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Differential Production of Intracellular Gamma Interferon in αβ and γδ T-Cell Subpopulations in Response to Peritonitis

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Flow cytometry was used to measure T-cell intracellular gamma interferon and surface interleukin 2 re-ceptor expression in response to peritonitis in rats. Interleukin 2 receptor expression levels were similar in the two T-cell subsets, but gamma interferon production was increased fivefold in γδ T cells compared with pro-duction in αβ T cells. Our results provide further evidence of an early and vigorous γδ T-cell response to bac-terial infection.

Natural immunity and tissue phagocytes play a key role in the immediate response to peritonitis, yet T-cell activation, differentiation, and cytokine production must occur to successfully and completely eradicate the infecting organism (6). There is evidence that a broadly reactive T-cell subset that is located primarily in the epithelial surfaces, lymph nodes, and intestinal lining and that expresses the γδ T-cell receptor (TCR) plays an important role in the early recognition of and resistance to infection (4). In vivo studies have demonstrated that γδ T cells appear an an early stage in the peritoneal cavities of mice infected with *Listeria monocytogenes* (2, 5, 8) and appear in the lymph nodes following infection with *M. tuberculosis* (1). Mice depleted of γδ T cells demonstrate an exaggerated multiplication of *L. monocytogenes* in the early stages of infection (5). Additionally, mice depleted of γδ T cells demonstrate an increased proliferative response of αβ T cells, suggesting a possible role for the former in the regulation of αβ T-cell activation in vivo (8). Most recently, γδ T cells in the spleens and peritoneal cavities of mice infected with *L. monocytogenes* and *Nippostrongylus brasilensis* have been demonstrated to respond earlier and with more pronounced Th1 and Th2 cytokine secretion patterns than αβ T cells (2). Strong Th1 and Th2 cytokine responses by γδ T cells may initiate as well as modulate the overall cellular and humoral immune response to infection (2). The goal of the present study was to measure intracellular gamma interferon production in γδ T cells in response to fecal bacterial peritonitis.

Forty Sprague-Dawley rats underwent anesthesia only or cecal ligation and puncture (CLP). Spleens were harvested 1, 2, 3, and 5 days later. All animals received fluid resuscitation with 5 ml of 0.9% NaCl per 100 g of body weight subcutaneously. Cell preparation was carried out as previously described by Ferrick et al. (2). Briefly, spleens were mechanically dissociated into single-cell suspensions by being pressed through a fine metal screen. The cell suspensions were incubated in 25-ml cell culture flasks for 3 h at 37°C with brefeldin A (10 μg/ml; Epicentre Technologies, Madison, Wis.) to disaggregate the Golgi complex, enabling the intracellular accumulation of proteins. Lymphocytes were isolated by density centrifugation with Lympholyte (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) and washed in phosphate-buffered saline (PBS). Cells were counted to obtain 2×10^6 cells per sample and were incubated for 20 min in 10% fetal goat's serum as a blocking agent. Cells were then incubated in either fluorescein isothiocyanate-conjugated anti-rat αβ TCR or fluorescein isothiocyanate-conjugated anti-rat γδ TCR (Pharmingen, San Diego, Calif.) for 15 min at 25°C. The cells were then washed, and half were incubated with PE-conjugated anti-rat interleukin 2 receptor (IL2R) (Pharmingen) for 15 min at 25°C, washed, and resuspended in PBS for analysis. The other half of the cells were fixed in 100 μl of solution A (Cell Perm & Fix kit; Caltag Laboratories, Inc., San Francisco, Calif.) for 15 min at 25°C, washed again, resuspended in 50 μl of permeabilization solution B (Caltag) containing purified mouse anti-rat gamma interferon (Gibco BRL, Gaithersburg, Md.), and then incubated with phycoerythrin (PE)-conjugated anti-mouse immunoglobulin G antibody (Pharmingen) for 15 min at 25°C. Control assays for gamma interferon staining were additionally performed with two CLP rats and one anesthesia animal 3 days after CLP. Irrelevant mouse immunoglobulin G isotype antibody and recombinant rat gamma interferon blocking at 10 μg/ml demonstrated 3% staining and 98% blocking, respectively. Cell fluorescence was measured with a Becton Dickinson FACScan flow cytometer. IL2R and gamma interferon levels were measured with and without gating on $\alpha\beta^+$ or $\gamma\delta^+$ lymphocytes by side scatter and FL2. Flow cytometry data were analyzed by using Lysis II software. Results are expressed as means ± standard deviations for the five CLP rats minus the values for the five control rats at each time point. IL2R and gamma interferon values for αβ and γδ T cells were compared at each time point by using unpaired two-tailed *t* tests with statistical significance defined at the $P < 0.01$ level.

Mortalities following CLP were 0, 20, 0, and 20% at days 1, 2, 3, and 5, respectively. γδ T cells constituted approximately 3% of all lymphocytes, with no significant difference between control and CLP rats. CLP rats showed significant and similar increases in surface IL2R expression in both αβ and γδ T cells in comparison with control rats on postoperative day 3, with the median IL2R fluorescence in αβ T cells being significantly higher than in γδ T cells (133 versus 98; $P < 0.001$) (Fig. 1). On post-operative day 5, CLP rats maintained significantly increased IL2R expression and median fluorescence in αβ T cells but now demonstrated decreased IL2R expression in γδ T cells in comparison with results for control animals. The median IL2R fluorescence was significantly higher in αβ T cells than in γδ T cells at all time points following CLP.

