Production of Macrophage-Activating and Migration-Inhibition Factors In Vitro by Serologically Selected and Cloned *Listeria monocytogenes*-Specific T Cells of the $\text{Lyt } 1^{+}2^{-}$ Phenotype

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Lyt-selected *Listeria*-immune T lymphocytes from peritoneal exudates and cloned T cells were cocultured with heat-killed *Listeria* and peritoneal macrophages from nonimmune donors. Supernatants were assayed for: (i) activation of macrophages for tumoristatic and tumoricidal activity via macrophage-activating factors and (ii) migration-inhibition factor activity. Peptone-induced peritoneal macrophages were activated by incubation with the supernatants for 24 h. For examination of cytotoxic activity, ^{51}Cr -labeled EL₄ tumor cells were subsequently added, and ^{51}Cr release was determined. Cytostatic activity was measured by adding unlabeled EL₄ tumor cells to the pretreated macrophages and determining [^3H]thymidine incorporation 24 h later. Migration-inhibition factor production was examined in an agar microdroplet assay. Only *Listeria*-specific T cells of the phenotype $\text{Lyt } 1^{+}2^{-}$ proved active in these assays, whereas T cells of the phenotype $\text{Lyt } 1^{-}2^{+}$ were not active. When T-cell clones were used, a single clone was capable of inducing macrophage-activating and migration-inhibition factor production at cell concentrations of ca. $10^3/\text{ml}$.

The current concept of immunity to facultative intracellular bacteria, such as *Listeria monocytogenes*, depends on the notion that specific T lymphocytes activate macrophages for bactericidal activity and induce granuloma formation (6). This interaction supposedly depends on various T-cell-derived soluble mediators. Attempts to reproduce these T-cell activities in vitro have yielded the discovery that peritoneal exudate T-lymphocyte-enriched cells (PETLEs) from *Listeria*-immune mice generate cytotoxic activity in macrophages when cocultured with the latter in the presence of heat-killed *Listeria* (HKL) (5). Supernatants of such cultures contain a macrophage-activating factor (MAF) which is able to render normal macrophages cytotoxic (5). Further investigations have shown that MAF activity is exerted by at least two different molecules, one probably being identical to γ -interferon (γIFN) (20, 22). In addition, a migration-inhibition factor (MIF) is supposed to influence inflammatory processes by focusing macrophages at sites of bacterial deposition. It indeed has been shown that *Listeria*-immune T cells are capable of MIF production (15). Furthermore, proliferation of *Listeria*-specific T cells and interleukin 2 production, both thought to be essential functions for the induction of an effective antibacterial defense, could be reproduced in vitro, and the phenotype of the responding cells was shown to be $\text{Lyt } 1^{+}2^{-}$ (12, 13). We demonstrate that selected native T-cell populations and cloned T cells with *Listeria* specificity which carry the $\text{Lyt } 1^{+}2^{-}$ phenotype produce MAF and MIF activity in vitro, further substantiating the role these mediators play in the defense against facultative intracellular bacteria.

MATERIALS AND METHODS

Mice. C57BL/6 and (C57BL/6 $\times$ DBA/2)F₁ mice (age, 8 to 10 weeks) of either sex, raised in our own breeding facilities, were used. Cloned *L. monocytogenes*-immune T cells originated from C57BL/6 mice.

EL₄ tumor cells. The EL₄ tumor line was generously provided by Jürgen Bier, Klinikum Steglitz, Freie Universität Berlin.

Bacteria and bacterial antigens. *L. monocytogenes* EGD was kept virulent by continuous mouse passage. Cultures were obtained by growing a sample of spleen homogenate from infected mice overnight in tryptic soy broth (Oxoid, Wesel, Federal Republic of Germany). *L. monocytogenes* was heat killed by incubation in a water bath at 61°C for 60 min (12). HKL was washed twice and suspended in phosphate-buffered saline at $10^9/\text{ml}$ and stored at -70°C until use.

Medium. The medium used in most of the cultures was Dulbecco minimal essential medium (DMEM) (Seromed, Munich, Federal Republic of Germany) supplemented with 10% fetal calf serum (FCS) (Seromed), antibiotics (50 IU of penicillin and 50 μg of streptomycin, or 50 μg of kanamycin per ml), and 2 mM L-glutamine. In experiments involving cultivation of cloned T cells, Click RPMI medium (4) supplemented with 10% FCS, 50 μg of kanamycin per ml, and 2 mM L-glutamine was used. Cultures employing DMEM were incubated at 10% CO_2 in air, whereas cultures employing Click RPMI medium were incubated at 5% CO_2 in air.

