4-1BB Ligand, a Member of the TNF Family, Is Important for the Generation of Antiviral CD8 T Cell Responses

Joyce T. Tan, Jason K. Whitmire, Rafi Ahmed, Thomas C. Pearson and Christian P. Larsen

*J Immunol* 1999; 163:4859-4868; 
http://www.jimmunol.org/content/163/9/4859

---

**References**
This article **cites 53 articles**, 31 of which you can access for free at:  
http://www.jimmunol.org/content/163/9/4859.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
4-1BB Ligand, a Member of the TNF Family, Is Important for the Generation of Antiviral CD8 T Cell Responses\textsuperscript{1}

Joyce T. Tan,\textsuperscript{*} Jason K. Whitmire,\textsuperscript{†} Rafi Ahmed,\textsuperscript{†} Thomas C. Pearson,\textsuperscript{2*} and Christian P. Larsen\textsuperscript{2*}

4-1BB (CD137) is a costimulatory molecule expressed on activated T cells and interacts with 4-1BB ligand (4-1BBL) on APCs. To investigate the role of 4-1BB costimulation for the development of primary immune responses, 4-1BBL-deficient (4-1BBL \textsuperscript{−/−}) mice were infected with lymphocytic choriomeningitis virus (LCMV). 4-1BBL \textsuperscript{−/−} mice were able to generate CTL and eliminate acute LCMV infection with normal kinetics, but CD8 T cell expansion was 2- to 3-fold lower than in wild-type (+/+ ) mice. In the same mice, virus-specific CD4 Th and B cell responses were minimally affected, indicating that 4-1BB costimulation preferentially affects CD8 T cell responses. This result contrasts with our earlier work with CD40L-deficient (CD40L \textsuperscript{−/−}) mice, in which the CD8 T cell response was unaffected and the CD4 T cell response was markedly impaired. When both 4-1BBL- and B7-dependent signals were absent, CD8 T cell expansion was further reduced, resulting in lower CTL activity and impairing their ability to clear LCMV. Altogether, these results indicate that T cells have distinct costimulatory requirements: optimal CD8 responses require 4-1BBL-absent, CD8 T cell expansion was further reduced, resulting in lower CTL activity and impairing their ability to clear LCMV. CD4 T cell responses were unaffected and the CD4 T cell response was markedly impaired. When both 4-1BBL- and B7-dependent signals were absent, CD8 T cell expansion was further reduced, resulting in lower CTL activity and impairing their ability to clear LCMV.

\textsuperscript{1} This work was supported by National Institutes of Health Grant AI/DK40519-01 (to C.P.L. and T.C.P.), the Carlos and Marguerite Mason Trust, and National Institutes of Health grants AI-30048 and NS-21496 (to R.A.).

\textsuperscript{2} Address correspondence and reprint requests to Drs. Christian P. Larsen and Thomas C. Pearson, Department of Surgery, Emory University School of Medicine, Room 510S, 1639 Pierre Drive, Atlanta, GA 30322.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{*} The Carlos and Marguerite Mason Transplantation Biology Research Center and Department of Surgery and \textsuperscript{†} Emory Vaccine Center and Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322.

Received for publication June 1, 1999. Accepted for publication August 24, 1999.

Copyright © 1999 by The American Association of Immunologists.
reduced the number of epitope-specific CD8 T cells 5- to 6-fold compared with +/+ mice. CTL responses were also significantly reduced and viral clearance was impaired in the absence of 4-1BBL- and B7-dependent signals. Our results indicate that 4-1BB costimulation is not required for normal mouse development and of immune system organogenesis, but identifies an important costimulatory interaction, along with B7-dependent signals, that can be targeted to improve CD8 T cell responses during vaccination.

Materials and Methods

Mice

(B6L29F/J; J1--/+, Tcr-β-/-, Jα18-/-)(H-2b), C57BL/6 (H-2b), and CD40L-/-/+ mice (33) were purchased from The Jackson Laboratory (Bar Harbor, ME). They were then stained with APC-conjugated mono- (for CD8 T cells) for 5 hi in vitro with Brefeldin A (GolgiPlug, PharMingen) or APC-conjugated anti-CD4 (for CD4 T cells) or NP396–404, GP33–41, GP276–286, or NP205–212 (35). Spleen cells were stimulated in vitro with medium or with GP61–80 and spleen cells from 4–8-wk-old mice were stained with anti-CD4 and anti-CD8 to determine the percentage of T cell subsets (4) and anti-B220 and anti-Ig(H & L) to measure the percentage of B cells (B). Numbers indicate the percentage of cells in the quadrant.

