Lymphocytic Choriomeningitis Virus-Induced Immune Dysfunction: Induction of and Recovery from T-Cell Anergy in Acutely Infected Mice

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Acute infection of immunocompetent mice by lymphocytic choriomeningitis virus induces a potent cytotoxic T-lymphocyte response that eliminates infectious virus. Concurrently and paradoxically, there is a general suppression of lymphocyte responses to mitogens and to other infectious agents. Splenocytes from infected mice released significant amounts of gamma interferon in response to mitogenic stimulation in vitro, but neither interleukin 2 nor interleukin 4 was similarly elevated relative to the amounts released by control cells. Early T-cell receptor-proximal signaling events were found to be intact, confirming that the cells were viable and had received the mitogenic stimuli in an appropriate manner. Acutely infected adult thymectomized mice regained concanavalin A responsiveness in parallel with euthymic mice, if T cells were left unmanipulated for several weeks after clearance of virus from the mice. Therefore, although acute lymphocytic choriomeningitis virus infection has the effect of disrupting proliferation when the T-cell receptor is ligated, this state is only temporary. In contrast, T cells from persistently infected adult mice reveal long-lasting alterations in concanavalin A responsiveness.

There are several examples of transient immune suppression or immune dysfunction that arise following acute virus infection of otherwise immunocompetent hosts (9). Peripheral infection of adult mice with lymphocytic choriomeningitis virus (LCMV) has been shown to suppress allograft reactions (14), antitumor reactions (10), immune responses to coinfecting agents (20), and in vitro T- and B-cell mitogen-induced proliferative responses (2, 6, 7, 11, 25, 27, 29). In contrast, however, the same peripheral LCMV infection of adult, immunocompetent mice induces strong delayed-type hypersensitivity responses that clear the virus within 7 to 14 days postinfection (5). There have been several recent investigations into the mechanism for this seemingly contradictory situation—generalized, although transient, lymphocyte suppression coinciding with a potent and biologically relevant anti-LCMV T-cell response (2, 6, 7, 21, 25, 27, 29). The interpretations of these previous studies are profoundly influenced by the strain of virus used. Infection of C57B1/6 mice with LCMV-WE strain results in a cytotoxic T-lymphocyte (CTL)-dependent loss of lymphoid tissue organization that has been associated with the elimination of antigen-presenting cells (25). Very large doses (2 × 10⁶ PFU) of the Armstrong strain of LCMV (LCMV-Arm) have also been shown to cause a similar loss of spleen cell architecture (2). It has been proposed that this disorganization prevents the proper priming of T and B cells and is responsible for the reduced immune responses of mice acutely infected with LCMV (25). However, in our studies with LCMV-Arm, any lack of priming could not be corrected by the addition of competent antigen-presenting cells in vitro (6, 7). Also, histological analysis of spleen and lymph nodes does not reveal LCMV-Arm-induced disruption of lymphoid tissue architecture (6, 20). It is presently unclear whether there are two totally distinct mechanisms, as exemplified by infections with the LCMV-WE and LCMV-Arm strains, to account for LCMV-induced immune dysfunction or whether the CTL-mediated lysis in the LCMV-WE infections is superimposed onto the generalized, but largely nonlytic, immune dysfunction induced by LCMV-Arm infection. Although LCMV is known to infect a wide variety of cell types, the vast majority of lymphocytes show no evidence of LCMV infection (1, 4, 8, 15, 33). This result suggests that indirect mechanisms affecting the T and B cells must account for the extensive changes in immune reactivity that are associated with LCMV infections. Neither prostaglandins (29) nor transforming growth factor β (31) appears to be responsible for the loss of T-cell functions.

When murine T cells are stimulated with polyclonal activators, naïve cells respond by secreting interleukin 2 (IL-2), Th1 cells respond by secreting IL-2 and gamma interferon (IFN-γ), and Th2 cells respond by secreting IL-4 and IL-10 (22). To begin to assess cytokine production by T cells collected from LCMV-infected mice, we cultured splenocytes from uninfected and infected BALB/c mice and assayed the supernatants for the production of cytokines.

**TABLE 1. Cytokine production by splenocytes from uninfected and LCMV-infected mice**

<table>
<thead>
<tr>
<th>Mice</th>
<th>IL-2 (U/ml)</th>
<th>IL-4 (U/ml)</th>
<th>IFN-γ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ConA +ConA</td>
<td>-ConA +ConA</td>
<td>-ConA +ConA</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 7</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>LCMV-infected, day 7</td>
<td>&lt;1</td>
<td>18 ± 1</td>
<td>1.6 ± 0.3</td>
</tr>
</tbody>
</table>

*Splenocytes from groups of three to five uninfected mice and LCMV-infected mice on day 7 were cultured in 24-well plates at 10⁶ cells per ml with (+) or without (−) 2 µg of ConA per ml. Supernatants were removed at 14 h and assayed for IL-2 and IL-4 and at 48 h for IFN-γ. The data are representative of five experiments. Data are the means and standard errors of triplicate samples.

