 to 25% of CD8+ T cells and 3 to 5% of total PBL, as compared with 0.4 and 0.05% of total Vβ10+ cells, respectively, in the CD8+ population and in the PBL of naive mice (Table I).

**Differential expansion of CW3-specific CTL following i.p. or i.d. immunization with P815 cells transfected with a CW3 minigene**

To address a potential role for a CD4 helper epitope in the development of the CW3-specific CD8+ T cell response, we used P815 tumor cells transfected either with the complete HLA-CW3 gene (P815-CW3 cells), or with a minigene coding only for the CD8 epitope 170–179 (P815 miniCW3 cells). As expected, both tumor cells were efficiently recognized and lysed by a CTL clone specific for the CW3 peptide 170–179 presented by H-2Kd (Fig. 3), but only the P815-CW3 cells expressed HLA-CW3 on the surface.

Contrary to the results obtained with P815-CW3 cells, the percentage of CW3-specific T cells increased only marginally after i.p. immunization with P815 miniCW3 cells (Fig. 2), although it was significantly different as compared with naive mice (Student’s t test, p < 10−4). In marked contrast, i.d. immunization with P815 miniCW3 cells led to a strong CW3-specific response, as on average, more than 40% of the CD8+CD62L− population expressed Vβ10+ (Fig. 2). These data suggest that development of the CW3-specific CD8 response is not strictly dependent on a potential CD4 epitope in the HLA-CW3 molecule when tumor cells are injected i.d.

**Kinetics of the CW3-specific CD8 response after i.p. or i.d. immunization**

Although i.d. immunization with P815 miniCW3 cells led to a very strong expansion of CW3-specific T cells, the response remained lower than with P815-CW3 cells injected i.p. or i.d. Kinetics experiments confirmed and extended these observations. As shown in Figure 4, i.p. or i.d. immunization with P815-CW3 cells was followed by a very rapid expansion of CW3-specific T cells, as the percentage of Vβ10+ cells reached 50% of the CD8+CD62L− population on day 8 after immunization, and it was maximum on day 12. Furthermore, with both routes of immunization, the level of CW3-specific T cells remained extremely high during a long period of time, with only a slight decrease 45 days after immunization. As compared with the response induced by P815-CW3 cells, i.d. immunization with P815 miniCW3 cells also led to a strong expansion of the CW3-specific population, but the increase was slower, the peak of the response was slightly delayed, and the
percentage of CW3-specific T cells decreased more rapidly after
the peak of the response.

Requirement for CD4 help differs according to the route of
immunization

As shown above (Figs. 2 and 4), immunization of DBA/2 mice with
P815-CW3, and activated CD8+ T cells were sorted on the basis of Vβ10
expression. Sorted CD8+CD62L-Vβ10+ (triangles) and Vβ10+ (circles) cells
were assayed directly for cytolytic activity on P815 (open symbols) or P815-
CW3 (closed symbols) target cells, at the indicated E:T ratio.

FIGURE 1. Direct cytolytic activity
of activated CD8+ cells expressing
Vβ10 in PEL and splenocytes from
HLA-CW3 immune mice. PEL and
splenocytes were isolated from DBA/2
mice 2 wk after i.p. immunization with
P815-CW3, and activated CD8+ T cells
were sorted on the basis of Vβ10 ex-
pression. Sorted CD8+CD62L-Vβ10+
(triangles) and Vβ10+ (circles) cells
were assayed directly for cytolytic ac-
tivity on P815 (open symbols) or P815-
CW3 (closed symbols) target cells, at the
indicated E:T ratio.

