BIOLOGICAL FUNCTIONS OF T CELL LINES WITH SPECIFICITY FOR THE INTRACELLULAR BACTERIUM *LISTERIA MONOCYTOGENES* IN VITRO AND IN VIVO

By STEFAN H. E. KAUFMANN* and HELMUT HAHN

From the Basel Institute for Immunology, Basel, Switzerland; and the Institut für Medizinische Mikrobiologie, Freie Universität Berlin, Berlin, Germany

Experimental infection of mice with *Listeria monocytogenes* has been widely used for studies of the cellular immune response to facultative intracellular bacteria (reviewed in 1). Mackaness (2, 3) has shown that acquired resistance to *L. monocytogenes* and delayed-type hypersensitivity (DTH)[1] to listerial antigens are mediated by specific lymphocytes that activate macrophages at the site of deposition of bacteria or of antigen. It was subsequently found that both protection and DTH are T cell dependent (4–6), and Lyt-1, as well as Lyt-1,2,3 T cells were shown to be involved in the immune response to *L. monocytogenes* (7, 8).[2] Also, protection against *L. monocytogenes* was found to be restricted by the H-2I locus of the major histocompatibility complex (MHC) (9), as was DTH of Jones-Mote-type to soluble protein antigens (10). Despite this parallel between DTH and protection, the issue whether the mechanism underlying protection to facultative intracellular bacteria and DTH to bacterial antigens involve identical cell reactions or not, remained unresolved (1, 11).

Recently, it has become feasible to analyze cellular immune functions by the use of cloned T cell lines that maintain antigen specificity as well as biological function for long periods of in vitro culture (reviewed in 12). Making use of this technical achievement, we have cloned *Listeria*-specific T cells and established from these clones continuously growing T cell lines specific for *L. monocytogenes*. These cell lines were restricted by the H-2IA locus of the MHC. They were capable of (*a*) *Listeria*-specific proliferation, (*b*) interleukin secretion, (*c*) "bystander help" for B cells in vitro, and, perhaps most important, (*d*) were able to confer DTH to listerial antigens and protection to live *L. monocytogenes* in vivo. The results of this report strongly support the idea that DTH and protection to facultative intracellular bacteria have a common cellular basis, being dependent on a single T cell population of helper type.

Materials and Methods

Mice. C57Bl/6 and BALB/c mice were obtained from Institut für Biologisch-Medizinische Forschung A. G., Füllingsdorf, Switzerland. B10.A(4R), B10.A(5R), and B10.MBR mice were

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; DTH, delayed-type hypersensitivity; FCS, fetal calf serum; [^{3}H]TdR, [^{3}H]thymidine; IMDM-ATL, Iscove's modified Dulbecco's medium supplemented with albumin, transferrin, and soybean lipids; MHC, major histocompatibility complex; PETLES, peritoneal exudate, T lymphocyte-enriched cells; PPD, purified protein derivative of tuberculin; SRBC, sheep erythrocytes; TCGF, T cell growth factor.

[2] Kaufmann, S. H. E., H. Hahn, M. M. Simon, M. Röllinghoff, and H. Wagner. Interleukin 2 induction by Lyt-1$^+$,2,3$^-$ T cells from *Listeria monocytogenes*-immune mice. Manuscript submitted for publication.

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bred at the Basel Institute for Immunology, Basel, Switzerland. Animals were bred and kept under specific pathogen-free conditions.

Bacteria and Bacterial Antigens. *L. monocytogenes* (strain EGD) was kept virulent by continuous mouse passage (2). Bacterial cultures were obtained by growing a sample of spleen homogenate from an infected mouse in trypticase-soy broth (Oxoid, Wesel, Federal Republic of Germany [FRG]), and appropriately diluted bacterial suspensions injected intravenously in a volume of 0.2 ml. The preparation of soluble antigens of *L. monocytogenes* has been described elsewhere (7). Heat-killed *L. monocytogenes* were obtained by incubating a bacterial suspension in phosphate-buffered saline at 60°C for 60 min, and were adjusted to 10^{10}/ml organisms and kept at −70°C.