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accumulation of secreted IL-2 and IL-4 in standard CTLL-2 and CT.4S proliferation assays. From the same cultures, supernatants were removed at 48 h for determination of IFN-γ content by a capture enzyme-linked immunosorbent assay. In agreement with the results of Saron et al. (29), we found that T cells from the acutely infected mice were deficient in IL-2 production (Table 1). No IL-4 was detected in the supernatants of the stimulated cells from the acutely infected mice at day 7, but these cells did secrete substantial quantities of IFN-γ. In all cases, cytokine production was dependent on concanavalin A (ConA) stimulation. It has been proposed that alteration of Th1/Th2 patterns can be responsible for loss of delayed-type hypersensitivity and the progression of induced immune suppression (30). Since the T cells from acutely infected mice were not making IL-4, a switch from Th1 to Th2 is unlikely to have occurred and such a change would not be consistent with the observed elevated levels of IFN-γ (Table 1).

The levels of T-cell receptor expression, as judged by anti-CD3 staining, were essentially normal on T cells from infected mice (6, 27), but one potential explanation for the altered response to mitogens is an uncoupling of the receptor from the normal signal transduction machinery. This possibility was assessed by measuring ConA-stimulated inositol phosphate turnover. T cells were prepared from groups of five uninfected mice and LCMV-infected mice on day 7. Cells were loaded overnight with [3H]myoinositol, and equivalent specific activities of cells were incubated with or without 10 μg of ConA per ml in the presence of 15 mM LiCl to block inositol phosphate recycling. Cells were lysed with methanol-CHCl₃, and inositol phosphates were recovered by ion-exchange chromatography. The results shown are representative of three experiments. The standard deviations of three (without ConA) or six (with ConA) samples are shown.

FIG. 1. Inositol phosphate accumulation in ConA-stimulated T-cells from uninfected mice and infected mice on day 7. T cells were prepared from groups of five uninfected mice (T) and LCMV-infected mice on day 7 (IT). Cells were loaded overnight with [3H]myoinositol, and equivalent specific activities of cells were incubated for the indicated time with or without 10 μg of ConA per ml in the presence of 15 mM LiCl. Cells were lysed with methanol-CHCl₃, and inositol phosphates were recovered by ion-exchange chromatography. The results shown are representative of three experiments. The standard deviations of three (without ConA) or six (with ConA) samples are shown.

There are conflicting reports as to whether the addition of exogenous IL-2 does (27) or does not (29) induce proliferation of T cells from LCMV-infected mice. However, cycling in IL-2 is known to predispose murine T cells to apoptosis upon TcR ligation (16). It may be that the elevated production of IFN-γ by T cells from acutely infected mice is a factor in the induction of apoptosis, since it has been identified as contributing to the apoptosis of classically anergic T cells (17). An increased susceptibility to apoptosis has also been observed in human T cells during acute viral infections and has been implicated as a mechanism in the loss of T cells associated with the progression of human immunodeficiency virus infection to AIDS (19).

Mitogen-driven proliferative responses can be restored in spleen cell preparations from acutely infected mice, if the mice are infected and retained for 3 to 6 weeks prior to analysis (6, 7, 27). In order to determine whether this phenomenon reflects the reacquisition of normal function by peripheral T cells or the emergence of new T cells from the thymus, we set out to examine the proliferative responses of spleen cells from adult thymectomized mice. Groups of four adult mice, thymecto-

FIG. 2. Proliferative responses to ConA stimulation of spleen cells prepared from individual adult thymectomized mice. Cells were prepared from uninfected mice (ATx [open symbols]) and mice at 7 (A) or 49 (B) days postinfection (iATx [closed symbols]). Each point represents the mean and standard error of triplicate samples, and where not apparent, error bars lie within the symbols. Similar results were obtained with a repetition of this experiment in which the recovery period was reduced to 31 days.
mized at 6 weeks of age (Taconic, Germantown, N.Y.), were either infected with LCMV or left uninfected as controls. At day 7 of infection, two mice from each group were sacrificed and spleen cell responses to ConA were determined (Fig. 2A). Virus infection reduced proliferation in the spleen cell populations by approximately 90%. After 49 days of infection, the remaining pairs of animals were sacrificed and ConA responsiveness was again determined (Fig. 2B). There was no significant difference between the proliferation of spleen cells from the mice that had been infected and uninfected controls. Mock-thymectomized animals were examined in parallel to confirm that the expected loss and recovery of ConA responsiveness had occurred (data not shown). In a second experiment, the mice were thymectomized at 8 weeks of age, and complete recovery of T-cell responsiveness was observed at 31 days postinfection (data not shown). When T cells were analyzed by fluorescence-activated cell sorting at day 7 of infection and at a later time point, coinciding with the observed recovery of mitogen responsiveness, there were no discernible differences in expression of CD3, CD4, CD8, CD2, CD25, or CD44 between the spleen cells of infected, thymectomized mice and infected, mock-thymectomized controls (data not shown). Virus plaque assays with serum collected at day 7 postinfection confirmed that both the infected adult thymectomized mice and the infected euthymic controls had cleared infectious virus below the limit of detection of the assay (200 PFU/ml).