Table I. Expansion of CW3-specific T cells following i.p. or i.d. immunization with P815-CW3 cells

<table>
<thead>
<tr>
<th>Immunization</th>
<th>CD8+</th>
<th>CD82L+ in CD8+</th>
<th>Vβ10+ in CD8+</th>
<th>CD82L+Vβ10+ in CD8+</th>
<th>CD8+CD82L+Vβ10+ in PBL</th>
<th>Vβ10+ in CD8+CD82L+</th>
</tr>
</thead>
<tbody>
<tr>
<td>P815-CW3, i.p.</td>
<td>19.6 ± 5.4</td>
<td>33.7 ± 14.2</td>
<td>70.1 ± 10.2</td>
<td>24.3 ± 12.3</td>
<td>5.1 ± 3.6</td>
<td>9.2 ± 0.9</td>
</tr>
<tr>
<td>P815-CW3, i.d.</td>
<td>16.9 ± 2.3</td>
<td>29.6 ± 5.9</td>
<td>73.1 ± 15.0</td>
<td>21.8 ± 6.3</td>
<td>3.8 ± 1.4</td>
<td>9.4 ± 0.8</td>
</tr>
<tr>
<td>Naive</td>
<td>13.4 ± 3.3</td>
<td>5.3 ± 4.2</td>
<td>7.2 ± 3.5</td>
<td>0.4 ± 0.3</td>
<td>0.05 ± 0.05</td>
<td>8.3 ± 0.9</td>
</tr>
</tbody>
</table>

* PBL were recovered from DBA/2 mice 2 wk after i.p. or i.d. immunization with P815-CW3 cells and triple stained with mAbs against CD8, CD62L,
and Vβ10. Results of each group are expressed as mean percentages ± SD (n = 27, 20, and 24 mice in each group, respectively).
As shown in Figure 5A, CD4 depletion abrogated the CW3-specific T cell response against P815-CW3 injected i.p. In contrast, the CW3-specific CD8 response after i.d. immunization with P815-CW3 or P815 miniCW3 was similar in the CD4-depleted mice as compared with the nondepleted controls (Fig. 5A). Indeed, statistical analysis of the percentage of V\textsubscript{b}10\textsuperscript{+} cells in the CD8\textsuperscript{+} CD62L\textsuperscript{−} population after i.d. immunization showed no significant difference between mice depleted or not of CD4\textsuperscript{+} T cells (Student's t test, p = 0.05), whereas the difference was highly significant after i.p. immunization (Student's t test, p < 10\textsuperscript{−6}).

Surprisingly, expansion of CW3-specific CD8\textsuperscript{+} T cells was increased significantly after CD4 depletion in mice injected i.d. with P815 miniCW3 cells (Student's t test, p = 10\textsuperscript{−4}), reaching the same level observed in mice injected with P815-CW3 cells (Fig. 5B).

We observed a good correlation between the CW3-specific CD8 response and tumor rejection in mice depleted of CD4\textsuperscript{+} T cells. Indeed, CD4 depletion prevented rejection of P815-CW3 tumor cells injected i.p., and mice died rapidly of ascitis growth (Fig. 6). In contrast, survival of mice injected i.d. with both types of tumor cells was not affected by CD4 depletion (Fig. 6 and data not shown). The clear correlation between the percentage of V\textbf{b}10\textsuperscript{+} cells in the CD8\textsuperscript{+} CD62L\textsuperscript{−} population and the ability to reject tumor cells confirms and strengthens the finding that CW3-specific CTL exclusively express the V\textsubscript{b}10 segment (Fig. 1) (13).

CD8 memory is achieved independently of CD4 help during the primary response

As mentioned above, the murine model used in this study allows us to monitor the specific CD8 response ex vivo by flow cytometry. We could thus analyze longitudinally in the same individual mice the CW3-specific CD8 primary response and the memory response to a secondary challenge, to determine whether the absence of CD4 help during the primary CD8 response would modify the establishment of CD8 memory. To standardize the readout, CD8 memory was always measured as the early response (6 days after injection of tumor cells) to an i.p. challenge with P815-CW3 cells. Groups tested included

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**FIGURE 2.** Magnitude of the CW3-specific CD8 response after immunization with P815-CW3 or P815 miniCW3 cells. DBA/2 mice were immunized i.p. or i.d. with P815 cells transfected with the complete HLA-CW3 gene (P815-CW3) or with a CW3 minigene (P815 miniCW3). Two weeks after immunization, PBL were triple stained with mAbs against CD8, CD62L, and V\textsubscript{b}10. The percentage of V\textbf{b}10\textsuperscript{+} cells in the CD8\textsuperscript{+} CD62L\textsuperscript{−} population is indicated for individual mice (n is the number of mice in each group). Dotted lines represent the mean value of each group.