Virus

Mice 6–10 wk old were infected i.p. with 2 × 10^5 PFU of the Armstrong CA 1371 strain of LCMV (34). Infectious LCMV in serum and tissues was quantitated by plaque assay on Vero cell monolayers, as described previously (34). CTL assay

MHC class I-restricted LCMV-specific CTL activity was determined ex vivo by ^51Cr release assay, as described (34). Target cells used were either infected with LCMV or coated with NP396–404 peptide (0.1 µg/ml). One lytic unit is the number of effector cells that gives 30% lysis in a CTL assay.

Flow cytometry

Characterization of lymphocyte populations in +/+ and 4-1BBL−/− was determined by staining thymus and spleen cells for the T cell markers CD4 and CD8, and the B cell markers B220 and Ig heavy and light chain (H & L), followed by FACS analysis, as previously described (17). T cell activation was determined by staining uninfected and day 8-infected mice for the T cell markers CD4 or CD8 and for the activation marker CD44, followed by FACS analysis. PE-conjugated anti-mouse CD8, PE-conjugated anti-mouse CD4, FITC-conjugated anti-mouse CD4, APC-conjugated anti-mouse B220, and FITC-conjugated anti-mouse CD44 were purchased from PharMingen. FITC-conjugated anti-mouse Ig(H & L) was purchased from Caltag (Burlingame, CA).

ELISA

LCMV-specific Ab titer was determined by solid-phase ELISA, as described previously (34, 36).

Fusion protein

CTLA4Ig fusion protein was a kind gift from Peter Linsley (Bristol Myers Squibb, Seattle, WA) (37). Mice were treated i.p. with 500 µg of CTLA4Ig at days 0, 2, 4, and 6 after infection (38). In some experiments, human IgG (Rockland, Gilbertsville, PA) was used as a control. Mice were treated with 500 µg of human IgG on days 0, 2, 4, and 6 after infection. There were no differences between the results obtained from human IgG-treated and untreated mice.

Results

4-1BBL−/− mice have normal tissue architecture and express normal levels of T cells and B cells

Histological analysis and gross inspection revealed that the size and tissue architecture of the thymus, spleen, and lymph node was normal in 4- to 8-wk-old 4-1BBL−/− mice (data not shown). To typically characterize lymphocyte populations in 4-1BBL−/− mice, thymus and spleen cells were stained with anti-CD4 and anti-CD8 or anti-B220 and anti-Ig(H & L) in the thymus and spleen and analyzed by flow cytometry. Fig. 1A shows that the thymus of both +/+ and 4-1BBL−/− mice contained ~15% CD4+CD8− cells, ~4% CD4+CD8+ cells, and ~80% CD4−CD8− cells. The spleens of both +/+ and 4-1BBL−/− mice had ~32% CD4+CD8− cells, ~17% CD4−CD8− cells, and ~2% CD4+CD8+ cells. Similarly, normal

---

percentages of B cells were observed in the thymus and spleen of 4-1BB−/− mice. Both +/+ and 4-1BB−/− mice had 3% B220− Ig(H & L)+ cells in the thymus. In the spleen, +/+ mice had ~50% B220− Ig(H & L)+ cells and 4-1BB−/− mice had 45% B220− Ig(H & L)+ cells (Fig. 1B). 4-1BB−/− mice therefore had no demonstrable differences in T cell and B cell profiles compared with +/+ mice.