CLP induced a significant increase in intracellular gamma

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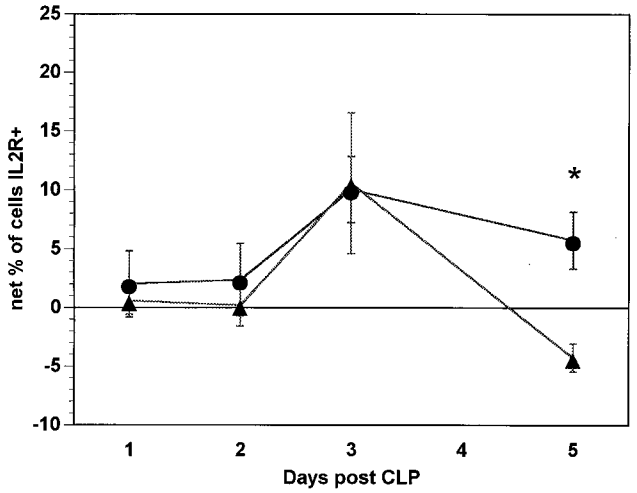

FIG. 1. Expression of surface IL2Rs in rat spleen αβ (●) and γδ (▲) T cells 1 to 5 days following CLP. Data are expressed as means ± standard deviations (error bars) for five CLP rats minus the means for five anesthesia-only control rats (negative datum points reflect lower values for CLP rats than for controls). *, $P < 0.05$ for αβ versus γδ T-cell subgroups.

interferon production over the level produced by control rats in both αβ and γδ T cells on postoperative day 3, but on postoperative day 5 this level only persisted in γδ T cells (Fig. 2). The magnitude of the difference in intracellular gamma interferon production levels between γδ T cells and αβ T cells on postoperative day 3 following CLP was marked (19 versus 4%; $P = 0.001$). The median gamma interferon fluorescence was also significantly higher in γδ T cells than in αβ T cells on postoperative days 1 and 3. It is of note that the percentage of γδ T cells producing intracellular gamma interferon was higher than the percentage of αβ T cells producing intracellular gamma interferon in both the control and the CLP groups on postoperative days 1, 3, and 5.

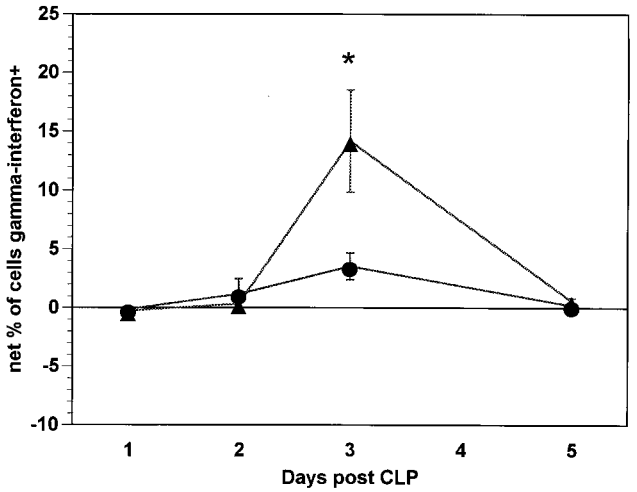

FIG. 2. Expression of intracellular gamma interferon in rat spleen αβ (●) and γδ (▲) T cells 1 to 5 days following CLP. Data are expressed as means ± standard deviations (error bars) for five CLP rats minus the means for five anesthesia-only control rats (negative datum points reflect lower values for CLP rats than for controls). *, $P < 0.05$ for αβ versus γδ T-cell subgroups.

The role of γδ T cells as early responders and modulators of the primary immune response to infection remains a subject of physiologic and clinical interest (1, 5). We measured the presence of intracellular gamma interferon in unstimulated cells in order to provide a minimally altered "snapshot" of in vivo intracellular cytokine production in response to peritonitis. We used surface IL2R expression as a parallel measure of cell activation (7). Our results demonstrate that both αβ and γδ T-cell populations exhibit an increase in expression of surface IL2R in response to peritonitis, although the IL2R response persisted only in αβ T cells. A more rapid decrease in surface IL2R expression in γδ T cells than in αβ T cells has not been previously reported and could reflect a shorter period of activation in γδ T cells or an early immunomodulatory response to limit further amplification of the immune response to infection once αβ T-cell activation is complete.

T cells produce gamma interferon in response to bacterial infection, and the presence of this cytokine during the early stages of infection is critical for the development of a strong Th1 T-cell response to infection (9). Our results demonstrate a significant increase in γδ T-cell intracellular gamma interferon levels in rats following CLP which persists through the fifth postoperative day. In contrast, αβ T-cells demonstrated a smaller increase in intracellular gamma interferon levels which did not persist. Thus, a fivefold-greater percentage of γδ T cells than of αβ T cells are producing gamma interferon at a higher cytokine level per cell during the early response to peritonitis. Our results are consistent with previous reports of γδ T cells producing gamma interferon in response to infection with *L. monocytogenes* (2, 5, 8) and group A streptococci (3). It has been suggested that the early and widespread production of gamma interferon by regional γδ T cells in response to infection directs an appropriate Th1 cellular immune response to peritonitis (2, 4). This hypothesis is supported by the observation that γδ T-cell-depleted mice exhibit an exaggerated bacterial multiplication following listerial infection (5). We conclude that γδ T cells may play an important role in the immune response to peritonitis by producing significant amounts of gamma interferon during the early phases of infection.

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