Isotopes. Sodium chromate (^{51}Cr) (specific activity, 240 mCi/mg of chrome; 0.5 mCi/ml in isotonic saline) was purchased from Amersham Buchler, Brunswick, Federal Republic of Germany. [^3H]methylthymidine ([^3H]TdR) (specific activity, 2 Ci/mM; 1 mCi/ml in aqueous solution) was obtained from Radiochemical Centre, Amersham, United Kingdom.

Cells. The preparation of macrophages and *Listeria*-immune PETLEs has been described previously (5). Briefly, *Listeria*-immune T cells were obtained from the peritoneal cavity of mice which had received an intravenous injection of 5×10^3 viable *L. monocytogenes* organisms 10 days previously. Exudate cells were collected 3 days after the injection of 10% protease-peptone intraperitoneally and enriched for T cells by one cycle of adherence on plastic dishes and subsequent passage of the nonadherent cells through nylon wool columns (8).

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Macrophages were obtained from normal mice which had received intraperitoneal injections of protease-peptone 3 days previously. After harvesting, peritoneal cells were cultured in either 16-mm-diameter or 6-mm-diameter well trays (MultiWell tissue culture plates; Becton Dickinson Labware, Oxnard, Calif.) for at least 1 h at 37°C and then washed extensively to remove nonadherent cells. More than 98% of the remaining adherent cells, which constituted 70 to 80% of the elicited peritoneal cell population, displayed morphological characteristics of macrophages.

Lymphocyte-macrophage cultures. *Listeria*-immune T cells from the peritoneal exudate together with HKL were added to cultures of adherent cells from nonimmune mice and cultured for 20 h. At that time, the culture supernatants were removed and frozen. For determination of MAF activity, the supernatants were added to freshly harvested adherent peritoneal exudate macrophages in a 1:1 dilution with fresh medium and incubated for another 20 h. These macrophage cultures were then assayed for their activity against EL₄ tumor cells by either of the following methods.

(i) **⁵¹Cr release.** EL₄ tumor cells were maintained in ascites by serial passage in C57BL/6 mice. After lavage of the peritoneal cavities of tumor-bearing mice with DMEM containing 10 U of heparin (Nordmark Werke, Hamburg, Federal Republic of Germany) per ml, the cells were washed twice with medium, and a sample was removed for labeling with ⁵¹Cr. Tumor cells (10⁷) that were suspended in 0.2 ml of medium were mixed with 0.2 ml of ⁵¹Cr in isotonic saline and incubated for 90 min at 37°C. The tumor cells were then washed by centrifugation (10 min; 200 × g), suspended in 0.5 ml of medium, and washed once more by centrifugation through FCS (10 min; 200 × g). The ⁵¹Cr-labeled cells were resuspended in 10 ml of medium and cultured at 37°C for 45 min. Cells were then washed again, resuspended, and added to the macrophage cultures. The final concentration was 10⁵ tumor cells per well in a volume of 0.8 ml. After 18 h of incubation, the tissue culture plates were centrifuged, 0.5 ml of supernatant was removed from each well, and ⁵¹Cr was determined in a gamma-counter (Auto-Gamma spectrometer model 5260; Packard Instruments International, Zurich, Switzerland). Specific ⁵¹Cr release was calculated as follows: $(E - C)/(FT - C) \times 100$, where E is the isotope release in wells containing target cells and macrophages preincubated with the culture supernatants to be assayed, C is the isotope release in wells containing target cells and macrophages preincubated with supernatants of macrophages and HKL alone, and FT is the maximum isotope release from target cells after two cycles of freezing and thawing. All results are expressed as percentages of specific release plus or minus one standard deviation.

Background release (E) of ⁵¹Cr was in the range of 25 to 35% of the maximum isotope release (FT) after freezing and thawing. ⁵¹Cr release of EL₄ tumor cells incubated without macrophages was significantly higher (50 to 60% of maximum isotope release). Similar observations have been previously reported by others (3) and are probably due to the low cell concentration in the wells with tumor cells incubated without macrophages. Spontaneous ⁵¹Cr release of EL₄ tumor cells alone could be markedly reduced by adding 10⁶ unlabeled EL₄ tumor cells to 10⁵ ⁵¹Cr-labeled cells per well.