**Primary CD8 T cell responses are diminished in 4-1BB−/− mice after LCMV infection**

To examine the role of 4-1BB costimulation in the development of primary CD8 T cell responses to a viral pathogen, we studied CD8 T cell responses of +/+ and 4-1BB−/− mice following infection with LCMV. Previous studies have shown that the expansion of virus-specific cells is maximal at day 8 after infection (35). Therefore, as an initial measure of the response of CD8 T cells to LCMV, we used flow cytometry to determine the ratio of CD44high to CD44low T cells and absolute number of activated (CD44high) CD8 T cells in the spleens of +/+ and 4-1BB−/− mice 8 days after infection. Fig. 2A shows that +/+ mice generated a vigorous CD8 T cell response, as evidenced by a dramatic increase in both the ratio of CD44high:CD44low and absolute number of activated CD44high CD8 T cells in the spleen of infected +/+ mice. The ratio of CD44high:CD44low T cells changed from 0.61 at day 0 to 11:1 at day 8 in +/+ mice. In 4-1BB−/− mice, less activation was observed as the ratio changed from 0.6:1 at day 0 to 2.8:1 at day 8 (Fig. 2A). The total number of activated CD8 T cells in the spleen of 4-1BB−/− mice was also lower than +/+ mice. Activated CD8 T cells expanded 8-fold in +/+ mice, but there was only a 3-fold expansion in 4-1BB−/− mice (Fig. 2B). The total number of nonactivated CD8 T cells (CD8+ CD44low) remained unchanged in both +/+ and 4-1BB−/− mice on day 0 and day 8 (Fig. 2B).

Next, the number of CD8 T cells specific to dominant (NP396–404, GP33–41) and subdominant (GP276–286, NP205–212) LCMV epitopes was 2- to 3-fold lower in 4-1BB−/− mice than in +/+ mice (see Fig. 9B), indicating that the CTL response is in part dependent upon 4-1BB costimulation. Nevertheless, both +/+ and 4-1BB−/− were able to clear the virus by day 8 postinfection because all the mice had <2.3 log10 PFU/g of virus in the liver and <1.7 log10 PFU/ml in the serum.

These results show that in the absence of 4-1BB costimulation there is lower CD8 T cell activation, and CD8 T cell expansion in response to subdominant epitopes was 2.5- to 3.5-fold lower in 4-1BB−/− than in +/+ mice. Similar results were found using the IFN-γ ELISPOT assay (data not shown).

Finally, we compared the ability of 4-1BB−/− mice to generate CTL. Spleen cells from +/+ and 4-1BB−/− mice were added to virus-infected target cells in an ex vivo 51Cr release assay. Table 1 shows that 4-1BB−/− mice had levels of CTL activity comparable with that observed in +/+ mice. However, the number of LU per spleen was 2-fold lower in 4-1BB−/− mice than +/+ mice (see Fig. 9B), indicating that the CTL response is in part dependent upon 4-1BB costimulation. Nevertheless, both +/+ and 4-1BB−/− were able to clear the virus by day 8 postinfection because all the mice had <2.3 log10 PFU/g of virus in the liver and <1.7 log10 PFU/ml in the serum.

**Primary CD4 T cell responses to LCMV do not require 4-1BB signals**

CD4 T cell activation after infection was measured by flow cytometry. Both +/+ and 4-1BB−/− mice generated strong CD4 T...
cell responses. In +/+ mice, the ratio of CD44<sup>high</sup> to CD44<sup>low</sup> CD4<sup>T</sup> cells increased from a baseline of 0.3:1 at day 0 to 1.8:1 at day 8. The increase in the ratio of CD44<sup>high</sup> :CD44<sup>low</sup> CD4<sup>T</sup> cells was comparable in 4-1BBL<sup>−/−</sup> mice with an increase from 0.3:1 to 1.2:1 on days 0 and 8, respectively (Fig. 4A). Likewise, an increase in the absolute number of activated CD4<sup>T</sup> cells in 4-1BBL<sup>−/−</sup> and +/+ mice was similar. By day 8 postinfection, the number of activated CD4<sup>T</sup> cells expanded 2-fold in both groups from

Table I. 4-1BBL<sup>−/−</sup> mice generate a primary effector CTL response and eliminate LCMV<sup>+</sup>

<table>
<thead>
<tr>
<th>LCMV-Specific CTL (% specific 51 Cr release at indicated E:T ratio)</th>
<th>LCMV Titer (Log&lt;sub&gt;10&lt;/sub&gt; PFU/g or ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Infected targets</td>
</tr>
<tr>
<td>+/+</td>
<td>50:1</td>
</tr>
<tr>
<td>+/+</td>
<td>35</td>
</tr>
<tr>
<td>+/+</td>
<td>43</td>
</tr>
<tr>
<td>+/+</td>
<td>55</td>
</tr>
<tr>
<td>+/+</td>
<td>60</td>
</tr>
<tr>
<td>4-1BBL&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>23</td>
</tr>
<tr>
<td>4-1BBL&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>28</td>
</tr>
<tr>
<td>4-1BBL&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>25</td>
</tr>
<tr>
<td>4-1BBL&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>36</td>
</tr>
</tbody>
</table>

