Developmental Interrelationship of Specific Lyt 123 and Lyt 1 Cell Sets in Expression of Antibacterial Immunity to *Listeria monocytogenes*

HELmut NÄHER,* UWE SPERLING, AND HELMUT HAHN

Institute of Medical Microbiology, Freie Universität Berlin, D-1000 Berlin 45, Federal Republic of Germany

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Lyt phenotypes of peritoneal exudate T lymphocytes from *Listeria monocytogenes*-immune mice were determined with respect to their capacity to confer protection upon local or systemic transfer. It is shown that, locally, Lyt 1 T cells provide protection, whereas only unselected native populations, containing adequate numbers of Lyt 123 T cells, were effective in transferring systemic protection. When Lyt 1 T cells were the crucial cell type (local transfer), admixture of Lyt 123-containing peritoneal exudate T-lymphocyte-enriched cells did not enhance protection, save for slight additive effects. Likewise, when Lyt 123-containing peritoneal exudate T lymphocyte-enriched cells were the crucial cell type (systemic transfer), admixture of various numbers of Lyt 1 cells was without marked effect. A change in Lyt phenotype from Lyt 123 to Lyt 1 paralleled by an increase of protective capacity upon local transfer was observed when sensitivity of early and late exudate T cells to anti-Lyt 2.2 antiserum plus complement treatment was investigated. The data suggest that (i) the propensity of specific T cells to enter exudates is associated with the Lyt 123 phenotype, (ii) the actual effector cell of antibacterial protection is an Lyt 1 T cell, and (iii) a developmental interrelation exists between Lyt 123 T cells and Lyt 1 T cells in T-cell-dependent protection of mice against *L. monocytogenes*.

As previously shown in this laboratory, specific Lyt 123 T cells are crucially involved in both protection and delayed-type hypersensitivity to the facultative intracellular bacterium *Listeria monocytogenes* and its antigens (9). This result was obtained when Lyt 123 T cells were injected systemically into cell recipients subsequently charged with *L. monocytogenes* infection or injected with soluble antigen, respectively.

On the other hand, in vitro experiments (7, 8) have yielded evidence that *Listeria*-specific T-cell functions, such as antigen-dependent interleukin release and specific T-cell proliferation, are functions of Lyt 1 T cells. Moreover, when cloned *Listeria*-specific T cells of the Lyt 1 phenotype were employed in adoptive transfer experiments, relatively high cell numbers were needed to successfully transfer protection as compared with heterogeneous peritoneal exudate T-lymphocyte-enriched cells (PETLEs) (6).

The discrepancies between the obligatory role of Lyt 123 cells in vivo and the in vitro performance of Lyt 1 T cells have prompted us to further analyze the relationship of these two cell phenotypes in protection against infection with facultative intracellular bacteria. Although Lyt 123 cells, when injected systemically, were confirmed to be crucial for protection, it was possible to achieve local transfer of protection using specific Lyt 1 T cells. It is concluded that a developmental relationship exists between the Lyt 123 and Lyt 1 T-cell subsets and that different homing patterns of the two subpopulations appear to account for the observed differences between the in vivo and in vitro data.

**MATERIALS AND METHODS**

**Mice.** C57BL/6 mice, 4 to 8 weeks old, were obtained from Bomholtgard, Ry, Denmark. Mice were sex matched for a given experiment.

**Bacteria.** *L. monocytogenes* EGD (50% lethal dose, $2 \times 10^5$) was kept virulent by continuous mouse passage. Suspensions of bacteria were prepared from 18-h tryptic soy broth (Oxoid, Wesel, Federal Republic of Germany) cultures of homogenates from infected mouse spleens. Bacteria were diluted in 0.15 M NaCl to appropriate concentrations for injection, and numbers of bacteria injected were confirmed by plate counts as previously described (13).

**Collection of peritoneal exudate cells and preparation of PETLEs.** Mice were injected intravenously (i.v.) with $5 \times 10^8$ live *L. monocytogenes* cells in a volume of 0.2 ml. On day 7, peritoneal exudates were induced with 1.5 ml of protease peptone (Difco Laboratories, Detroit, Mich.) injected intraperitoneally, and 3 days later peritoneal exudate cells were collected. PETLEs were obtained after incubation of peritoneal exudate cells on nylon wool columns by the method of Julius et al. (4).

