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T-Cell Subsets in Delayed-Type Hypersensitivity, Protection, and Granuloma Formation in Primary and Secondary *Listeria* Infection in Mice: Superior Role of Lyt-2$^+$ Cells in Acquired Immunity

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Immunity to *Listeria monocytogenes* was studied in mice treated with rat monoclonal antibodies (MAbs) specific for the Thy-1.2, L3T4, and Lyt-2 T-cell markers. Three characteristic T-cell-mediated phenomena were investigated. Delayed-type hypersensitivity (DTH) to listerial antigen was totally abolished in mice treated with anti-Thy-1.2 or anti-L3T4 MAbs, whereas anti-Lyt-2 MAb treatment had no effect, regardless of whether the MAb was given during the induction or the expression of DTH. On the other hand, the elimination of bacteria from the spleens of infected animals was inhibited only by the application of either anti-Thy-1.2 MAb or anti-Lyt-2 MAb. This could be shown most impressively during the secondary infection of immune mice with a normally lethal dose of listeriae. In this situation, treatment with anti-Lyt-2 MAb sufficed to completely abolish immunologic memory, whereas anti-L3T4 MAb had only a marginal effect on antibacterial protection. However, the accelerated development of mononuclear cell foci in the livers of immune mice was inhibited by the application of both anti-L3T4 MAb and anti-Lyt-2 MAb. It is concluded that in murine listeriosis, DTH and acquired immunity to reinfection are dissociable phenomena. Although DTH is a function of L3T4$^+$ T lymphocytes, Lyt-2$^+$ T cells are necessary and sufficient for the expression of acquired resistance to *L. monocytogenes*. The roles of the different T-cell subsets in granuloma formation warrant further investigation.

Infections caused by facultative intracellular bacteria are characterized by macrophage activation, delayed-type hypersensitivity (DTH), granulomatous lesions in infected organs, and long-term immunity to reinfection (10). These phenomena are all mediated by thymus-dependent lymphocytes (2, 18, 23). The close temporal relationship between the emergence of DTH and the clearance of bacteria from infected tissues, as well as the cellular nature of both phenomena, have long been taken to indicate that allergy and protection both depend on the same mechanisms (19), although a few authors (11, 24) have stated that DTH and protection are not causally related.

The discovery that T cells can be assigned to different subsets has provided an opportunity to assess whether protective immunity and DTH are mediated by the same or different types of T cells. Data obtained with in vitro systems originally led to the assumption that T lymphocytes of the helper phenotype play a predominant role in both phenomena because of their ability to secrete migration inhibition factor and macrophage activation factor. In addition, cloned listeria-specific T cells of the Lyt-1$^+$23$^-$ phenotype have been shown not only to mediate these in vitro functions (25) but also to mediate DTH in a local transfer system and provide some degree of systemic protection in vivo (12, 13).

The concept of an exclusive role for Lyt-1$^+$ helper T cells in cell-mediated antibacterial immunity, however, could not be reconciled with the earlier observation (17) that in an adoptive cell transfer system, the lysis of selected T-cell subsets by antibodies against either the Lyt-1 or the Lyt-23 markers abolished the capacity of listeria-immune T cells to transfer protection. This finding was subsequently substantiated by in vitro preselection using antibodies against the mutually exclusive markers L3T4 and Lyt-2 (16). Experiments by Cheers and Sandrin (3), who showed that protection against *Listeria monocytogenes* in mice is a class-I-restricted phenomenon, made an important role for Lyt-2$^+$ T cells in protection likely and cast doubt on the concept of an exclusive H-2I restriction of immunity to listeriosis as postulated by Zinkernagel et al. (26). Trying to solve these discrepancies, Näher et al. (21) entertained the possibility that, whereas class II-restricted helper T cells are primarily responsible for macrophage activation, as shown by Farr et al. (8, 9), class I-restricted Lyt-2$^+$ T cells are critically involved in the induction of granulomas. Indeed, granuloma formation was shown by these investigators (21) to be an H-2K-restricted, Lyt-2$^+$ T-cell-dependent phenomenon.