\(a\) +/+ and 4-1BBL<sup>−/−</sup> mice were injected i.p. with 2 \(\times 10^7\) pfu of the Armstrong strain of LCMV. At day 8 postinfection, viral titers and CTL activity were measured. Viral titers were determined by plaque assay on Vero cells. LCMV-specific CTL activity was measured by direct ex vivo CTL assay.
FIGURE 4. CD4 T cell activation in 4-1BBL−/− mice. Spleen cells at day 0 and day 8 postinfection from +/+ and 4-1BBL−/− mice were stained with anti-CD4 and anti-CD44. A representative flow-cytometric analysis is shown in A. An increase in the proportion of CD44-expressing CD4 T cells is seen in both +/+ and 4-1BBL−/− mice after infection. Numbers indicate the percentage of cells in the quadrant. The number of activated (CD4+CD44high) and nonactivated (CD4+CD44low) cells in the spleen of +/+ and 4-1BBL−/− mice is shown in B. Note that the expansion of activated CD4 T cells in 4-1BBL−/− mice was similar to +/+ mice.

10^6 to 10^6 (Fig. 4B). The number of nonactivated CD4 T cells remained unchanged in +/+ and 4-1BBL−/− mice on day 0 and day 8 (Fig. 4B).

The level of virus-specific CD4 T cell expansion was also quantitated by intracellular IFN-γ staining of CD4 T cells responding to the MHC class II-restricted LCMV peptide (GP61–80). The percentage of GP61–80-specific CD4 T cells was 8% in +/+ and 5% in 4-1BBL−/− mice (Fig. 5A). The number of GP61–80-specific CD4 T cells in +/+ and 4-1BBL−/− mice was also similar (1.2 × 10^6 in +/+ vs 0.9 × 10^6 in 4-1BBL−/− mice) in Fig. 5B. Similar results were found using the IFN-γ ELISPOT assay (data not shown). In addition, the number of LCMV-specific IL-2-secreting CD4 T cells in the spleen was similar between +/+ and 4-1BBL−/− mice, as determined by IL-2 ELISPOT. These data indicate that in contrast to CD8 T cell responses, CD4 T cell priming was minimally affected by the absence of 4-1BB signals.

Since previous reports have shown that CD4 T cell responses are impaired in the absence of CD40-CD40L interaction, we compared CD8 and CD4 T cell responses in +/+ and 4-1BBL−/− mice. Table II shows that while CD8 T cell responses were reduced 2-fold in 4-1BBL−/− mice compared with +/+ mice, CD8 T cell responses in CD40L−/− mice were similar to +/+ mice. Interestingly, when CD4 T cell responses were analyzed in the same mice, it was found that while 4-1BBL−/− mice generated CD4 T cell responses similar to +/+ mice, CD40L−/− mice had a 10-fold lower CD4 T cell response. These results indicate that CD8 and CD4 T cells have distinct costimulatory requirements in which optimal CD8 responses to LCMV require 4-1BB costimulation and CD4 responses require CD40-CD40L costimulation.

4-1BBL−/− mice generate normal B cell responses

Ig class switching from IgM to IgG is a Th-dependent process. To examine the role of 4-1BB costimulation in the generation of Th-dependent B cell responses, serum was taken from mice at day 8 postinfection, and the amount of LCMV-specific IgG was measured by ELISA. Fig. 6 shows that both +/+ and 4-1BBL−/− mice generated high levels of LCMV-specific Abs, because both mice had a titer of ~15 log_{2}. Furthermore, the subclasses of anti-LCMV IgG were indistinguishable between +/+ and 4-1BBL−/− mice. As shown in the legend of Fig. 6, both +/+ and 4-1BBL−/− mice had similar percentages of IgG1, IgG2a, IgG2b, and IgG3. 4-1BBL−/− mice therefore have no defect in their ability to generate Th-dependent B cell responses.

