Interleukin 2 Induction in Lyt $1^+23^-$ T Cells from Listeria monocytogenes-Immune Mice

STEFAN H. E. KAUFMANN,1* HELMUT HAHN,1 MARKUS M. SIMON,2 MARTIN RÖLLINGHOFF,3 AND HERMANN WAGNER3

Institut für Medizinische Mikrobiologie, Freie Universität Berlin, D-1000 Berlin 45,1 Deutsches Krebsforschungszentrum, D-6900 Heidelberg, 2 and Institut für Medizinische Mikrobiologie, Johannes Gutenberg Universität, D-6500 Mainz, 3 Federal Republic of Germany

Received 16 February 1982/Accepted 7 May 1982

Peritoneal exudate T lymphocytes from mice experimentally infected with the intracellular bacterium Listeria monocytogenes secreted high interleukin 2 activities after interaction with syngeneic normal macrophage presenting listerial antigen in vitro. L. monocytogenes-immune cells secreting IL 2 were radioresistant and bore the phenotype Thy $1^+23^-$. The T-cell system of mice can be divided into distinct subpopulations discernible by their Lyt phenotype (1, 8). For adequate immune responses to occur, different Lyt T-cell subpopulations have to interact with each other and with accessory cells (H. Cantor and R. K. Gershon, Fed. Proc. 38:2058-2064, 1979). Recently, it has been shown that different stimuli can activate T cells to secrete interleukin 2 (IL 2) (9, 13), and evidence has been accumulated that Lyt $1^+$ helper T-cell-derived IL 2 fulfills an important mediator function in the generation or differentiation of antigen-primed Lyt $23^+$ cytotoxic T cells (CTLs) (3, 12, 14, 15).

Cell interactions in acquired antibacterial immunity are much less well understood (2). However, it has been shown that Lyt $1^+$ and Lyt $123^+$ T cells participate in the immune response to the intracellular bacterium Listeria monocytogenes (2a, 5, 6; S. H. E. Kaufmann, H. Hahn, and M. M. Simon, Scand. J. Immunol., in press).

In the present study, we show that L. monocytogenes-immune Lyt $1^+$ T cells induced in vivo are potent secretors of IL 2 upon restimulation with macrophage-associated listerial antigen in vitro, indicating a role for IL 2 in acquired immunity against intracellular bacteria. C57BL/6 mice were infected with approximately $4 \times 10^3$ to $6 \times 10^3$ live L. monocytogenes organisms (strain EGD) (5, 7). After 7 days, peritoneal exudates were induced by intraperitoneal injection of 1.5 ml of 10% protease peptone, and peritoneal exudate cells (PECs) were collected after another 3 days. Peritoneal exudate T lymphocytes (PETLs) were enriched by two passages of cells over nylon wool columns (4). As a source of normal macrophage, PECs from nonimmunized syngeneic mice were harvested 3 days after intraperitoneal injection of 1.5 ml of 10% protease peptone. PECs ($10^6$/ml) from nonimmunized mice were incubated in macrotiter plates at 37°C under 5% CO$_2$ for 2 h, and nonadherent cells were removed by extensive washing. Adherent PECs were cocultured with $5 \times 10^7$ heat-killed L. monocytogenes organisms for another 2 h, and then the supernatants were discarded. Different numbers of L. monocytogenes-immune PETLs were added to the antigen-pulsed PECs, and the cells were cultured in a total volume of 1 ml of RPMI 1640 medium containing 5% fetal calf serum, L-glutamine, penicillin, and streptomycin. After 24 h, supernatants were collected and tested for IL 2 activity by their ability to substitute for the requirements of helper T cells during the induction of Lyt $123^+$ thymocytes to become alloreactive CTLs, as described previously (14).

The release of IL 2 activity required the presence of L. monocytogenes-immune T cells, normal syngeneic macrophage, and listerial antigen (Table 1). The amount of IL 2 activity depended upon the number of immune PETLs present in the culture; $10^5$ PETLs was sufficient. Furthermore, IL 2 secretion appeared to be rather insensitive to X-irradiation (at 2,000 roentgens), although at low concentrations and at low effector-to-target cell ratios, some differences became manifest (Table 1).