Most of the data in the experimental model system of murine listeriosis had been obtained by the passive transfer of in vitro-depleted or positively selected T-cell subsets from immune donors to syngeneic naive recipients and thus may not reflect the actual situation of actively immunized hosts deficient in one T-cell subset. We have, therefore, reevaluated the relative contributions of T-cell subpopulations to DTH, granuloma formation, and bacterial clearance by the approach of in vivo serotherapy with monoclonal antibodies (MAbs) against T-cell surface markers recently shown to be effective in depleting T-cell subsets in vivo (6). Our data clearly demonstrate that DTH and protective immunity are critically dependent on phenotypically distinct T-cell subsets; DTH to listerial antigen is attributable to the T-helper subset, whereas the capacity for effective bacterial clearance, especially in animals mounting a secondary immune response during reinfection, is vested almost entirely in the Lyt-2$^+$ T-cell pool.

MATERIALS AND METHODS

Mice. Female C57BL/6 mice raised in our own breeding facilities were used at the age of 10 to 16 weeks.

Bacteria and bacterial antigens. *L. monocytogenes* EGD was kept virulent by continuous mouse passages. Cultures

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were obtained by growing samples of spleen homogenates from infected mice overnight in tryptic soy broth (Oxoid, Wesel, Federal Republic of Germany), dispensed in vials of 0.2-ml lots, and stored at −70°C until use. For each experiment, the appropriate number of vials from *L. monocytogenes* stock suspension was thawed and diluted in phosphate-buffered saline (PBS) for intravenous inoculation. Soluble antigen was prepared by culturing *L. monocytogenes* in tryptic soy broth for 18 h, washing it in PBS, and subsequently subjecting it to ultrasonication. One gram (wet weight) of bacterial cells was suspended in 10 ml of PBS and sonicated five times for 1 min each time (87.5% output, degree 7 on a sonifier [model S-125; Branson Sonic Power Co., Danbury, Conn.]) on ice. The sonicated suspension was centrifuged at 39,000 × *g* for 50 min, and the supernatant was filter sterilized (pore size, 0.45 μm) and stored at −20°C at a dilution of 1:100 in PBS.

MAbs. Five rat MAbs, obtained from ascitic fluid from pristane-primed nude mice intraperitoneally injected with the relevant hybridoma line, were used. The hybridoma lines 30-H12 (anti-Thy-1.2, rat immunoglobulin G2b [IgG2b]), 2.43 (anti-Lyt-2, rat IgG2b), and GK 1.5 (anti-L3T4, rat IgG2b) were purchased from the American Type Culture Collection, Rockville, Md. (Tumor Immunology Bank [TIB] 107, 210, and 207). The MAb designated 23-7 (unrelated specificity, rat IgG) was kindly provided by T. Diamantstein, Department of Immunology, Freie Universität Berlin, Berlin, Federal Republic of Germany, and served as a control for nonspecific effects of ascitic fluid. Ascitic fluid was cleared and delipified by centrifugation. The concentration of antibodies was determined by an enzyme-linked immunosorbent assay with specificity for rat IgG and calculated with a commercially available standard of rat IgG2b (catalog no. 1330, Becton Dickinson and Co., Paramus, N.J.) as a reference. Dilutions containing 500 and 200 μg of MAb per ml in PBS were stored at −20°C until use. All experiments were performed with portions of the same batch of the respective MAb preparation. MAbs were injected into the peritoneal cavity on days −3 and 0 and on every other day until termination of the experiment.

Infection with *L. monocytogenes*. Primary infection with *L. monocytogenes* was performed by an intravenous injection of 3×10^4 viable bacteria in a volume of 0.2 ml of PBS. Bacterial growth in the spleen was determined on day 8 postinfection by plating 10-fold serial dilutions of the spleen homogenates on tryptic soy agar. The detection limit of this procedure was 10^2 listeria organisms per spleen. Colonies were counted after 24 h of incubation at 37°C. For secondary infection, mice were injected with 10^6 listeriae on day 28 after primary infection. The number of bacteria in the spleen was determined 48 h after reinfection. Numbers of bacteria are given as $\log_{10}$ figures.

DTH response to *L. monocytogenes* antigen. For determination of DTH responsiveness, 50 μl of soluble *L. monocytogenes* antigen diluted 1:100 in PBS was injected into the left hind footpads of mice on day 7 of the primary infection. Twenty-four hours later, thicknesses of the left and right footpads of individual mice were measured with a dial-gauge caliper (Kröplin, Schlüchtern, Federal Republic of Germany). DTH was calculated by subtracting the mean differences between left and right footpad thicknesses of injected normal control mice from those of immune mice. Nonspecific footpad swelling never exceeded 0.2 mm.