Blockade of both B7- and 4-1BBL-dependent signals reduces the number of virus-specific T cells

Although some have shown that costimulation of CD28 or 4-1BB alone was sufficient to induce immunity for some tumors (32, 39–41), both 4-1BBL and B7 need to be expressed on the poorly
immunogenic tumor AG104A to induce CTL generation and clearance of this tumor (42). In addition, soluble 4-1BBL and 4-1BBL-expressing cell lines induce IL-2 secretion from T cells in the absence of CD28 (27, 30). We therefore examined the role of both 4-1BBL- and B7-dependent signals on T cell responses to LCMV. For these studies, we examined four groups of mice 8 days after LCMV infection. The first group of mice was untreated +/- mice. The second group was untreated 4-1BBL-/- mice. To block B7-dependent signals, the third group consisted of +/- mice treated with the fusion protein CTLA4Ig (500 μg on days 0, 2, 4, and 6). Finally, to block both 4-1BBL- and B7-dependent signals, the fourth group consisted of CTLA4Ig-treated 4-1BBL-/- mice.

The number of virus-specific T cells generated was visualized directly by MHC tetramer staining for NP396–404-specific T cells and intracellular IFN-γ staining. The percentage of tetramer+ CD8 T cells in +/- mice increased from a basal level of <1% in uninfected mice to 24% 8 days after infection (Fig. 7A and data not shown). Interruption of 4-1BB signaling (4-1BBL-/- mice) or B7-dependent signaling (CTLA4Ig treatment) resulted in percentages of tetramer+ CD8 T cells of 19% and 17%, respectively. The percentage in the absence of both B7- and 4-1BBL-dependent signals was 15%. The number of NP396–404-specific T cells in the spleen was also analyzed (Fig. 7B). The number of tetramer+ CD8 T cells was 13.6 × 10^6 in +/- mice. 9.7 × 10^6 in 4-1BBL-/- mice, and 9 × 10^6 cells in CTLA4Ig-treated +/- mice. The greatest reduction was seen in CTLA4Ig-treated 4-1BBL-/- mice, which generated 3.8 × 10^6 tetramer+ CD8 T cells.

Next, we studied CD4 T cell responses in the absence of 4-1BBL- or B7-dependent responses. In addition, CD4 T cell responses in the absence of both 4-1BBL- and B7-dependent signals were also examined. Untreated 4-1BBL-/- and untreated +/- mice generated ~1 × 10^6 epitope-specific CD8 T cells after infection (Fig. 8F). However, expansion of CD4 T cells specific to GP61–80 was reduced 10-fold in both +/- and 4-1BBL-/- mice treated with CTLA4Ig (Fig. 8F). This result shows that B7-dependent signals are critical for the generation of CD4 T cell responses.
Finally, we compared the ability of the four groups of mice to generate CTL and clear the virus. CTL responses in 4-1BBL−/− or CTLA4Ig-treated +/+ mice were indistinguishable from untreated +/+ mice. In contrast, treatment of 4-1BBL−/− mice with CTLA4Ig significantly reduced CTL development, resulting in an 8-fold lower CTL than untreated +/+ or untreated 4-1BBL−/− mice (Fig. 9A). Untreated 4-1BB−/− mice generated 2-fold fewer LU/spleen than untreated +/+ mice, again indicating that part of the CTL response is dependent upon 4-1BB signals (Fig. 9B). Part of the CTL response was also dependent upon B7 interactions, because treatment of +/+ mice with CTLA4Ig reduced the number of LU/spleen by 2-fold. When both 4-1BB-4-1BBL− and B7-dependent interactions were blocked, the number of LU/spleen was diminished to lower than detectable levels (<30) (Fig. 9B). Analysis of viral clearance in the four groups mirrored CTL function. +/+ , 4-1BBL−/− , and CTLA4Ig-treated +/+ mice had all cleared LCMV from the liver by day 8 postinfection. In contrast, CTLA4Ig-treated 4-1BBL−/− mice had not completely cleared LCMV by day 8 postinfection (Fig. 9C). Furthermore, these results demonstrate that the absence of both

