Adoptive Transfer of Murine Host Protection to Salmonellosis with T-Cell Growth Factor-Dependent, Salmonella-Specific T-Cell Lines

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A spent medium antigen was prepared from the avirulent RIA strain of Salmonella typhimurium. Lymph node cells isolated from female BALB/c mice injected subcutaneously with the spent medium antigen exhibited antigen-specific proliferation. By using these cells and T-cell growth factor, continuous spent medium antigen-specific, Thy 1.2-sensitive lines were generated. These cells exhibited antigen-specific proliferation in vitro and were effective in inducing significant (P < 0.01) host protection when adoptively transferred to naive syngeneic mice.

The reduction in numbers of Salmonella typhimurium, a facultative intracellular pathogen in mice, requires the interaction of sensitized T lymphocytes and macrophages (9, 16). Lymphokines are released by sensitized T cells in response to a specific antigenic stimulus, yet their action is characterized by enhancing a nonspecific bactericidal function of macrophages (9, 16, 17). In a normal immunocompetent animal the small percentage of available T cells specific for a given antigen (Ag) is small, 0.2 to 0.3% (8). A technique or procedure by which this small population of cells could be expanded would be useful in the examination of the cell-mediated immune response.

Recently, Kaufmann and Hahn (14) and Kearns and DeFreitas (15) have demonstrated that it is possible to isolate and maintain Ag-specific T cells in vitro and that such cells are capable of providing a significant degree of protection against the facultative intracellular pathogen Listeria monocytogenes. The isolation, characterization, and availability of T-cell growth factor (TCGF) has made possible the long-term maintenance of Ag-specific T cells in vitro (11, 22). It has been shown that T lymphocytes maintained in vitro in the presence of TCGF can retain their Ag specificity and reactivity (2, 23, 25, 27). Therefore, as a means of examining the role(s) of Ag-specific T lymphocytes in murine salmonellosis, we isolated, maintained, and characterized T lymphocytes derived from mice which were immunized with a spent medium antigen (SMA) from S. typhimurium.

In this report, mice were immunized with SMA from S. typhimurium, and the draining lymph nodes were used as a source of Ag-specific T cells. These T cells were used to demonstrate that (i) such an immunization and cell collection protocol could yield SMA reactive cells, based upon a blastogenesis assay; (ii) such cells could be maintained indefinitely in vitro in the presence of TCGF; (iii) T cells continuously cultured can elicit positive Ag-specific blastogenesis in vitro; and (iv) T cells can induce host protection when reintroduced into a syngeneic mouse, subsequently challenged with virulent S. typhimurium SR-11.

MATERIALS AND METHODS

Antigen preparation. SMA was prepared from S. typhimurium RIA (avirulent strain) as described by Cohn (3) and modified by Collins and Mackaness (4) and Plant and Glynn (21). The protein concentration of SMA was determined by the Bio-Rad assay. The antigen was divided into portions and stored at –20°C. A lysate of Escherichia coli was also prepared to serve as a negative control for the blastogenesis assay.

Generation of Ag-specific T cells. T lymphocytes were obtained and cultured essentially as described by Corradin et al. (7). Briefly, 6- to 8-week-old female BALB/c mice (Harlan, Indianapolis, Ind.) were injected subcutaneously at the base of the tail with 10 to 20 μg of either SMA (based upon protein content) or ovalbumin (OVA; Sigma Chemical Co., St. Louis, Mo.) emulsified in complete Freund adjuvant in a volume of 100 μl. Ten days later mice were sacrificed, and the inguinal and peri-aortic lymph nodes were removed. Lymph node cells (LNC) were suspended in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 100 μg of gentamycin per ml and placed on ice for 15 min. Cells were centrifuged at 100 × g for 10 min and resuspended in RPMI 1640 supplemented with 100 μg of gentamycin per ml, 2 mM l-glutamine, 10% heat-inactivated horse serum (GIBCO Laboratories), and 5 × 10−5 M 2-mercaptoethanol (GIBCO Laboratories). and 5 × 10−5 M 2-mercaptoethanol (GIBCO Laboratories). and 5 × 10−5 M 2-mercaptoethanol (GIBCO Laboratories).