Adoptive transfer of immunity. Adoptive transfer of immunity was carried out by infusing spleen cells from donor mice immunized 10 days previously into syngeneic naive recipients that had been infected with 5×10^4 listeriae 1 h earlier. Donor spleens were gently teased with two tweezers to prepare single-cell suspensions. The cells were then washed twice in PBS, and lysis of erythrocytes was performed by hypotonic shock for 15 s. Cells were washed again and divided into five groups, each containing five splenic equivalents (5×10^8 spleen cells) in 2 ml of either PBS containing the appropriate MAb at a concentration of 500 μg/ml or PBS alone. Cells were incubated for 1 h at 4°C before the suspensions were injected intravenously (400 μl per mouse). The numbers of viable listeriae in spleens were determined 3 days after bacterial challenge.

Granuloma formation. Livers were removed in parallel with spleens for histologic examination. Organs were Formalin fixed, embedded in paraffin, sectioned (3 μm), and stained with hematoxylin and eosin by standard procedures. Cellular infiltrations were reported according to the predominant cell type.

Analysis of T-cell subsets in spleens of MAb-treated mice. Spleen cells from infected and noninfected mice treated with MAbs in vivo were incubated with anti-Thy-1.2, anti-L3T4, anti-Lyt-2, and 23-7 (control antibody) MAbs (25 μg/ml) in 100 μl of PBS supplemented with 5% fetal calf serum and 0.02% NaN_3 on ice for 30 min. After being washed, cells were treated with fluorescein isothiocyanate-labeled goat anti-rat IgG (code no. 6270, Medac) for 30 min on ice. Afterwards, cells were washed three times and analyzed on a cytofluorograph (Becton Dickinson).

Each experiment was repeated at least once and accepted as valid only if the trials showed similar results.

All data were analyzed statistically by Student's *t* test.

RESULTS

Effects of systemic application of MAbs before the induction of DTH to soluble listerial antigen. To determine the relative contributions of different T-lymphocyte subsets in DTH to listerial antigen, mice were treated with MAbs to T-cell surface markers during primary infection. The results of one representative experiment are shown in Fig. 1. DTH could not be elicited in animals treated with anti-Thy-1.2 or anti-L3T4 MAb during primary infection, whereas the administration of anti-Lyt-2 MAb had no effect on the response compared with the effect of no treatment or treatment with control ascitic fluid. Similar results were obtained when MAbs were administered during the expression of DTH only (data not shown).

Effects of MAbs on the clearance of listeriae from spleens of primarily infected animals. Since it was possible to abolish DTH reactions by the in vivo administration of anti-L3T4 MAb, but not by anti-Lyt-2 MAb, we sought to determine whether the course of a primary infection was affected in a corresponding manner. The clearance of listeriae from spleens of infected animals was strongly inhibited when the latter had been treated with anti-Thy-1.2 MAb, the mean bacterial count being approximately 3,000-fold higher than in the control group (Fig. 2). The application of anti-Lyt-2 MAb alone still had a considerable effect (resulting in an 80-fold increase of bacterial load), whereas treatment with anti-L3T4 MAb alone had only a marginal influence on bacterial clearance. Moreover, the combined application of anti-Lyt-2 and anti-L3T4 MAbs only slightly enhanced the effect of anti-Lyt-2 alone and was far from attaining the level of inhibition caused by anti-Thy-1.2.

Effects of MAbs on anamnestic immunity to *L. monocytogenes*. The following experiments were designed to evaluate