Culture Medium. Iscove's modified Dulbecco's medium (IMDM) was used for long-term cultures as well as for in vitro assays. The medium was supplemented with 1 mM glutamine (Gibco Europe, Glasgow, United Kingdom [UK]), 5×10^{-5} M 2-mercaptoethanol (Merck, Darmstadt, FRG), kanamycin (Gibco, Europe, Glasgow, UK), 1 mg/ml purified serum albumin (Behring-Werke, Marburg, FRG), and soybean lipids (Nattermann & Co., Köln, FRG) according to (13) (IMDM-ATL). IMDM-ATL was used without addition of serum in all in vitro assays.

Listeria-specific T cell lines were kept in long term cultures in the presence of 5% fetal calf serum (FCS) of batches selected for good T cell growth (Gibco Europe) and 5% T cell growth factor (TCGF). For TCGF production, 5×10^6/ml rat spleen cells were cultivated for 1 d in IMDM plus bovine serum albumin (0.5 mg/ml) in the presence of 5 μg/ml concanavalin A (Con A) in tissue-culture flasks (Costar, Data Packaging, Cambridge, MA) at 37°C, 10% O_2, 7% CO_2, 83% N_2 (14). Cell-free supernatants were concentrated by saturation with 80% ammonium sulfate, and the resulting precipitate collected by centrifugation (10,000 rpm, 30 min) (DuPont Instruments-Sorvall Biomedical Div., Newtown, CT). The pellet was dissolved in 10 ml 0.9% NaCl, 0.01 M Hepes, and dialyzed overnight against the same buffer. Insoluble material was removed by centrifugation (10 min, 10,000 rpm), and the resulting supernatant was applied to a Sephadex G-100 column (100 × 5 cm; Pharmacia, Uppsala, Sweden) equilibrated with the above buffer. Fractions of 10 ml were collected at 4°C and sterilized through 0.45-μm filters (Millipore Corp., Bedford, MA).

Immunization and Enrichment for Peritoneal Exudate T Lymphocyte-enriched Cells (PETLES). Mice were immunized with 5×10^4 live *L. monocytogenes*. 7 d later, peritoneal exudates were induced with 1.5 ml 10% proteose peptone. Peritoneal exudate cells were harvested 3 d thereafter and enriched for PETLES by incubation in nylon wool columns (15).

Accessory Cells. Spleen cells from unimmunized mice were irradiated with 2,200 rad using a Philips 305 x-ray machine (Philips Electronic Instruments, Inc., Mahwah, NJ). Irradiated spleen cells were used as accessory cells.

Cloning of Listeria-specific T Cells. Cloning was essentially done according to Sredni et al. (16). PETLES (5×10^6) from *Listeria*-immune C57Bl/6 mice were cultured together with 5×10^6 syngeneic accessory cells and 5×10^8 heat-killed *L. monocytogenes* in 5 ml IMDM-ATL in 25-cm^2 tissue-culture flasks (Falcon Labware, Becton, Dickinson & Co., Oxnard, CA) at 37°C in 10% O_2, 7% CO_2, 83% N_2 for 3 d. Cells were washed and cloned in double-layer agar in 30-mm dishes (Falcon Labware). The lower layer consisted of 1.25 ml twofold-concentrated Click's medium (17), 0.5 ml FCS, 0.375 Agar Nobel (3.3%, Difco Laboratories, Detroit, MI), and 2×10^8 heat-killed *L. monocytogenes*. The lower layer was equilibrated at 37°C in 5% CO_2 over night. The upper layer consisted of 0.3 ml FCS, 0.15 ml Agar Nobel (1.8 %), and 0.1 ml Click's medium containing 1×10^6 *Listeria*-immune PETLES and 1×10^5 syngeneic accessory cells. Cultures were incubated at 37°C, 5% CO_2 for 5 d.