**Adoptive transfer of antibacterial protection.** Recipient mice were injected either i.v. or subcutaneously (s.c.) (footpad) with cell preparations together with $5 \times 10^4$ *L. monocytogenes* cells in a final volume of 0.2 or 0.05 ml, respectively. Alternatively, recipients were infused i.v. with cells, and *L. monocytogenes* was injected s.c. Sixty hours later, spleens of i.v.-infected recipients were removed or infected footpads were cut off and homogenized. Samples of spleen or footpad homogenates (0.1 ml each) were plated at appropriate dilutions on tryptic soy agar (13). Colony counts were made after 24 h of incubation at 37°C.

**Treatment of PETLEs with antisera.** Monoclonal anti-Lyt 1.2 and anti-Lyt 2.2 antisera were commercially obtained (NEN, Dreieich, Federal Republic of Germany). For complement, rabbits were bled, and sera selected for low cytotoxicity against mouse thymocytes and high specific complement activity were used. To 1 ml of cell suspension (concentration, $10^8$/ml) 100 μl of antiserum was added, resulting in a final antibody dilution of 1:1,000. Cells were incubated at 4°C for 60 min, washed twice, and suspended in 1 ml of complement (final dilution, 1:8). The cells were then
RESULTS

Listeria-specific T cells of the Lyt 1 phenotype responsible for local transfer of protection and cells of the Lyt 123 phenotype responsible for systemic transfer of protection.

When 3 x 10⁶ Listeria-specific T cells selected for Lyt phenotype by treatment with anti-Lyt 2.2 or anti-Lyt 2.2 antisera plus complement were injected i.v. and recipients were charged with systemic infection, neither cell population was able to mediate adoptive protection, thus confirming earlier published results (9) (Fig. 1).

On the other hand, when T cells treated with anti-Lyt 2.2 antisera plus complement were injected locally, marked protection resulted in infected footpads (Fig. 2), whereas T cells treated with anti-Lyt 1.2 antisera plus complement failed to provide local protection (Fig. 2). In contradiction to data obtained after systemic transfer, treatment with anti-Lyt 2.2 antisera plus complement did not abolish the ability of PETLEs for local transfer of protection. The admixed cell preparations, as expected, yielded effects similar to those seen with the anti-Lyt 2.2 plus complement-treated cell population (Fig. 2). The failure of Lyt 2 cells (T cells left after treatment of PETLEs with anti-Lyt 1.2 antisera plus complement) to transfer local protection rules out contaminating macrophages as playing a role in adoptive transfer of local protection.

The requirement for different T-cell sets in local and systemic transfer of protection could be due to the route immune T cells have to take after transfer. To exclude the alternative possibility that different cellular requirements at different anatomical sites of bacterial implantation (spleen or footpad) account for the observed differences, the Lyt phenotype of specific T cells which, after systemic transfer, protected against a local infection in the footpad was determined (Fig. 3). Specific T cells treated with anti-Lyt 1.2 antisera plus complement or anti-Lyt 2.2 antisera plus complement did not provide local protection. Admixture of the selected T cells did not reconstitute the effect. Thus, Lyt 123 T cells are involved in systemic transfer of protection.

FIG. 1. Effect of anti-Lyt antisera plus complement treatment on the capacity of Listeria monocytogenes-immune PETLEs to transfer systemic protection. Unselected PETLEs (3 x 10⁶) treated with complement alone (A), equivalent numbers of anti-Lyt 1.2 antisera plus complement-treated PETLEs (B) or anti-Lyt 2.2 antisera plus complement-treated PETLEs (C), or a mixture of (B) and (C) and a ratio 1:1 (D) were injected i.v. together with 5 x 10⁶ L. monocytogenes cells. Means ± standard deviation of five mice per group are shown.

FIG. 3. Effect of anti-Lyt antisera plus complement treatment on the capacity of i.v.-injected Listeria monocytogenes-immune PETLEs to transfer protection against a bacterial inoculum into the footpad. Unselected PETLEs (10 x 10⁶) treated with complement alone (A), equivalent numbers of anti-Lyt 1.2 antiserum plus complement-treated PETLEs (B) or anti-Lyt 2.2 antiserum plus complement-treated PETLEs (C), or a mixture of (B) and (C) at a ratio 1:1 (D) were injected i.v. simultaneously with 5 x 10⁶ L. monocytogenes cells injected s.c. into one footpad. Means ± standard deviation of five mice per group are shown.
regardless of the site at which the immune reaction occurs.