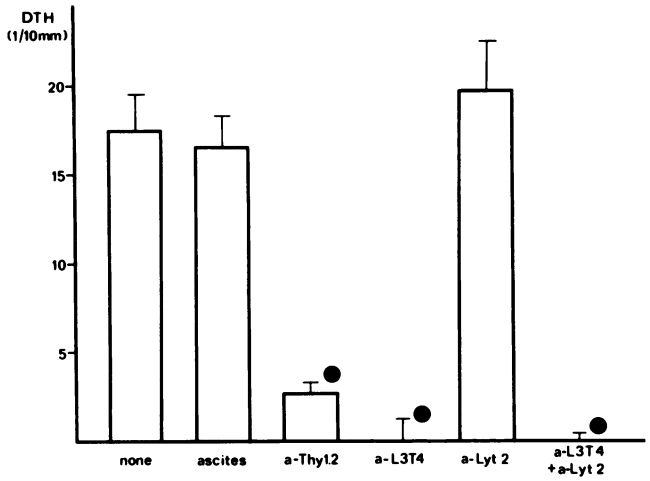

FIG. 1. Effects of MAbs on DTH to listerial antigen. Mice infected with 3×10^4 live listeriae on day 0 were either left untreated or treated with control antibody, anti-Thy-1.2 MAb, anti-L3T4 MAb, or anti-Lyt-2.2 MAb by injection of 500 μg of MAb intraperitoneally on day −3 and 200 μg of MAb on days 0, +2, +4, and +6. DTH was elicited on day +7 by intrafootpad injection of 50 μl of soluble listerial antigen and was examined 24 h later. Results indicate means of specific footpad swelling of five mice each ± standard error of the mean. Significant differences between treated and control mice ($P \leq 0.005$) are indicated (●).

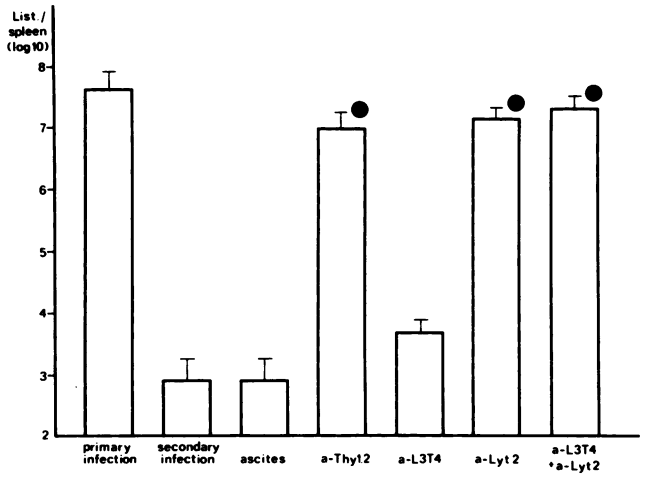

FIG. 3. Effects of MAbs on the clearance of bacteria from spleens of secondarily infected animals. Mice were immunized with 3×10^4 live listeriae and reinfected 4 weeks later with an acute lethal dose (1×10^6) of viable listeriae. Three days before, and on the day of secondary infection, mice were either not treated or treated with an intraperitoneal injection of 500 and 200 μg of the appropriate MAb, respectively. Untreated primarily infected mice served as an additional control. Results are expressed as the mean numbers of listeria ($\log_{10}$ CFU) recovered from the spleens of five mice per group 2 days after reinfection. Significant differences between treated and control mice ($P \leq 0.005$) are indicated (●).

the roles of T-cell subsets during the recall of immunologic memory to *L. monocytogenes* (Fig. 3). Immunized mice were protected against an otherwise lethal challenge infection, the bacterial load in spleens of such mice 48 h after infection being approximately 0.002% of that in primarily infected animals. The eradication of listeriae was severely impaired not only in mice treated with anti-Thy-1.2, but also in those treated with anti-Lyt-2 MAb; bacterial numbers in the spleens of these animals approximated the levels seen in primarily infected animals. In contradistinction, the clearance of listeriae from the spleens of mice treated with anti-L3T4 MAb essentially remained unimpaired, although the same treatment resulted in complete abolition of the normally accelerated expression of DTH during reinfection (data not shown). In conclusion, Lyt-2^+ T cells appear to play the supreme role in the expression of protective immunity to *L. monocytogenes* during the response to reinfection.