Recloning of Listeria-specific T Cell Lines. For recloning of *Listeria*-specific T cell lines, 200 cells were seeded in double-layer soft agar, and 5 d later colonies were counted. Some colonies were picked and expanded as described below.

Culture of Listeria-specific T Cell Lines. Colonies were picked with a pasteur pipet and transferred into flat-bottomed microculture plates (Costar, Data Packaging) containing 100 μl IMDM-ATL with 5% FCS, 5% TCGF, 10^4 accessory cells, and 10^7 heat-killed *L. monocytogenes*. T cells grown to near confluence were transferred to 16-mm flat-bottomed culture plates (Costar, Data Packaging) and cultured in 1 ml IMDM-ATL with 5% FCS, 5% TCGF, 10^5 accessory cells, and 10^8 heat-killed *L. monocytogenes*. Cultures were kept at 37°C in 10% O_2, 7%

CO_2, 83% N_2, and fed every 3–4 d. Cells were kept between 1×10^5 and 1×10^6 cells/ml. They could be expanded to as many as 10^8 cells. *Listeria*-specific T cell lines have so far been propagated for up to 5 mo.

Proliferative Responses of Listera-specific T Cell Lines. 2×10^4 T cells were co-cultured with 2×10^5 accessory cells and 5×10^7 heat-killed *L. monocytogenes* for 4 d in flat-bottomed microculture plates containing 0.2 ml IMDM-ATL at 37°C in 10% O_2, 7% CO_2, 83% N_2. 18 h before cell harvest 1 μCi [^{3}H]thymidine ([^{3}H]TdR) (2 Ci/mmol; Radiochemical Centre, Amersham, UK) was added to the cultures, and incorporation of radioactivity was measured.

Interleukin Secretion by Listeria-specific T Cell Lines. 2×10^4 T cells were cultured in the presence of 2×10^5 accessory cells and 5×10^7 heat-killed *L. monocytogenes* in 0.2 ml IMDM-ATL in flat-bottomed microculture plates at 37°C in 10% O_2, 7% CO_2, 83% N_2. After 18 h, cell-free supernatants were collected and tested for mitogenic activity on thymocytes and Con A-activated spleen cells as follows.

Thymocyte Proliferation. 1×10^6 thymocytes from 3–4-wk-old BALB/c mice were cultured in 0.2 ml IMDM-ATL together with 25% supernatant in round-bottomed microculture plates (Greiner, Nürtingen, FRG) at 37°C in 10% O_2, 7% CO_2, 83% N_2 for 3 d, and for the last 18 h, 0.1 μCi [^{3}H]TdR was added (18).

Proliferation of Con A-activated Spleen Cells. 5×10^6/ml spleen cells from C57Bl/6 mice were incubated in tissue-culture flasks (Falcon Labware) in the presence of 2.5 μg/ml Con A at 37°C for 2 d (19). Afterwards, cells were washed, and 2×10^4 Con A-activated cells were cultured for 2 d in 0.2 ml IMDM-ATL together with 25% supernatant in flat-bottom microculture plates at 37°C in 10% O_2, 83% N_2. For the last 2 h, 1 μCi [^{3}H]TdR was added.

Helper Activity for B Cells of Listeria-specific T Cell Lines. 1×10^4 *Listeria*-specific T cells were co-cultured for 4 d in 0.2 ml IMDM-ATL with 1×10^5 small resting B cells and 4×10^5 accessory cells in the presence of 5×10^7 heat-killed *L. monocytogenes* and 5×10^5 sheep erythrocytes (SRBC) using flat-bottomed microculture plates. Small resting B cells were obtained by velocity sedimentation at unit gravity of spleen cells pretreated with monoclonal anti-Thy-1.2 antiserum plus complement as described (20). Helper activity of Listeria-specific T cell lines for the bystander antigen, SRBC, was determined by the direct SRBC-specific plaque assay (21, 22).