To determine the approximate molecular weight of IL 2, supernatants were passed over a Sephadex G-75 column (Fig. 1). In accordance
alloreactive CTLs, killed L. monocytogenes organisms served as substitute for specific lysis assays in the absence of either antigen or Listeria-immune PETLs, the percent specific lysis of target cells was 0.

With the known molecular weight of IL-2, the helper activity resided in the 30,000-to-35,000 molecular weight fraction; no activity was demonstrable in the 18,000 molecular weight fraction.

Next, the Lyt phenotype of the IL-2-secreting T-cell subset was determined. PETLs (3 x 10^6/ml) from L. monocytogenes-immune mice were treated at room temperature with anti-Lyt antisera for 30 min, washed, and incubated in selected rabbit complement as described previously (5). Anti-Lyt antisera had been prepared according to the method of Shen et al. (10) as described previously (11). Treatment of PETLs was repeated once. After treatments, 10^6 unselected PETLs or corresponding numbers of selected PETLs were cocultured with 10^6 L. monocytogenes-pulsed macrophage. After 24 h, supernatants were collected and tested for IL-2 activity by their ability to induce clonal expansion of alloreactive CTLs as described previously (14). L. monocytogenes-immune T cells responsible for IL-2 secretion bore the phenotype Thy 1^+ Lyt 1^+23^+ (Table 2).

Our experiments demonstrate that L. monocytogenes-immune Lyt 1^+ T cells are potent secretors of IL-2 after interactions with syngeneic macrophage presenting listerial antigens in vitro. Earlier studies have shown that proteins mitogenic for thymocytes are produced in cocultures of L. monocytogenes-immune Lyt 1^+ T cells with macrophage and listerial antigen (16; Kaufmann et al., in press). These mitogenic activities mainly resided in the molecular weight range around 18,000 and presumably were a macrophage product (IL-1) (Kaufmann et al., in press). Thus, L. monocytogenes-immune Lyt 1^+ T cells are involved in secretion of IL-1 and IL-2, indicating that both interleukins might exert mediator functions in the generation of the immune response to intracellular bacteria.
TABLE 2. IL 2 secretion by Listeria-immune Lyt 1+23- T cells

| Group no. | Primary culture* | Treatment of PETLs | T cell subset | % Specific lysis of 51Cr-labeled P 815 with following % (vol/vol) of supernatant assayed:
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>Unselected</td>
<td>None</td>
<td>10:1</td>
<td>5:1</td>
<td>0.4:1</td>
<td>10:1</td>
<td>5:1</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>Unselected</td>
<td>None</td>
<td>70</td>
<td>34</td>
<td>11</td>
<td>63</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>Anti-Thy 1.2 + C</td>
<td>None</td>
<td>Lyt 1+23+</td>
<td>63</td>
<td>40</td>
<td>14</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Anti-Lyt 1.2 + C</td>
<td>Lyt 1+23-</td>
<td>Lyt 1+23-</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Anti-Lyt 2.2, 3.2 + C</td>
<td>Lyt 1+23-</td>
<td>Lyt 1+23+</td>
<td>67</td>
<td>35</td>
<td>18</td>
<td>69</td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>Mixture of groups 4 + 5 (1:1)</td>
<td>Lyt 1+23- + Lyt 1+23+</td>
<td>68</td>
<td>48</td>
<td>14</td>
<td>72</td>
<td>47</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>10^2 untreated PETLs</td>
<td>Unselected</td>
<td>Lyt 1+23-</td>
<td>66</td>
<td>34</td>
<td>5</td>
<td>41</td>
<td>13</td>
</tr>
</tbody>
</table>

* Equivalents of 10^6 Listeria-immune PETLs were cocultured with 10^6 syngeneic macrophage and 5 x 10^7 heat-killed L. monocytogenes organisms for 24 h. Supernatants were tested for IL 2 activity by their ability to induce clonal expansion of alloreactive CTLs, as described previously (14). C, Complement.

* Ratio of effector to target cells.

We thank Brigitte Brokmeier and Petra Holzweg for helpful technical assistance and Annelore Häusler for typing the manuscript.

LITERATURE CITED