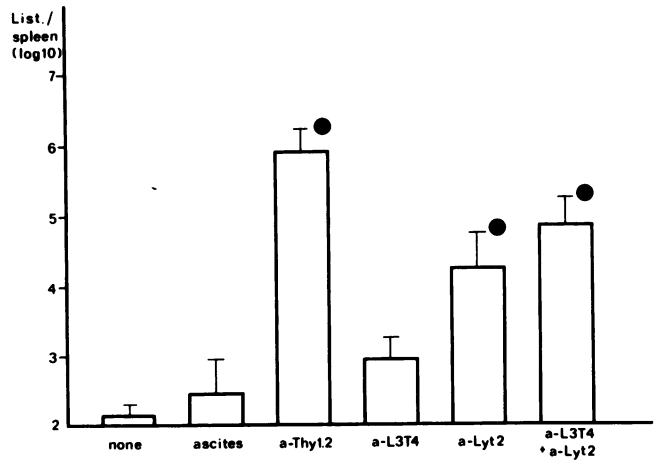

FIG. 2. Effects of MAbs on the clearance of listeriae from spleens of primarily infected animals. Mice infected with 3×10^4 live listeriae on day 0 were treated with the appropriate MAb on days −3, 0, +2, +4, and +6 (see the legend to Fig. 1). Bacterial counts in the spleens were determined on day 8. Results indicate the mean numbers of listeriae ($\log_{10}$ CFU) recovered from the spleens of five mice per group. Significant differences between treated and control mice ($P \leq 0.005$) are indicated (●).

The efficiency of MAb treatments was evaluated by cytofluorometric analysis of the proportion of the relevant T-cell subsets in the spleens of treated versus untreated mice. The described regimen (500 μg of MAb per mouse intraperitoneally followed 3 days later by 200 μg of MAb) resulted in the complete specific depletion of T-cell subsets in normal (data not shown) and infected (Fig. 4) mice.

Because of the unexpected finding that anti-L3T4 treatment in vivo had little effect on antilisterial immunity in primary and secondary infections, we next analyzed the influence of anti-Thy-1.2, anti-L3T4, and anti-Lyt-2 MAbs on the protective capacity of adoptively transferred immune spleen cells from mice with a 10-day-old infection. Appropriately treated spleen cells were incubated for 1 h in vitro at 4°C with the appropriate MAb without any further manipulation such as cell separation or complement treatment. Spleen equivalents were intravenously injected into syngeneic recipients which had received 5×10^4 viable listeriae 1 h previously. The results are shown in Fig. 5. Recipients of PBS-treated immune spleen cells were as well protected as mice which had been actively immunized 10 days previously, whereas similarly infected mice receiving spleen cells from noninfected donors showed no protection compared with primarily infected animals. As expected, the protective capacity of transferred cells was totally abolished by treatment with anti-Thy-1.2 MAb. Again, the reduction of the protective capacity of immune spleen cells by treatment with anti-L3T4 MAb was very small compared with the marked