Adoptive Protection by Listeria-specific T Cell Lines. Mice were infected with $5–10 \times 10^4$ live *L. monocytogenes*, and 2 h later received various numbers of T cells intravenously. After 2 d, viable bacteria in spleens were determined by plating 0.1-ml samples of tissue homogenate at appropriate dilutions on trypticase-soy agar as described (2, 7).

Adoptive DTH by Listeria-specific T Cell Lines. Mice were injected intravenously with varying numbers of T cells and immediately thereafter challenged for DTH by subcutaneous injection of 50 μl soluble antigen of *L. monocytogenes* into one hind footpad. Alternatively, T cells were mixed with soluble antigen of *L.* monocytogenes and injected together subcutaneously in a volume of 50 μl into one hind footpad. DTH reactions were measured 24 h later using a dial gauge caliper (Kröplin, Schlüchtern, FRG) as described (7).

Results

Proliferation and Interleukin Induction by Listeria-specific T Cell Lines. The specificity of cloned T cells induced in vivo by *L. monocytogenes* was analyzed in vitro by their response to listerial antigen presented by syngeneic accessory cells. Proliferation and interleukin induction by six established cell lines (propagated for 6 wk) are summarized in Table I. In the presence of heat-killed *L. monocytogenes* and histocompatible accessory cells, all lines were stimulated to proliferate and secrete interleukins. In the absence of either accessory cells or antigen, as well as in the presence of the nonrelated antigens, purified protein derivative of tuberculin (PPD) or SRBC, proliferation was negligible. Thus, we conclude that all T cell lines shown in Table I are specific for *L. monocytogenes*. Proliferation as well as interleukin induction both depended on the concentration of T cells present in the culture, 5,000 cells still being sufficient for significant responses (Figs. 1 and 2).

TABLE I

Proliferation and Interleukin Induction by Listeria-specific T Cell Lines

T cell line	Proliferative response* ([^{3}H]TdR uptake [cpm/2 × 10^4 T cells])	Interleukin activity‡	
		[^{3}H]TdR uptake (cpm/10^6 thymocytes)	[^{3}H]TdR uptake (cpm/2 × 10^4 Con A-blasts)
9-1	105,900	27,300	71,800
9-2	58,400	20,500	58,900
9-3	38,000	25,800	81,300
9-4	92,300	35,000	74,100
9-5	32,100	32,200	82,400
9-6	15,000	30,500	75,700
—	120	4,300	8,700

* In the absence of either accessory cells or antigen, proliferative responses were < 1,000 cpm. In the presence of the unrelated antigens, PPD (5 μg), or SRBC (2 × 10^5), proliferative responses were <1,500 cpm. Means of three experiments; SD < 20%. For details see Materials and Methods.

‡ In the absence of either accessory cells or antigen, the T cell lines develop negligible quantities or interleukin activity (<2,000 cpm [thymocytes] <6,000 cpm [Con A-blasts]). Means of three experiments; SD < 15%. For details see Materials and Methods.

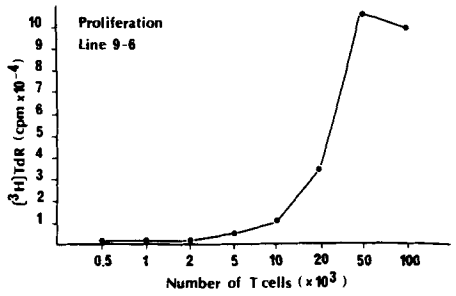

FIG. 1. Dose dependence of *Listeria*-specific proliferation. Graded numbers of *Listeria*-specific T cells were cultured together with 2 × 10^5 syngeneic accessory cells and 1 × 10^8 heat-killed *L. monocytogenes* for 4 d, the last 18 h in the presence of 1 μCi [^{3}H]TdR. Representative data of line 9-6; similar data were obtained with other cell lines. Means of three experiments, SD < 10%.