Proliferation assay. LNC were tested for their ability to proliferate in the presence of Ag or mitogen by the method of Alkan (1). LNC obtained as described above were plated directly into 96-well microculture plates (Costar; Hyclone Laboratories, Logan, Utah) at 4 × 105 cells per well. Ag, mitogen, or complete medium alone was added to each well in a volume of 20 μl. After 54 h in culture, 0.5 μCi of [methyl-3H]thymidine was added to each well in a volume of 20 μl. Incubation was continued for 18 h, after which cells were harvested and counted. To test these culture conditions for the ability to support proliferation, concanavalin A (ConA; Sigma Chemical Co.) was added at a concentration of 5 μg/ml. Conditions for the proliferation assay involving the TCGF-dependent T-cell lines were as follows. SMA-sensitive LNC (as described) were cultured in 24-well Costar microculture plates (Hyclone Laboratories) at a concentration of 5 × 105 cells per well along with SMA. Four days later the T cells were recovered by running the well contents on a Ficol-Hypaque gradient (20). The recovered cells were cultured as before, except that the SMA was omitted and...
TABLE 1. Blastogenic responses* of LNC from Salmonella SMA-injected and OVA-injected mice

<table>
<thead>
<tr>
<th>LNC</th>
<th>Antigen (μg)</th>
<th>cpm ± SD</th>
<th>Stimulation index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>PPD (2.5)</td>
<td>754 ± 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SMA (8)</td>
<td>17,273 ± 205</td>
<td>29.9*</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>14,441 ± 335</td>
<td>19.2*</td>
</tr>
<tr>
<td></td>
<td>lysate (10)</td>
<td>999 ± 289</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>ConA (0.5)</td>
<td>7,428 ± 654</td>
<td>9.9*</td>
</tr>
<tr>
<td>OVA</td>
<td>OVA (5)</td>
<td>1,188 ± 375</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SMA (7.5)</td>
<td>4,785 ± 511</td>
<td>4.0*</td>
</tr>
<tr>
<td></td>
<td>ConA (0.5)</td>
<td>1,174 ± 575</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9,096 ± 102</td>
<td>7.7*</td>
</tr>
</tbody>
</table>

* LNC, cultured at 2 × 10⁶ cells per well in microculture plates, were pulsed with 0.5 μCi of [³H]thymidine at 54 h and harvested at 72 h.

RESULTS

Proliferation assay. To verify the Ag specificity of the T cells isolated from the lymph nodes, a proliferation assay was performed. The data in Table 1 show typical results of this assay. Note that the LNC from mice previously injected with the Salmonella SMA only exhibited blastogenesis to the Salmonella Ag but not to the E. coli lysate. Also, the Salmonella LNC responded to purified protein derivative as a result of the Ag being incorporated in complete Freund adjuvant. These results demonstrate that the proliferative response (Table 1) reflects Ag-specific stimulation rather than nonspecific proliferation. Again note that heterologous Ag was not stimulatory to either the Salmonella SMA or OVA LNC, whereas ConA stimulated both cell populations to proliferate. The data (Table 2) show the results of a proliferation assay in which the TCGF-dependent SMA T-cell line underwent blastogenesis when cultured with SMA. This T-cell line also responded to ConA.

Adoptive transfer of SMA-specific T cells. To assess the in vivo functional capacity of the in vitro-maintained T cells, these T cells were adoptively transferred to naive BALB/c mice. Three hours after the intravenous injection of 10⁶ SMA-specific or OVA-specific T cells, the mice were challenged with 10 or 20 LD₅₀ of virulent S. typhimurium strain SR-11. Also, a group of normal controls were challenged in an identical fashion. The results show that mice receiving the SMA-specific T-cell lines exhibited significant host protection (P < 0.01) to a challenge infection with 10 LD₅₀ of S. typhimurium SR-11 (Table 3). In one challenge experiment of 20 mice receiving the TCGF-dependent OVA T cells survived the challenge infection; however, no normal control mice survived 10 or more LD₅₀ of virulent salmonellae. In addition, of the mice that died, the mean time to death of the mice receiving SMA T cells was significantly (P < 0.05) greater (10.5 days) than that of control mice (7.6 days). When a challenge dose of 20 LD₅₀ was used, only mice receiving the SMA T cells survived. Of the mice dying from the 20 LD₅₀ challenge, mice receiving the SMA T cells survived significantly (P < 0.05) longer (11.5 days) than did control mice (7.3 days). All control and OVA T-cell mice were dead within 2 weeks after challenge (Table 3).

TABLE 2. Blastogenic responses* of TCGF-dependent SMA T-cell line

<table>
<thead>
<tr>
<th>T cells</th>
<th>Antigen (μg)</th>
<th>cpm ± SD</th>
<th>Stimulation index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>100 ± 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMA</td>
<td>SMA (2.5)</td>
<td>1,430 ± 125</td>
<td>14.3</td>
</tr>
<tr>
<td>SMA</td>
<td>SMA (1.3)</td>
<td>1,720 ± 140</td>
<td>17.2</td>
</tr>
<tr>
<td>ConA</td>
<td>ConA (0.5)</td>
<td>7,500 ± 454</td>
<td>7.5</td>
</tr>
</tbody>
</table>

* Continuous T lymphocytes were cultured at 10⁶ cells per well in a Costar microculture plate. Each well received 0.5 μCi of [³H]thymidine 54 h later, followed by an additional 18 h of incubation and then harvesting.