(ii) **Decreased proliferation of EL₄ tumor cells.** EL₄ cell proliferation was assessed by measurement of [³H]TdR incorporation (2, 16, 17, 23). EL₄ tumor cells were produced in the same way as described for the ⁵¹Cr release assay. Cells were resuspended and added to the macrophage cultures. The final concentration was 5 × 10⁴ tumor cells per well in a

volume of 0.2 ml. After 20 h of incubation, 2 μCi of [³H]TdR was added to each well; 60 min later, the cells were collected on glass-fiber filters (Skatron multiple cell culture harvester; Flow Laboratories, Inc., Irvine, United Kingdom). Radioactivity was determined with a liquid scintillation analyzer (LSA PW 4540; Philips, Hamburg, Federal Republic of Germany). Data were expressed as percent reduction of [³H]TdR incorporation (wells with the supernatants to be assayed compared with control wells containing supernatants of macrophages incubated with HKL). In analogy to the ⁵¹Cr release assay, [³H]TdR incorporation of EL₄ tumor cells alone was 40 to 60% lower than incorporation of the isotope by EL₄ cells incubated with macrophages pretreated with control supernatants.

MIF assay. Supernatants were tested for their ability to inhibit macrophage migration in an agar microdroplet assay (1, 7). Mouse peritoneal exudate cells were obtained from mineral oil (EGA-Chemie, Steinheim, Federal Republic of Germany)-stimulated peritoneal cavities of nonimmune mice. Cells were washed three times and suspended at 5 × 10⁸ cells per ml in fully supplemented DMEM containing 0.2% low-gelling-temperature agar (Sigma Chemical Co., Munich, Federal Republic of Germany). A 1-μl droplet of the cell suspension was placed in the center of each well of a 96-well flat-bottomed culture plate (microtest II; Becton Dickinson) by means of a 50-μl Hamilton syringe equipped with an automatic dispenser and a 22-gauge blunt-tipped needle. The droplets were allowed to gel at 4°C for 10 min. Then, 100 μl of the medium to be assayed was added to each well. Cultures were incubated at 37°C and 10% CO₂ in air for 18 to 24 h. The migration was quantified with an inverted microscope equipped with an ocular micrometer (Carl Zeiss, Oberkochen, Federal Republic of Germany). The distance from the edge of the droplet to the periphery of the migration front was measured at four sites. The averages of the migration distances were taken as the migration distance of a particular sample. The results shown in this paper are the differences of the migration distances between each experimental group and the migration distance determined by using control medium, plus or minus one standard deviation (percent inhibition of migration).

Determination of Lyt phenotype. Anti-Lyt antisera were kindly provided by M. M. Simon (Max-Planck-Institut für Immunbiologie). They were prepared and adsorbed to remove autoantibodies as previously described (24). Antiserum treatment of PETLEs was done as previously described (14). In short, 3 × 10⁷ PETLEs per ml was treated at room temperature for 30 min with anti-Lyt 1.2 antiserum (final dilution, 1:20) or anti-Lyt 2.2 antiserum (final dilution, 1:10). Cells were washed, resuspended at a concentration of 3 × 10⁷/ml in selected rabbit serum as a source of complement (final dilution, 1:11), and kept at 37°C for 30 min. Treatment of cells with anti-Lyt antisera and complement was repeated once. In this way, cells of Lyt 1⁺2⁻ or Lyt 1⁺2⁺ phenotype were negatively selected. After treatment, cell samples were adjusted to volumes equivalent to those of control cultures treated with complement alone. Populations of Lyt 1⁺2⁻ and Lyt 1⁺2⁺ cells were a 1:1 mixture of lymphocytes treated with either anti-Lyt 1.2 or anti-Lyt 2.2 antisera plus complement. Negatively selected cells were added to macrophage cultures, and supernatants were produced as described above.

Accessory cells. Spleen cells from normal mice were irradiated with 2,200 rads with a Philips 303 X-ray machine (Philips Electronic Instruments, Mahwah, N.J.). Irradiated spleen cells were used as accessory cells.