H-2 Restriction of Listeria-specific T Cell Lines. Proliferation and interleukin induction by *Listeria*-specific T cell lines (C57Bl/6) only occurred in the presence of accessory cells from C57Bl/6 and B10.A(5R) mice (Table II). In the presence of accessory cells from B10.A(4R) and B10.MBR mice, no responses were observed. We conclude that histocompatibility within the H-2IA locus of antigen-presenting cells is required and sufficient for interactions between the *L. monocytogenes*-specific T cell lines, antigen, and accessory cells.

Helper Activity of Listeria-specific T Cell Lines for B Cells. In the presence of syngeneic small B cells and accessory cells as well as the specific (*L. monocytogenes*) and bystander (SRBC) antigen, *Listeria*-specific T cell lines provided help for proliferation and maturation to Ig-secreting SRBC-specific B cells (Fig. 3). At high concentrations of T cells, a well-documented (23) inhibition of B cells was observed. Bystander help was restricted by the H-2IA locus of the MHC (Figs. 3 and 4).

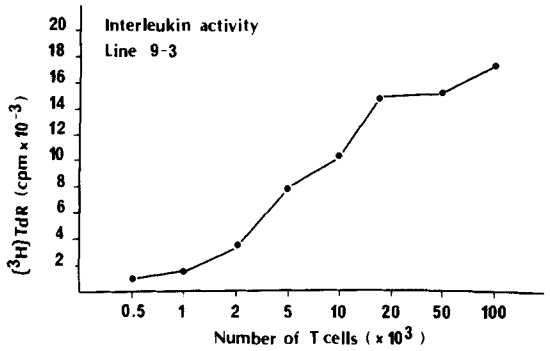

FIG. 2. Dose dependence of interleukin secretion. Graded numbers of *Listeria*-specific T cells were cultured together with 2×10^5 syngeneic accessory cells and 1×10^8 heat-killed *L. monocytogenes* for 24 h. Supernatants (25%) were incubated with 10^6 thymocytes from young BALB/c mice for 3 d for the last 18 h in the presence of 0.1 μCi [^{3}H]TdR. Representative data of line 9-3; similar data were obtained with other lines. Means of three experiments, SD < 15%.

TABLE II

H-2 Restriction of Listeria-specific T Cell Proliferation and Their Capacity to Induce Interleukin Secretion (T Cell Line 9-3)

Accessory cell	H-2-complex					Proliferative response* ([^{3}H]TdR uptake [cpm/2 × 10^4 cells])	Interleukin activity*	
	K	I-A	I-E	I-C	D		[^{3}H]TdR uptake (cpm/10^6 thymocytes)	[^{3}H]TdR uptake (cpm/2 × 10^4 Con A blasts)
C57Bl/6	b	b	b	b	b	48,700	28,100	64,400
B10.A(4R)	k	k	b	b	b	1,350	700	4,100
B10.A(5R)	b	b	k	d	d	51,200	25,300	65,500
B10.MBR	b	k	k	k	q	1,420	960	3,900
BALB/c	d	d	d	d	d	900	1,220	2,700
None	—	—	—	—	—	1,230	710	3,000

* In the absence of either accessory cells or antigen, proliferative responses of T cells were <2,000 cpm, and interleukin activity induced by these cells <1,000 cpm (thymocytes) <4,000 cpm (Con A blasts). Means of three experiments; SD < 15%. For details see Materials and Methods.

Listeria-specific T Cell Lines Confer DTH to Soluble Listerial Antigens and Protection to Live L. monocytogenes In Vivo. *Listeria*-specific T cell lines conferred DTH reactions locally (Table III). In some cases, only poor DTH reactions were obtained. This might have been caused by the relatively small number of cells (2×10^4) transferred. For example, 1×10^5 cells of line 9-2 initiated high DTH reactions as compared with 1×10^4 cells, which did not induce significant responses (Fig. 5).

Systemic intravenous transfer of 5×10^4 *Listeria*-specific T cells did not result in adoptive DTH responses, nor in protection to live *L. monocytogenes*. Therefore, lines 9-6 and 9-16 were expanded, and 2×10^6 or 5×10^6 cells, respectively, were injected intravenously into syngeneic recipient mice. Using these higher cell numbers, *Listeria*-specific T cell lines were capable of systemically conferring DTH to listerial antigen as well as protection to live *L. monocytogenes* (Table IV).