* Based on protein content.

* Stimulation index = Counts per minute with Ag or mitogen/counts per minute without Ag or mitogen.
DISCUSSION

Protection of hypersusceptible mouse strains against facultative intracellular parasites resides mainly in the activation of cell-mediated immune responses (6, 12). Collins (5) and Smith and Bigley (24) have demonstrated that the passive transfer of immune serum will not protect nonimmune mice from a lethal challenge infection. Recipients of the immune serum will exhibit increased survival times before succumbing to the lethal effects of the challenge infection, compared with control nonimmune mice. However, it should be noted that Morris et al. (19) concluded that humoral immunity was of greater importance than cell-mediated immunity in mice vaccinated with a GalE mutant of S. typhimurium. Also, Hochadel and Keller (13) demonstrated that purified B lymphocytes induced greater adoptive protection in C32F hybrid mice than did purified T cells isolated from immune mice. Collins (6), Smith and Bigley (24), and Venneman and Berry (26) demonstrated that mice adoptively transferred with Salmonella immune lymphocytes or peritoneal exudate cells will survive a lethal challenge infection. McGregor et al. (18) demonstrated that Listeria immune T lymphocytes were effective in transferring host protection to nonimmune rats challenged with a lethal dose of L. monocytogenes.

Recently, Kaufmann and Hahn (14) and Kearns and DeFreitas (15) induced continuous interleukin 2 (IL2)-dependent T-cell lines specific for L. monocytogenes. Both groups of investigators demonstrated that protective host immunity could be adoptively transferred to naive syngeneic mice with the respective Ag-specific T-cell lines. Of particular interest was the finding that a single anti-listerial T-cell clone was active in adoptively transferring both host protection and positive footpad swelling (delayed hypersensitivity) (14). This same clone of T cells underwent blastogenesis in vitro when cultured in the presence of heat-killed L. monocytogenes.

In this present study we have reported the successful induction of TCGF-dependent continuous T-cell lines specific for S. typhimurium. Naive mice adoptively transferred with these T cells exhibited significant host protection to 10 LD50 of virulent salmonellae (Table 3). In this particular experiment no normal control mice survived, although two of the OVA TCGF-dependent T-cell recipient mice did survive the challenge infection. This is the first instance in which we have observed nonsalmonellae immune BALB/c mice surviving a challenge infection with 10 LD50 of virulent salmonellae. Two possible explanations may exist. First, actively proliferating continuous T-cell lines or clones released IL2 (14). It is possible that OVA T cells were releasing IL2 in vivo, thus permitting some degree of non-Ag-driven T-cell activation. Second, Fukazawa et al. (10) showed that supernatants from ConA-activated spleen cells could induce host protection to salmonellae-challenged mice, indicating that lymphokines alone may afford some degree of host protection.

Although we demonstrated that the freshly isolated LNC taken from SMA-injected mice will proliferate in T-cell blastogenic assays in response to SMA, the IL2-dependent T-cell lines were inconsistent in this assay. Stimulation indices of 14 to 17 (Table 2) were obtained when the SMA T cells were used, but we also found that cells responsive in a blastogenic assay on one occasion may not be responsive later on. This problem may in part reflect the mixed population of IL2-dependent T cells in our long-term salmonellae-specific line. When we used cloned SMA-specific T cells in the blastogenesis assay, more consistent results should be obtained, since these cells will be proliferating in response to a single antigenic determinant of the SMA. Alternatively, Wilde and Fitch (28) demonstrated that Ag-specific T cells cultivated as a continuous culture will go through phases of unresponsiveness.

ACKNOWLEDGMENTS

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LITERATURE CITED


 TABLE 3. Adoptive transfer* of host protection to experimental salmonellosis with SMA continuous T-cell lines

| Cell transferred | Challenge dose (LD50) | No. surviving/
|------------------|-------------------|-----------------
| None             | 10                | 0/20           |
| OVA              | 10                | 2/20           |
| SMA              | 10                | 13/20          |
| None             | 20                | 0/10           |
| OVA              | 20                | 0/10           |
| SMA              | 20                | 5/10           |

* Mice received 10^6 T cells intravenously, followed 3 h later with an intraperitoneal challenge of virulent S. typhimurium SR-11 (LD50 = three bacteria).
* Mice surviving for 30 days after the challenge infection.
* P < 0.01 compared with control challenged animals by the X^2 test.
passively transferred immune T or B lymphocytes in mice infected with Salmonella typhimurium. J. Infect. Dis. 135: 813–823.