**FIGURE 3.** Expression of HLA-CW3 and lysis by a CW3-specific CTL clone of P815-CW3 and P815 miniCW3 cells. Control P815 cells (circles) and transfectants P815-CW3 (squares) and P815 miniCW3 (diamonds) were assayed in a 4-h \textsuperscript{51}Cr-release test with the CTL clone 1.1 specific for the CD8 epitope 170–179 of HLA-CW3, at the indicated E:T ratio. Expression of HLA-CW3 on the surface of transfectants and control P815 is shown in the insert, after staining with anti-HLA-A,B,C mAb B9.12.1.

**FIGURE 4.** Kinetics of the CW3-specific response after i.p. or i.d. immunization. DBA/2 mice (four in each group) were immunized i.p. (open symbols) or i.d. (closed symbols) with P815-CW3 (squares) or P815 miniCW3 (diamonds) cells. Control mice (circles) were not immunized. PBL were harvested at various times after immunization, and triple stained with mAbs against CD8, CD62L, and V\textbf{b}10. Mean percentage of V\textbf{b}10\textsuperscript{+} cells in the CD8\textsuperscript{+}CD62L\textsuperscript{−} population is indicated for each group. Results are representative of three independent experiments.
mice immunized 3 mo before with P815-CW3 or P815 miniCW3 injected i.p. or i.d., and depleted or not of CD4 T cells throughout the primary response. Nonimmunized mice used as controls for the primary response were divided into two groups. One group was retained as naive controls, while the other group was injected i.p. with P815-CW3 cells at the same time and in the same conditions as the immune mice. As expected, no CW3-specific response could be detected in control mice 6 days after the primary immunization, whereas a strong CW3-specific expansion was observed after 14 days (Fig. 7, inset).

At the time of i.p. challenge with P815-CW3 cells (i.e., 3 mo after primary immunization), the level of CW3-specific CD8+ cells decreased to close to background levels in all groups of immune mice. However, in all groups of mice, a strong CW3-specific response was observed 6 days after challenge, which rapidly decreased thereafter (Fig. 7). The strongest CW3-specific memory response was observed in mice immunized with P815-CW3 cells during the primary response. This response was similar between groups of mice immunized i.p. or i.d., and CD4 depletion during the primary response (after i.d. immunization) did not alter the magnitude of the memory response (Fig. 7). Furthermore, the percentage of CW3-specific memory T cells observed 6 days after challenge in these groups of mice was comparable with the CW3-specific response in control mice 14 days after primary i.p. immunization with P815-CW3 cells. Mice immunized during the primary response with P815 miniCW3 i.p. or i.d. also displayed an efficient CW3-specific CD8 memory response, although it was lower than in mice immunized with P815-CW3 cells (Fig. 7). Finally (similarly to what was observed during the primary response (Fig. 5B)), CD4 depletion during the primary response to P815 miniCW3 cells injected i.d. led to a higher CD8 memory response as compared with nondepleted mice.
Discussion

The major finding in the present study is that the CD8 T cell response to P815-CW3 tumor cells injected i.p. or i.d. with P815-CW3 tumor cells, after in vivo depletion of CD4 T cells by repeated injections of anti-CD4 mAb GK1.5 (or PBS in controls). Survival was assessed every day in each group until 45 days after injection.

FIGURE 6. Effect of CD4 depletion on the survival of DBA/2 mice injected i.p. or i.d. with P815-CW3 tumor cells. DBA/2 mice were injected i.p. or i.d. with P815-CW3 cells, after in vivo depletion of CD4+ T cells by repeated injections of anti-CD4 mAb GK1.5 (or PBS in controls). Survival was assessed every day in each group until 45 days after injection.