TABLE 1. Production of MAF by *Listeria*-immune PETLEs^a

Group	No. of <i>Listeria</i> -immune PETLEs/well	HKL	% Specific ⁵¹ Cr release	% Reduction of [³ H]TdR incorporation
1	3×10^5	—	-3.3 ± 1.1	-8.3 ± 4.9
2	3×10^5 (nonimmune)	+	-1.4 ± 1.4	17.3 ± 12.9
3	3×10^5	+	79.8 ± 2.8	97.5 ± 0.6
4	1×10^5	+	75.8 ± 6.0	92.6 ± 3.5
5	3×10^4	+	32.8 ± 8.2	68.7 ± 4.9
6	1×10^4	+	1.3 ± 3.7	3.0 ± 9.0

^a Specific ⁵¹Cr release and [³H]TdR incorporation by EL₄ tumor cells were determined after coculture with normal peritoneal exudate macrophages (10^6 in 0.8 ml for ⁵¹Cr release, 3×10^5 in 0.2 ml for [³H]TdR incorporation) activated by culture supernatants obtained by incubating *Listeria*-immune PETLEs with 1×10^6 macrophages and 2×10^5 HKL in a total volume of 0.8 ml or with 3×10^5 macrophages and 4×10^5 HKL in a total volume of 0.2 ml, respectively. Values are the means of at least three determinations $\pm$ standard deviations.

Cloning of *Listeria*-specific T cells and culture of T cell lines.

Cloning and recloning was essentially done according to Sredni et al. (25) and has been described previously (10). Recloned T cells were cultured in 16-mm flat-bottomed culture plates (Costar, Cambridge, Mass.) in 1 ml of Click RPMI with 5% FCS, 5% T-cell growth factor, 10^5 accessory cells, and 10^8 HKL. The production of T-cell growth factor has been previously described (10). Cultures were incubated at 5% CO₂ and fed every 3 to 4 days. Cells were kept at a concentration of between 10^5 and 10^6 /ml. For production of MAF and MIF, cloned *Listeria*-specific T cells together with HKL were added to macrophage cultures in appropriate concentrations.

RESULTS

Production of MAF by *Listeria monocytogenes*-immune heterogeneous T-cell populations. In keeping with earlier observations (5), nonimmune peritoneal macrophages, assayed immediately after harvesting, were not cytotoxic. Culture supernatants, produced by PETLEs from *Listeria*-immune mice in the presence of nonimmune peritoneal macrophages and HKL, were capable of inducing cytotoxic activity in freshly harvested nonimmune peritoneal macrophages. In the absence of HKL or in the presence of nonimmune T cells, the culture supernatants did not activate normal macrophages for tumoricidal or tumoristatic activity. MAF activity could be determined by either ⁵¹Cr release or a reduction of [³H]TdR incorporation (Table 1) by EL₄ tumor cells added to the macrophages. For the ⁵¹Cr release assay, 10^6 macrophages per well was optimal, whereas in the [³H]TdR incorporation assay, 3×10^5 macrophages per well

proved to be the optimum number of effector cells. For production of supernatants, 3×10^5 *Listeria*-immune T cells per well in both cases was found to represent the optimal concentration.

Lyt phenotype of MAF-producing T cells. The following experiment was done to examine the ability of *Listeria*-immune T-lymphocyte subpopulations of different Lyt phenotypes to produce MAF under the in vitro culture conditions described. T-cell subsets of different Lyt phenotypes were selected with anti-Lyt antisera plus complement as described above. Culture supernatants were produced by culturing T-cell subpopulations produced by treatment with anti-Lyt antisera and complement together with HKL and peritoneal exudate macrophages. These supernatants were assayed for their ability to induce cytotoxic macrophages by determining the ⁵¹Cr release and reduced [³H]TdR incorporation (Table 2) by EL₄ tumor cells.

The subpopulation treated with anti-Lyt 2 antiserum and complement, consisting of $\text{Lyt } 1^{+}2^{-}$ T lymphocytes, was capable of MAF production. There was no significant reduction of MAF activity compared with the complement group. On the other hand, the subpopulation treated with anti-Lyt 1 antiserum and complement, consisting of $\text{Lyt } 1^{-}2^{+}$ T lymphocytes, did not produce MAF activity. The recombination group, consisting of pretreated lymphocytes of both populations, was also capable of MAF production. These data reveal that in infection with *L. monocytogenes*, the $\text{Lyt } 1^{+}2^{-}$ T-lymphocyte subpopulation is the main effector population for MAF production in vitro. This could be demonstrated by ⁵¹Cr release as well as with the [³H]TdR incorporation assay.