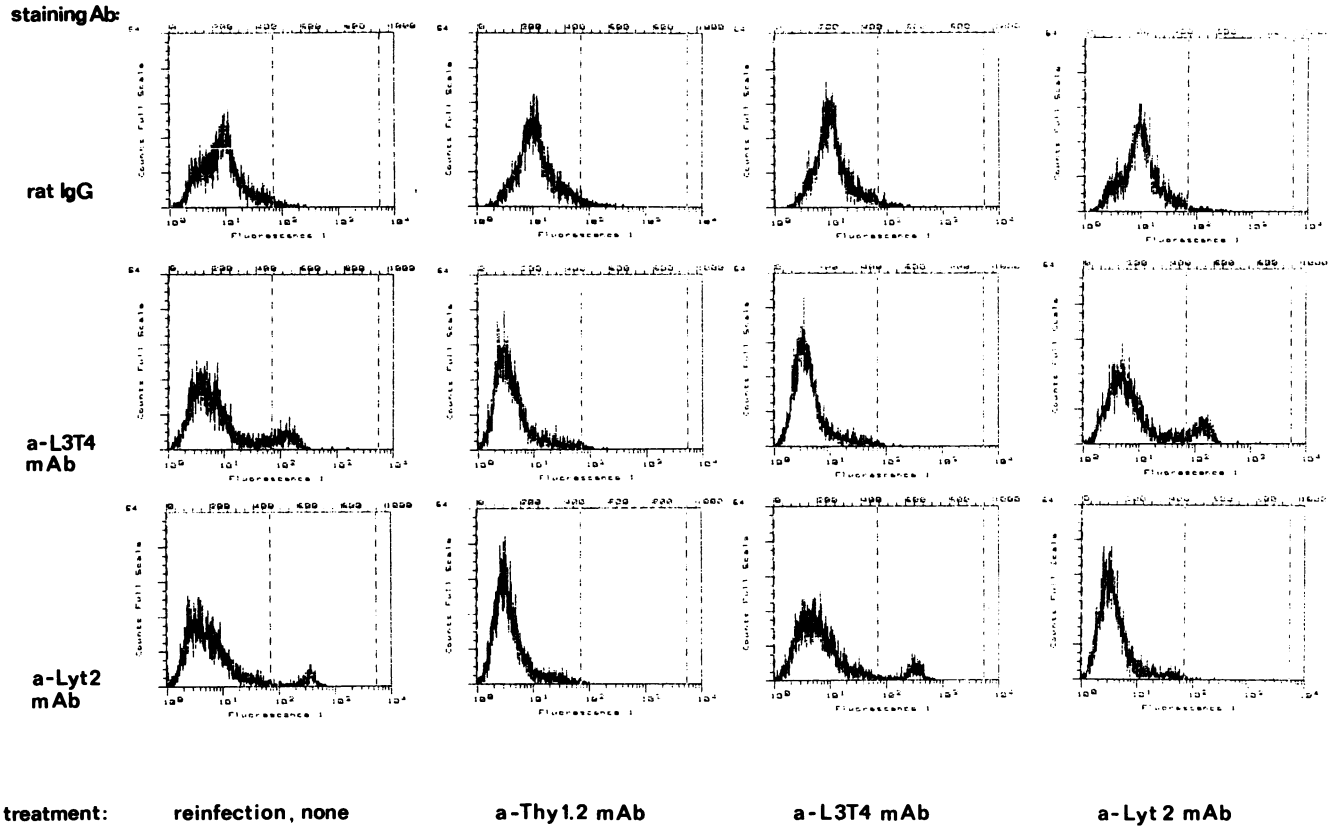

FIG. 4. Efficiency of T-cell subset depletion by MAb treatment of listeria-infected mice assayed by cytofluorometric analysis. Four-week immune mice were treated with MAb on days −3 and 0 of reinfection with a normally lethal dose of listeriae. Two days later, spleens were removed and spleen cells were analyzed for T-cell marker expression with a fluorescence-activated cell sorter. Ab, Antibody.

effect of anti-Lyt-2 MAb treatment. The combined treatment with both anti-L3T4 and anti-Lyt-2 MAbs only slightly enhanced the effect of treatment with anti-Lyt-2 alone.

Effects of MAbs on accelerated granuloma formation in immune mice. Granuloma formation is an essential feature of immunity to facultative intracellular bacteria. We therefore investigated the effect of MAb treatment on the composition of listeria-induced cellular infiltrations in the livers of secondarily infected animals. The predominant lesion in early primary infection consisted of loosely arranged polymorphonuclear infiltrates, whereas in immune animals the characteristic lesion was a compact and orderly arranged infiltrate of mononuclear cells (Table 1). As expected, the typical lesions in the livers of immune animals treated with anti-Thy-1.2 MAb looked identical to those in primarily infected mice. Treatment with either anti-L3T4 or anti-Lyt-2 MAb also resulted in a severe reduction in the capacity of the animals to form mononuclear foci at the usual accelerated rate. This observation provided evidence that both T-cell subsets must cooperate in granuloma formation in nonlymphoid tissues.

DISCUSSION

This paper summarizes experiments done with the aim of assessing the differential role of T-cell subsets in immunity to *L. monocytogenes* in mice. Rat MAbs with specificity to Thy-1.2, L3T4, and Lyt-2 were administered in vivo to determine the relative contributions of T-cell subsets to DTH, protection, and granuloma formation. DTH was sup-