**FIGURE 8.** The number of epitope-specific CD8 and CD4 T cells is reduced in the absence of 4-1BBL- and B7-dependent signals. The number of epitope-specific T cells, as measured by intracellular IFN-γ staining, is shown. Note the 5- to 6-fold reduction in the number of epitope-specific CD8 T cells in CTLA4Ig-treated 4-1BBL−/− mice compared with +/+ mice (A). The number of GP61–80-specific CD4 T cells in CTLA4Ig-treated 4-1BBL−/− and CTLA4Ig-treated +/+ mice was 10- to 20-fold below +/+ mice (B). The data shown are from three independent experiments. Mice treated with human IgG responded the same as untreated animals. Each circle represents one mouse. The line indicates the average.
4-1BBL and B7-dependent signals reduce LCMV-specific CTL responses and impair clearance of LCMV infection by day 8 postinfection.

Discussion

In this study, 4-1BBL−/− mice were used to study the role of 4-1BB costimulation in T cell and B cell responses to LCMV. 4-1BBL−/− mice developed normally and had normal lymphocyte profiles in the spleen and thymus at 4–8 wk of age. Following LCMV infection, 4-1BBL−/− mice generated LCMV-specific CTL and cleared the virus. Although sufficient to control the infection, expansion of LCMV-specific CD8 T cell responses was 2- to 3-fold lower in the absence of 4-1BB signals, whereas CD4 T cell responses were minimally affected. 4-1BBL−/− mice did not have a defect in their ability to generate Th-dependent B cell responses. In addition, blockade of B7-dependent signals in 4-1BBL−/− mice reduced expansion of LCMV epitope-specific CD8 T cells and CTL generation, impaired clearance of LCMV, and diminished CD4 T cell expansion.

Previous studies using 4-1BB fusion protein, soluble 4-1BBL protein, or 4-1BBL-expressing cell lines indicated that 4-1BB costimulation stimulates anti-CD3 cross-linked T cells (23, 25, 27–30). Additional reports using agonistic Abs to 4-1BB have also indicated that 4-1BB costimulates T cells (31) and that it induces T cell responses to eliminate tumors from mice (32). This study shows that 4-1BB signals were not required to clear LCMV, as 4-1BBL−/− mice generated a strong CTL response. However, 4-1BB signals did appear to exert some costimulatory effects on CD8 T cell responses to LCMV, because the number of virus-specific CD8 T cells generated in 4-1BBL−/− mice was 1.1 × 10^7 cells compared with 2.4 × 10^7 in +/+ mice at day 8 postinfection.

An interesting dichotomy in the requirement of 4-1BB costimulation for T cell responses was identified. Virus-specific CD8 T cell numbers were reduced 2- to 3-fold in 4-1BBL−/− mice, whereas virus-specific CD4 T cell numbers were minimally affected. This result contrasts with what is seen after LCMV infection in the absence of CD40-CD40L interaction (43, 44). In CD40L−/− mice, the primary CD8 T cell response is minimally affected, but the CD4 T cell response is reduced 10-fold (Table II). These results suggest that CD8 T cells and CD4 T cells may have distinct costimulatory requirements: some CD8 T cells depend upon 4-1BB costimulation, whereas the majority of CD4 T cells depend upon CD40-CD40L interaction. This differential effect of 4-1BB costimulation on CD8 T cells is consistent with other in vitro studies, in which agonistic anti-4-1BB Abs were used (31). In addition, we have shown in an allogeneic model that agonistic anti-4-1BB mAbs exerted a strong proliferative effect on CD8 T cells, but a weaker effect on CD4 T cell proliferation (our unpublished observations). A possible explanation for these findings is that CD4 and CD8 T cells express different amounts of 4-1BB. Forty-one percent of alloreactive CD4 T cells express 4-1BB, whereas 71% of alloreactive CD8 T cells express 4-1BB (unpublished observations). Another possibility is that 4-1BB may bind to intracellular signaling molecules that are present in CD8 T cells, but not in CD4 T cells. 4-1BB has been shown to bind p56(lck) (45) and TRAF1, TRAF2, and TRAF3 (30, 46, 47). TRAF2 is required for effective 4-1BB signaling (30), and 4-1BB induces NF-kB activation in a TRAF-dependent manner (46, 47). However, no work has been done on differential signaling of 4-1BB in CD4 and CD8 T cells.