Lyt phenotype of MIF-producing T cells. Besides macrophage activation, the concentration of macrophages at the focus of inflammation presumably due to the action of chemotactic factor and MIF (15) is thought to be of essential importance in the elimination of *L. monocytogenes*. In the following experiments, we were concerned with the T-cell subpopulation producing MIF. T lymphocytes pretreated with anti-Lyt 2 antiserum and complement ($\text{Lyt } 1^{+}2^{-}$ subset) were capable of MIF production, whereas T cells pretreated with anti-Lyt 1 antiserum and complement ($\text{Lyt } 1^{-}2^{+}$ subset) were unable to do so. The recombination group consisting of cells from both subsets also showed MIF production. Thus, MIF production, too, is mainly due to T cells of the $\text{Lyt } 1^{+}2^{-}$ phenotype.

Examination of production of MAF and MIF by cloned T cells with specificity for *L. monocytogenes*. As treatment with anti-Lyt antisera and complement does not lead to complete destruction of the T-cell subset to be eliminated, the results just described were confirmed by experiments with cloned *Listeria*-specific T cells of the $\text{Lyt } 1^{+}2^{-}$ phenotype (9;

TABLE 2. Production of MAF by antisera-treated *Listeria*-immune PETLEs^a

Group	No. of <i>Listeria</i> -immune PETLEs/well	Treatment	% Specific ⁵¹ Cr release	% Reduction of [³ H]TdR incorporation
1	3×10^5	None	59.9 ± 0.1	91.3 ± 2.3
2	1×10^5	None	60.9 ± 1.8	57.9 ± 17.8
3	3×10^5	Complement	48.4 ± 1.0	88.2 ± 1.2
4	3×10^5	$\alpha\text{Lyt } 1 + \text{complement}$	-2.2 ± 2.7	3.1 ± 1.1
5	3×10^5	$\alpha\text{Lyt } 2 + \text{complement}$	53.3 ± 7.1	70.2 ± 4.5
6	Recombination of group 4 and 5		65.0 ± 5.4	81.5 ± 7.7

^a Specific ⁵¹Cr release and [³H]TdR incorporation by EL₄ tumor cells cocultured with normal peritoneal exudate macrophages (10^6 in 0.8 ml for ⁵¹Cr release, 3×10^5 in 0.2 ml for [³H]TdR incorporation) activated by culture supernatants obtained by incubating antisera-treated or untreated *Listeria*-immune PETLEs with 10^6 macrophages and 2×10^6 HKL in a total volume of 0.8 ml or with 3×10^5 macrophages and 4×10^5 HKL in a total volume of 0.2 ml, respectively. Values are the means of at least three determinations $\pm$ standard deviations.

TABLE 3. Production of MIF by antisera-treated *Listeria*-immune PETLEs^a

Group	Treatment	Migration inhibition (%) at the following concn of PETLEs/ml:		
		5 × 10 ⁵	1.5 × 10 ⁵	5 × 10 ⁴
1	None	37.9 ± 6.4	36.2 ± 6.5	23.3 ± 3.5
2	Complement	40.5 ± 4.7	41.4 ± 7.9	34.5 ± 12.1
3	αLyt 1 + complement	13.4 ± 2.6	10.8 ± 5.2	11.6 ± 3.8
4	αLyt 2 + complement	34.9 ± 3.0	31.4 ± 3.8	21.1 ± 8.2
5	Recombination of group 3 and 4	40.5 ± 5.6	16.8 ± 4.7	6.5 ± 4.8

^a Antiserum-treated or untreated *Listeria*-immune PETLEs were cocultured with 3 × 10⁵ peritoneal exudate macrophages and 4 × 10⁵ HKL in a total volume of 0.2 ml. Supernatants were tested for MIF activity undiluted. The values are the differences of the migration distances between each experimental group and the migration distance determined by using control medium (± standard deviations) (percentage of the migration distance with control medium). Values are the means of at least three determinations.

S. H. E. Kaufmann, unpublished observation). Cloned T cells in the presence of peritoneal macrophages and HKL in vitro produce both MAF and MIF activity (Tables 4 and 5); cloned T lymphocytes (10³/ml) were still capable of performing marked mediator production.