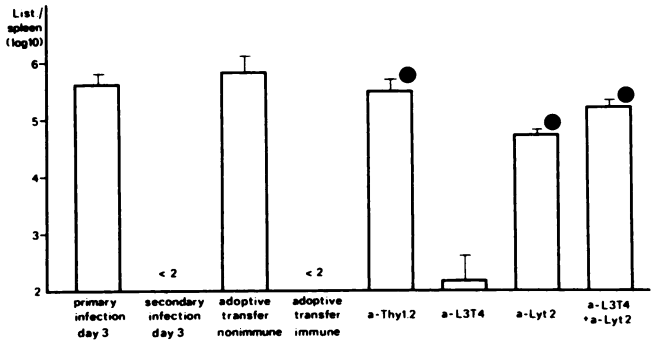

FIG. 5. Effects of MAbs on the protective capacity of transferred spleen equivalents of immune mice. Ten days after immunization of mice with 5×10^3 viable listeriae, spleens from these and noninfected control donors were removed. Spleen cells were suspended in ascitic fluid dilutions in either PBS containing 500 μg of the appropriate MAb per ml or PBS alone and were incubated for 1 h at 4°C. Cell recipients were intravenously infected with 5×10^4 viable listeriae, and 1 h later received MAb-treated spleen cells in a volume of 0.4 ml intravenously together with 200 μg of the appropriate MAb. One spleen equivalent per mouse (nonimmune, 0.7×10^8 spleen cells per mouse; immune, 1.3×10^8 spleen cells per mouse) was transferred. Results are given as the mean numbers of listeriae ($\log_{10}$ CFU) recovered from the spleens of five mice 3 days after transfer. Significant differences between treated and nontreated mice ($P \leq 0.005$) are indicated (●).

TABLE 1. Effects of MAb on the composition of listeria-induced cellular infiltrations in the livers of secondarily infected mice[a]

Infection type and treatment	Infiltration by:	
	Polymorphonuclear cells	Mononuclear cells
Primary	+++	0
Secondary	(+)	+++
Control ascites	(+)	+++
Anti-Thy-1.2	+++	0
Anti-L3T4	+	(+)
Anti-Lyt-2	+	++

[a] Cellular infiltrations were reported according to the predominant cell type, and a semiquantitative scale ranging from (+) (lowest) to +++ (highest) was used to represent the number of lesions per section.

pressed by the application of either anti-Thy-1.2 or anti-L3T4 MAb, whereas anti-Lyt-2 MAb had no effect or even slightly increased DTH. These results are in keeping with results in previously published reports on related systems, such as in the rat model of listeriosis (5) and mycobacterial infections in mice (24), thus strengthening the view that DTH reactions, including those in murine listeriosis, are exclusively mediated by T cells of the helper phenotype. Moreover, Kaufmann and Hahn (13) provided evidence that Lyt-1^+23^- *L. monocytogenes*-specific T cells are capable of conferring DTH even at the clonal level. In addition, in testing a panel of *Mycobacterium lepraemurium*-specific T-cell clones of various phenotypes, Hussein et al. (11) demonstrated that only the $L3T4^+$ clones were capable of conferring DTH.

The interrelatedness of DTH and protection against facultative intracellular bacteria has long been a subject of considerable controversy. The fact that animals can be desensitized without loss of resistance or, conversely, can be rendered hypersensitive without causing a corresponding increase in resistance argued against the widely accepted view that both phenomena depend on the same mechanism (20). Our finding that DTH can be abolished in vivo by serotherapy with anti-L3T4 MAb but not with anti-Lyt-2 MAb provided the opportunity to investigate whether DTH and protection are indeed mediated by the same or different T cells. Our data demonstrate that in primary infection, bacterial clearance is inhibited by anti-Thy-1.2 MAb treatment and, to a lesser extent, by anti-Lyt-2 MAb treatment. Surprisingly, anti-L3T4 MAb treatment had no significant effect. Even when given together with anti-Lyt-2 MAb, anti-L3T4 MAb was far from approaching the inhibitory effect of anti-Thy-1.2 alone. Treatment with MAbs shortly before reinfection of animals immunized 4 weeks previously yielded a comparable pattern. However, the effect of the anti-Lyt-2 MAb treatment was much more pronounced. Both anti-Thy-1.2 and anti-Lyt-2 MAb treatment completely abolished established protective immunity, whereas anti-L3T4 MAb treatment had a negligible effect. It is tempting to speculate that the difference in the magnitude of the observed effect of anti-Lyt-2 MAb treatment compared with that of anti-Thy-1.2 MAb treatment on bacterial clearance during primary versus secondary infection is based on maturation events occurring during primary infection and resulting in the enhanced expression of the Lyt-2 molecule on the surface of Thy-1^+ antigen-specific cells.

Further evidence for the superior role of Lyt-2^+ cells in antilisterial protection was obtained by transferring spleen equivalents from immune donors treated only by coating the cells with the appropriate MAb in vitro. In this experiment, the very strong protective capacity of splenic equivalents was abolished by anti-Thy-1.2 or anti-Lyt-2 MAb treatment, whereas there was only a slight inhibition by anti-L3T4 MAb treatment. Likewise, in the rat model of listeriosis, Chen-Woan et al. (4) have shown that the mediators of acquired resistance are encompassed within an $OX8^+$ T-cell subset equivalent to murine Lyt-2^+ T cells and that $W3/25^+$ helper cells contribute little if anything to the clearance of listeriae after adoptive transfer of immunity.