Infection of BALB/c mice with LCMV-Arm at birth results in a lifelong persistent infection, and although such mice are overtly normal, they are specifically deficient in an anti-LCMV CTL response (5, 32). We have found that the mitogen responsiveness of T cells from adult BALB/c mice persistently infected with LCMV-Arm is intermediate between uninfected and acutely infected mice (Fig. 3A), whereas the B-cell proliferative response to lipopolysaccharide appears to be normal (Fig. 3B). Since the dose-response curve to ConA is not shifted (Fig. 3A), it can be inferred that the proliferating T cells from persistently infected mice are as sensitive to the mitogen as the cells from the uninfected mice but that fewer cells are proliferating. The disruption of T-cell proliferation for cells harvested from persistently infected mice correlates with an intermediate level of IL-2 production in vitro cultures (Table 2). These results suggest that the events inducing altered T-cell responses are ongoing in the persistently infected mice and/or that other mechanisms become activated during persistent LCMV infection to disrupt normal T-cell proliferation.

Reduced IL-2 production, conserved IFN-γ production, and apoptosis (27) upon stimulation are all features that bear superficial resemblance to descriptions of T-cell anergy (17, 26, 28). As classically defined, anergy results from T-cell receptor engagement in the absence of costimulatory signals (23). However, it seems unlikely that that up to 90% of the T cells in a mouse would have receptors capable of engaging LCMV antigens or LCMV infection-stimulated self antigens, and LCMV does not appear to have any direct activity as a superantigen or polyclonal activator. Instead, the loss of lymphocyte proliferation following acute infection by LCMV may reflect a degenerate or incomplete state of activation resulting from exposure of uninfected T cells in these mice to factors designed to mobilize immune responses during a disseminated infection. Cells in this state would not be competent for cell division, and following receipt of signals by the T-cell receptor, they commit unsuccessfully to division and undergo apoptosis (27) as the default option.

<p>| Table 2. Proliferation and IL-2 secretion by splenocytes from mice persistently infected with LCMV* |
|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Expt no. and mice</th>
<th>Amt (U/ml) of IL-2</th>
<th>Proliferation (% of uninfected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>105</td>
<td>100 (51,553 cpm)</td>
</tr>
<tr>
<td>Persistently infected</td>
<td>48</td>
<td>77</td>
</tr>
<tr>
<td>Acutely infected, day 7</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Expt II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>20</td>
<td>100 (32,174 cpm)</td>
</tr>
<tr>
<td>Persistently infected</td>
<td>14</td>
<td>58</td>
</tr>
<tr>
<td>Acutely infected, day 7</td>
<td>2.4</td>
<td>28</td>
</tr>
</tbody>
</table>

* Splenocytes were prepared from groups of three control mice and LCMV-infected mice on day 7, and congenitally LCMV-infected mice. Cells were cultured with 1 μg of ConA per ml pulsed with [3H]thymidine after 48 h and harvested 12 to 14 h later. In the absence of ConA, background proliferation was <750 cpm for all samples. Data are the means of triplicate samples. Standard deviation was less than 10%. IL-2 levels were determined from the proliferation of CTL lines cells by using triplicate samples of tissue culture supernatant, removed after 18 to 24 h of culture.
An alternative possibility is that the altered mitogen responsiveness may reflect a kind of systemic feedback inhibition. LCMV-induced immunosuppression coincides with the peak of anti-LCMV CTL and delayed-type hypersensitivity activity (3, 5, 13) and the fraction of T cells responding to LCMV approaches 2% of the total population (24). Many other cell types are also recruited into infected tissues (12, 18). Mechanisms may have evolved to down-regulate vigorous immune responses to limit immunopathologic damage to the host. It is conceivable that such a mechanism might also impinge upon the functional capabilities of cells not directly involved in the ongoing immune response. Thereafter, a time-dependent recovery might allow restoration of normal responsiveness to the affected population but only after clearance of the eliciting antigens and subsidence of altered cytokine levels. The ability to dissociate the acute infection from the immune response to LCMV might allow discrimination between these possibilities.

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REFERENCES