DISCUSSION

MAF and MIF are assumed to be essential effector mechanisms of specific T lymphocytes in the immune response against facultative intracellular bacteria, such as *L. monocytogenes*. It has been demonstrated that *Listeria*-immune T lymphocytes are able to produce both mediators in vitro when cocultured with HKL and peritoneal macrophages from nonimmune donors (5, 15). In this paper, we have shown that the T-lymphocyte subpopulation capable of producing both lymphokines is Lyt 1⁺2⁻. This could be demonstrated with serologically selected T cells, as well as with cloned T lymphocytes of the Lyt 1⁺2⁻ phenotype with specificity for *L. monocytogenes*. T cells of the phenotype Lyt 1⁻2⁺ from *Listeria*-immune mice were not capable of performing these functions. In accordance with these findings, it has been shown previously that other in vitro functions of *Listeria*-immune T cells, such as lymphocyte proliferation and production of interleukin 2 and γ-IFN, are due to the Lyt 1⁺2⁻ T-lymphocyte subpopulation (11–13). MAF activity is closely correlated with γ-IFN production (18, 20, 22), and one molecule with MAF activity is probably identical to γ-IFN. The T-cell clone examined in this study,

TABLE 4. Production of MAF by cloned *Listeria*-specific T cells^a

Group	No. of cloned T cells/ml	No. of normal macrophages/ml	HKL	% Specific ⁵¹ Cr release	% Reduction of [³ H]TdR incorporation
1	3 × 10 ⁴	10 ⁶	+	43.1 ± 7.2	91.8 ± 2.1
2	1 × 10 ⁴	10 ⁶	+	45.6 ± 1.6	96.8 ± 0.7
3	3 × 10 ³	10 ⁶	+	50.0 ± 2.9	96.1 ± 0.9
4	1 × 10 ³	10 ⁶	+	40.5 ± 4.8	92.2 ± 0.6
5	3 × 10 ⁴	10 ⁶	—	-2.5 ± 4.4	-0.6 ± 12.9
6	3 × 10 ⁴	10 ⁶	+	2.6 ± 2.8	-17.4 ± 20.5

^a Specific ⁵¹Cr release and [³H]TdR incorporation of EL₄ tumor cells (5 × 10⁴ cells per well) cocultured with 3 × 10⁵ normal peritoneal exudate macrophages activated by culture supernatants in a total volume of 0.2 ml. Culture supernatants were obtained by incubating cloned T cells with 10⁶ normal macrophages and 10⁷ HKL in a total volume of 1.0 ml. Values are the means of at least three determinations ± standard deviations.

TABLE 5. Production of MIF by cloned *Listeria*-specific T cells^a

Group	No. of cloned T cells/ml	No. of normal macrophages/ml	HKL	% Inhibition of migration
1	3 × 10 ⁴	10 ⁶	+	36.7 ± 4.5
2	1 × 10 ⁴	10 ⁶	+	44.1 ± 12.9
3	3 × 10 ³	10 ⁶	+	48.9 ± 6.8
4	1 × 10 ³	10 ⁶	+	49.8 ± 1.6
5	3 × 10 ⁴	10 ⁶	—	10.0 ± 4.2
6	3 × 10 ⁴	10 ⁶	+	10.6 ± 4.2

^a Cloned *Listeria*-specific T cells were cocultured with 10⁶ peritoneal exudate macrophages and 10⁷ HKL in a total volume of 1.0 ml. Supernatants were tested for MIF activity undiluted. The values are the differences of the migration distances between each experimental group and the migration distance determined by using control medium (± standard deviations) (percent inhibition of migration). Values are the means of at least three determinations.

being of the phenotype Lyt 1⁺2⁻, previously has been shown to be capable of proliferation and interleukin 2 production in the presence of HKL and accessory cells (9). Kühner et al. (19), investigating the MIF production by BCG-immune T lymphocytes, have shown that in this case the Lyt 1⁺2⁻ subpopulation is of essential importance. Taken together, the findings point to the Lyt 1⁺2⁻ T-lymphocyte subpopulation as the main effector T-cell population in immunity against *Listeria* infection when in vitro assays are employed.

The in vitro functions exerted by *Listeria*-immune T cells of the phenotype 1⁺2⁻ are in contradistinction to the known requirement for Lyt 1⁺2⁺ T cells in mediating protection (14). T cells of the clone examined in this study, although capable of producing MAF and MIF, were poorly suited for systemic adoptive protection (9). There are indications of a developmental relationship between Lyt 1⁺2⁺ and Lyt 1⁺2⁻ T cells (21), but further examination of their role in the immune response to facultative intracellular bacteria seems to be necessary.

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