Lack of communicative interrelations between Lyt 123 and Lyt 1 PETLEs. In admixture experiments (Fig. 4), i.v. injection of $2 \times 10^6$ Lyt 1 T cells (T cells left after treatment of PETLEs with anti-Lyt 2.2 antiserum plus complement) resulted in marginal protection (groups A, B, and C). In Fig. 1, group C, Lyt 2 T cells were eliminated after treatment of cells with anti-Lyt 2.2 antiserum plus complement since these Lyt 1 cells acted poorly in transferring protection systemically. Injection of unselected PETLEs in a dose-dependent fashion resulted in significant protection (groups D, E, and F), and this was not materially enhanced when $10^6$ Lyt 1 T cells were added to each injection group (groups G, H, and I). Vice versa, when the number of i.v.-injected specific PETLEs was kept constant at $10^6$ per mouse, admixture of Lyt 1 cells in numbers ranging from $0.5 \times 10^6$ to $2 \times 10^6$ per mouse did not appreciably change the degree of protection obtained. Thus, there was no indication that a communicative relationship, e.g., resulting in potentiating effects, plays a role in this system. In keeping with this notion, local transfer of Lyt 1 cells in a dose-dependent fashion resulted in substantial local protection (Fig. 5, groups A, B, and C). As expected, injection of $10^6$ unselected PETLEs also caused local protection (Fig. 5, group D). When $10^6$ unselected PETLEs were added to graded numbers of Lyt 1 T cells in the local transfer system (Fig. 5, groups E, F, and G), a similar pattern was observed as with groups A, B, and C, except for a parallel increase of protection. This is best explained by assuming that an additive effect is exerted by the admixed unselected PETLEs. Again, no synergistic effects were seen.

Change of Lyt phenotype over time in peritoneal exudates paralleled by an increase of local protective capacity. When 3 to $10^6$ PETLEs obtained from 1-, 3-, or 5-day-old peritoneal exudates were employed for local transfer of protection, cells harvested late protected better than earlier cells. In contrast, optimum systemic protection was obtained with PETLEs collected on day 3 after exudate induction and decreased thereafter (Fig. 6). Treatment with anti-Lyt 1.2 antiserum plus complement abrogated the capacity of T cells from 1-, 3-, and 5-day-old peritoneal exudates to protect locally. Treatment with anti-Lyt 2.2 antiserum plus complement abrogated the capacity of T cells to protect locally in 1-day-old peritoneal exudates almost completely, whereas little effect was observed with T cells from 3- and 5-day-old peritoneal exudates. This result indicates that the enhancement of local protective capacity cannot simply have resulted from loss of irrelevant cells during the 5-day period of the peritoneal inflammatory response, but that T cells of antibacterial immunity immigrate into an inflammatory exudate as Lyt 123 T cells and there mature to Lyt 1 cells, the latter being the actual effector cells. These obviously have lost their ability to migrate into inflammatory exudates.

DISCUSSION

Earlier work by North and Spitalny (16), McGregor and Logie (14), Koster et al. (10), and North et al. (15) has stressed the importance of homing patterns of effector T cells in the buildup of an effective defense against facultative intracellular bacteria. These authors have demonstrated that it is the recently formed, blast-like T cells which enter inflammatory exudates and there differentiate into more mature cells (10, 16). Later, with the discovery of Lyt differentiation antigens and the association of T-cell Lyt phenotypes with different functions (3), the Lyt phenotype of T cells essential in protection against facultative intracellular bacteria could be established. Thus, Lyt 123 T cells were found to be crucially involved in protection and delayed-type hypersensitivity to L. monocytogenes (9). This was confirmed by other authors using the brucellosis model (17). Also, in antivirus immunity, as exemplified by lymphocytic choriomeningitis virus infection of mice, Lyt 123 T cells are essential (19). In all models cited, the Lyt 123 T cells used in cell transfer experiments had been injected systemically.

However, when effector functions were studied in vitro, Lyt 1 T cells in the listeriosis model caused T-cell proliferation and proved to be active in interleukin (7, 8) as well as migration inhibitory factor and macrophage activation factor production (Sperling et al., submitted for publication).