These data are in keeping with recent evidence provided by Bishop and Hinrichs (1), who also reported that adoptive transfer of antilisterial resistance by large numbers of listeria-immune T cells was markedly reduced only when spleen cells were pretreated with anti-Lyt-2 MAb and complement but not with anti-L3T4 MAb. When lower numbers of cells were transferred, a clear synergistic effect could be shown. In our view, this latter finding provides an explanation for the otherwise conflicting results, reported by Kaufmann et al. (16), who transferred low numbers of in vitro-preselected immune peritoneal exudate T-lymphocyte-enriched cells and came to the conclusion that treatment with either anti-L3T4 or anti-Lyt-2 MAb completely eradicates the protective capacity of the transferred cells. These findings seem to point to a role for a quantitative factor in protection apart from the qualitative requirement for specific Lyt-2^+ cells. Thus, it could be argued that whenever the absolute number of immune T cells is low, the Lyt-2^+ subset alone is incapable of mediating protection, possibly because additional interleukin-2 generated by the helper cells amplifies the functions of the critical Lyt-2^+ cell. Once a threshold number of Lyt-2^+ cells is present, only a small augmentation of its protective capacity is attained by additional helper T cells.

At the clonal level, evidence for a superior role of an Lyt-2^+ cell in protective immunity is as yet only anecdotal. Local protection in *M. lepraemurium* infections by transfer of a Lyt-2^+ clone, but not by transfer of $L3T4^+$ clones, has been reported (11). On the other hand, Kaufmann et al. (15) have shown local and systemic protection after adoptive immunization with high numbers of cloned $L3T4^+$ T cells specific for *L. monocytogenes*. These authors have also established Lyt-2^+ T-cell clones specific for *L. monocytogenes* (14), but no data on the systemic protective capacities of these clones are available to date. For its in vitro functions (such as gamma interferon production) to occur, the described Lyt-2^+ T-cell clone requires the presence of interleukin-2 in the culture medium and likewise may need considerable quantities of interleukin-2 for its in vivo functions.

Finally, on the basis of the observation made with nude mice (7) that despite strong macrophage activation, adequate protection cannot be achieved in the absence of granuloma formation, we investigated the effects of MAb treatment on this essential feature of the host response. The examination of liver sections from immune animals 48 h after reinfection showed complete absence of mononuclear foci in anti-Thy-1.2-treated animals. The typical listeria-induced lesions in such treated animals were polymorphonuclear infiltrations similar to the ones that can be observed in the very early phase of primary infection. This kind of infiltration also persisted in both anti-Lyt-2- and anti-L3T4-treated animals, although the formation of mononuclear cell foci as seen in immune mice was not totally suppressed in either group (Table 1). These data suggest a synergy of both T-cell subsets in granuloma formation, at least in nonlymphoid organs, which is the subject of a forthcoming paper

(M. E. A. Mielke et al., manuscript in preparation). This assumption is consistent with immunohistological findings reported by Näher et al. (22), who examined the typical round-cell infiltrations in adoptively immunized mice challenged with *L. monocytogenes* and demonstrated that T lymphocytes of both phenotypes were present. This morphological finding seems to be at variance with published data (21) which suggest that H-2K-restricted Lyt-2^+ T cells are necessary and sufficient for granuloma formation. However, this result was based on adoptive transfer experiments performed with animals compatible at the K locus only but incompatible at all other loci of the *H-2* gene complex, including I-A, so that an allogeneic effect may have circumvented the need for bacterial antigen to induce helper cell activity in the recipient.

In summary, we propose the following role for T-cell subsets in murine listeriosis. The cell ultimately responsible for bacterial clearance, especially during recall of established immunity, is an Lyt-2^+ listeria-specific T cell. In situations in which its absolute number is low, such as during early primary infection or after transfer of low numbers of cells, its function seems to be dependent on cooperation with helper cells. The concomitant DTH response, usually measured by skin reaction, which is itself entirely dependent on L3T4$^+$ cells, could be a reflection of a function of these cells in focusing Lyt-2^+ cells (e.g., at the site of granuloma formation in nonlymphoid tissues).

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