The precise nature of the putative HLA-CW3-specific helper epitope recognized by CD4 T cells on P815-CW3 remains to be determined. In this regard, i.p. injection of P815 miniCW3 cells cotransfected with the intact HLA-B7 gene did not restore the CW3-specific CD8 T cell response in DBA/2 mice (data not shown), suggesting that the putative helper epitope is not shared between HLA-CW3 and HLA-B7. Additional experiments using synthetic peptides derived from nonhomologous regions of these two HLA molecules will be required to formally demonstrate the existence of a CW3-specific helper epitope.

In our model system, the development of CW3-specific CD8 memory also does not appear to require CD4 T cell help during the primary response, i.e., during the activation and expansion

FIGURE 7. Effect of CD4+ T cell depletion during the primary response on the establishment of CW3-specific CD8 memory. Immunization and PBL staining were as in Figure 5. The same individual mice were analyzed at day 14 and day 91 after primary immunization (day 0). All groups of mice were challenged with an i.p. injection of P815-CW3 cells 6 days after analysis, and PBL were harvested for triple staining after an additional 6 and 14 days. Inset, Nonimmunized mice used as controls for the primary response were immunized i.p. with P815-CW3 cells at the same time (day 98) and in the same conditions as the immune mice. Results are representative of two independent experiments.
phase of CW3-specific naive precursors. Signals necessary to rescue CW3-specific effector CD8 T cells from activation-induced cell death can thus be provided in the absence of CD4 help. This result further strengthens the hypothesis that CD4 T cell help in the CW3 system is not mediated by a direct effect on CW3-specific CD8 T cells. In addition, the number of secondary effector CD8 T cells decreases much more rapidly after the peak of the response as compared with primary effectors. This phenomenon could reflect a reduced tumor load in the secondary response. Indeed, the CD8 response after boosting is much faster than the primary response, so that tumor cells could undergo a smaller number of divisions before their clearance. Alternatively, this result could reflect a differential susceptibility to activation-induced cell death of primary effectors derived from naive CD8 T cells and secondary effectors derived from the memory pool.

Whereas in vivo depletion experiments showed that CD4 help was not required for the i.d. route of immunization with P815-CW3 cells, similar immunization with P815 miniCW3 cells revealed an increased expansion of specific CD8 T cells after CD4 depletion. To explain this surprising result, we propose the following hypothesis: both an HLA-CW3-encoded helper epitope and a regulatory epitope encoded by the P815 cell itself, respectively recognized by helper and regulatory CD4 T cells, could coexist in the P815-CW3 cells. The very effective CD8 response following i.p. immunization, in which CD4 help is necessary, further suggests that the HLA-CW3 helper epitope would be dominant over the putative P815 regulatory epitope. In P815 miniCW3 cells, only the P815 regulatory epitope remains, which would explain the increased CW3-specific CD8 response after CD4 T cell depletion in the i.d. route of immunization, insofar as CD4 help is not required. Our hypothesis is also supported by previous reports of CD4 suppressor T cells specific for the P815 tumor cells (30, 31), as well as evidence of regulatory CD4 T cells in several diseases including chronic experimental autoimmune encephalomyelitis (32), inflammatory bowel disease (33, 34), and contact sensitivity (35).

Our finding that an efficient Ag-specific CD8 T cell response can develop without CD4 help after i.d. immunization might have important implications for vaccination strategies aiming at stimulating antitumor immunity in humans, since the skin represents a very convenient injection site for clinical purposes. In this regard, it is of interest that Sampson et al. (36) showed that s.c. vaccination with irradiated granulocyte-macrophage CSF-producing tumor cells increased survival of mice bearing preestablished tumors in the brain, conceivably by stimulating uptake and presentation of tumor Ags by cutaneous dendritic cells. The tumor cells used in this particular study were poorly immunogenic, and the beneficial effect observed with granulocyte-macrophage CSF-producing tumor cells was not dependent on CD4 T cells, thus suggesting that our results obtained with the immunogenic tumor P815-CW3 might be extended to other less immunogenic tumors. Several other studies based on expression of costimulating molecules such as B7-1 or B7-2 and ICAM-1 (37–39), or administration of cytokines such as IL-12 (40), which all play a fundamental role in the establishment of an effective helper cell-independent CTL response by cross-priming requires cognate CD4 T cell help. J. Exp. Med. 186:65.

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