Likewise, in the lymphocytic choriomeningitis virus infection model, the more mature Lyt 23 T cells were cytolytically active in vitro, yet, upon systemic transfer, they failed to eliminate virus from infected tissues (19). Moreover, when cloned T cells of the Lyt 1 or Lyt 23 phenotype, respectively, were injected systemically, comparatively high numbers, approaching those required when native unFractionated T cells were used, were needed for adoptive protection (6, 12). In view of these discrepancies between the requirement for specific Lyt 123 T cells in systemic transfer of protection and the performance of Lyt 1 and Lyt 23 T cells, respectively, in vitro, the hypothesis was developed by us that different migration patterns are associated with different Lyt phenotypes. Accordingly, Lyt 123 T cells would constitute the cell

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FIG. 5. Capacity of mixtures at different ratios of L. monocytogenes-immune PETLEs treated with anti-Lyt 2.2 antiserum plus complement and unselected PETLEs to transfer local protection. Means ± standard deviation of five mice per group are shown.

FIG. 6. Capacity of L. monocytogenes-immune PETLEs obtained on the days indicated from exudates to confer local (square) or systemic (circles) protection. Effect of anti-Lyt 1.2 antiserum plus complement treatment (triangle) or of anti-Lyt 2.2 antiserum plus complement treatment (circle) on the capacity of these PETLEs to confer local protection is shown. Means ± standard deviation of five mice per group are shown.
type capable of entering exudates, and Lyt 1 T cells and Lyt 23 T cells, respectively, represent the actual effector cell population after having been differentiated from Lyt 123 precursors in inflammatory sites.

In this study, employing local and systemic transfer of adoptive protection, we have demonstrated that locally, Lyt 1 T cells provide protection, whereas systemic transfer is critically dependent on Lyt 123 T cells. Differences with respect to the Lyt phenotype of T cells do not reflect different cellular requirements depending on whether systemic or local infection with L. monocytogenes was done, since, regardless of the site of bacterial implantation, i.e., transfer of protection requires Lyt 123 T cells (Fig. 1 and 3). The propensity of specific Lyt 123 T cells to enter exudates seems therefore to account for the differences. Thus, not only the blast-like state, but the Lyt 123 phenotype as well, appears to be associated with the propensity of effector T cells to enter exudates.

Since the relationship between Lyt 123 and Lyt 1 T cells can be either developmental or communicative (18), the experiments summarized in Fig. 4, 5, and 6 were done. No potentiation of protective capacity was seen when Lyt 123- and Lyt 1-containing cell populations were admixed. Thus, when Lyt 1 cells were the essential cell type (local transfer), admixture of Lyt 123-containing PETLEs did not materially enhance protection, save for slight additive effects. Likewise, when i.v.-injected Lyt 123-containing PETLEs were the essential cells (systemic transfer), admixture of various numbers of Lyt 1 T cells was without marked effect. Thus, in protection against L. monocytogenes, communicative interrelations between Lyt 123 T cells and Lyt 1 T cells do not appear to play a role in the expression phase of immunity.

On the other hand, there was evidence for developmental interrelations. Protection by local transfer of early (day 1) exudate T cells was sensitive to both anti-Lyt 1.2 antiserum and anti-Lyt 2.2 antiserum and therefore due to Lyt 123 T cells, whereas late (day 5) exudate T cells were sensitive to anti-Lyt 1.2 antiserum but resistant to anti-Lyt 2.2 antiserum and therefore must have consisted of Lyt 1 T cells. This is in keeping with the increase over time in refractoriness of peritoneal exudate cells to mitotic poisons reported earlier (16). A change in the Lyt phenotype of PETLEs was paralleled by an increased protective capacity in the local transfer system, whereas late exudate T cells were poor performers in systemic protection. This is in keeping with the notion that the Lyt 1 T cell is the actual effector cell of antibacterial protection, as outlined by the results of in vitro experiments (7, 8; Sperling et al., submitted).

Effector cells in cell-mediated immunity to facultative intracellular bacteria and specific cytotoxicity against tumor, virus-infected, or histo-incompatible cells originate from Lyt 123 T cells (2, 20). Lyt 123 T cells have been considered by many as precursors in the sense that they had yet to acquire a functional state. Our data suggest that the propensity of Lyt 123 T cells to enter inflammatory exudates is an important independent function toward immune reactions in vivo.

This notion becomes apparent in the relatively limited capacity of cloned Lyt 1 or Lyt 23 T cells to exert their functions upon systemic transfer in vivo (6, 12). Substituting this, L. monocytogenes-specific T-cell clones, upon local transfer, provide protection at distinctly lower cell numbers than heterogeneous T-cell populations (5).

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