Although blockade of 4-1BB signals reduced LCMV-specific CD8 T cell responses, ~50% of the CD8 T cell response was 4-1BB independent. It is possible that these cells received enough stimulation through their TCR that they did not need costimulatory help. Viola et al. showed that ~1500 TCRs were necessary to push a human T cell clone to their threshold value of activation in the presence of CD28 costimulation. In contrast, ~8000 TCRs were required when no costimulation was provided (48). It is therefore possible that when a high Ag load or strong ligands are provided for the TCR on CD8 T cells, as is the case during an LCMV infection, T cell activation thresholds are reached. Costimulatory molecules would therefore not be necessary to push these T cells.
to threshold values of activation. It would be interesting to examine cases in which only low levels of Ag are present to determine whether compensatory molecules such as 4-1BB play more prominent roles in CD8 T cell activation.

An effective Ag-specific immune response must be generated to eliminate a viral infection. Our results show that 4-1BB costimulation was required to generate approximately half of the CD8 T cell response, but that the other half of the CD8 T cell response was 4-1BB independent. This suggests that 4-1BB-independent CD8 T cell responses either do not require costimulation or that compensatory costimulatory molecules act to stimulate these cells. To determine whether compensatory costimulatory molecules synergize to activate 4-1BB-independent CD8 T cell responses, we performed experiments blocking B7-dependent signals, the dominant costimulatory pathway by which T cells are activated (49). Studies examining CD28 costimulation have shown that CD4 T cell responses required this signal, but CD8 T cell responses do not require this signal (19). However, B7-dependent signals may be involved in generating CTL responses under certain circumstances, such as for CD4 Th-dependent CTL against cross-priming Ags, H-Y Ags, or certain viral infections (50–52). In addition, CD8 T cells from CD28−/− mice proliferate poorly in an in vitro allogeneic model (our unpublished results), which suggests that at least some CD8 T cells may have a requirement for CD28 signals. Other studies have shown that both B7 and 4-1BB expression on tumors were required to elicit a CTL response and clearance of these poorly immunogenic tumors from mice (42, 53). The results in this study indicate that expansion of virus-specific CD8 T cells was lowered a further 2- to 3-fold when signals from either costimulatory interaction were missing, indicating that part of the CD8 T cell response was B7 dependent and part was 4-1BB dependent. In addition, blockade of both molecules reduced the number of LCMV-specific CD8 T cells even more, suggesting that these pathways, at least in part, operate independently of one another. In addition, both B7- and 4-1BB-dependent signals are important for effective CD8 T-cell expansion and expansion of CD8 T cells.

Our results show that 4-1BB costimulation contributes to the generation of CD8 T-cell responses. In addition, 4-1BB- and B7-dependent signals induce expansion of CD8 T cells, generation of CTLs, and clearance of virus. Future studies should address the role of this interaction in T cell memory and their requirement during chronic viral infections. The importance of this interaction should also be examined in Th-dependent CTL responses to determine whether its expression depends upon other CD4-APC interactions. Recent studies looking at Th-dependent CTL responses (50–52) have shown that activation of these CTLs require CD40-CD40L interaction. Activation of the APC by CD40-CD40L interaction conditions the APC to activate CTLs. Although there is some evidence that CTL activation after APC conditioning involves B7 (especially B7.2), it may be possible that 4-1BB costimulation is also involved. It would also be interesting to determine in Th-independent infections, whether 4-1BB is upregulated on APCs and contributes to the generation of CTL responses in the absence of CD4 T cells.

In summary, our study suggests that in contrast to the CD40 costimulatory pathway, which affects the CD4 response, the 4-1BB pathway preferentially regulates the expansion and differentiation of CD8 T cells. In addition, simultaneous blockade of B7- and 4-1BB-dependent signals reduces both CD8 and CD4 T cell responses and points to these signals as good targets for immuno-therapy to boost T cell responses for vaccination or to block and prevent allograft rejection.

Acknowledgments

4-1BB−/− mice were kindly provided by Jacques Pechon, Immunex (Seattle, WA). H-2Db NP396–404 tetramers were kindly provided by John Altman, Emory University. We thank Rose Hendrix, Shannon Cowan, Carol Tucker-Burden, Mary Hagler, Mary Kathryn Large, and Kaja Madhvari Khanna for excellent technical assistance.

References