As shown in Table V, *Listeria*-specific T cells of C57Bl/6 haplotype were capable of adoptively mediating protection against live *L. monocytogenes* only in C57Bl/6 or

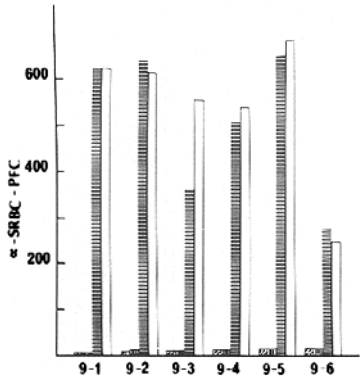

FIG. 3. Bystander helper effect of *Listeria*-specific T cell lines. 1×10^4 *Listeria*-specific T cells of lines 9-1 to 9-6 were cultured together with 1×10^5 small resting B cells and 4×10^5 accessory cells of different haplotypes in the presence of 5×10^7 heat-killed *L. monocytogenes* and 5×10^5 SRBC. After 5 d anti-SRBC-IgM were determined. B10.MBR accessory cells and B cells (▨); B10.A(4R) accessory cells and B cells (▥); B10.A(5R) accessory cells and B cells (▤); C57Bl/6 accessory cells and B cells (□).

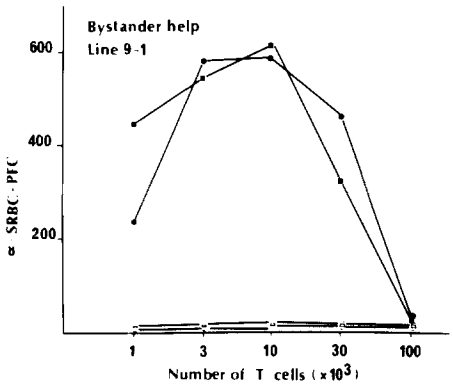

FIG. 4. Dose dependence of the bystander helper effect. Graded numbers of *Listeria*-specific T cells were co-cultured with 1×10^5 small resting B cells and 4×10^5 accessory cells of different haplotypes in the presence of 5×10^7 heat-killed *L. monocytogenes* and 5×10^5 SRBC. After 5 d anti-SRBC-IgM were determined. Representative data of line 9-1; similar data were obtained with other lines. B10.MBR accessory cells and B cells (□); B10.A(4R) accessory cells and B cells (○); B10.A(5R) accessory cells and B cells (■); C57Bl/6 accessory cells and B cells (●).

B10.A(5R) recipient mice, but not in B10.A(4R) or B10.MBR mice. Thus, histocompatibility at the H-2IA locus between T cells and recipients is required and sufficient for successful adoptive protection by *Listeria*-specific T cell lines.

Biological Functions of Recloned Listeria-specific T Cells. When a *Listeria*-specific T cell line (9-2) was recloned, a high cloning efficiency (80%) was observed. Antigen-induced proliferation and interleukin secretion in vitro, as well as adoptive mediation of DTH (subcutaneous cell transfer) and antibacterial protection (intravenous cell transfer) were comparable to those of the original T cell line (Tables VI and VII). These data strongly suggest that the original T cell lines already consisted of one homogeneous T cell population.

TABLE III

Local (Subcutaneous) DTH Transfer with Listeria-specific T Cell Lines *

T cell line	DTH (0.1 mm)‡	
	Antigen present	Antigen absent
9-1	5.0	0.7
9-2	2.3	1.0
9-3	5.0	0.7
9-4	5.0	0.3
9-5	2.7	0.7
9-6	6.3	0.7
—	0.3	—

* 2×10^4 T cells were injected subcutaneously into one hind footpad of syngeneic recipient mice.

‡ Means of three experiments. For details see Materials and Methods.

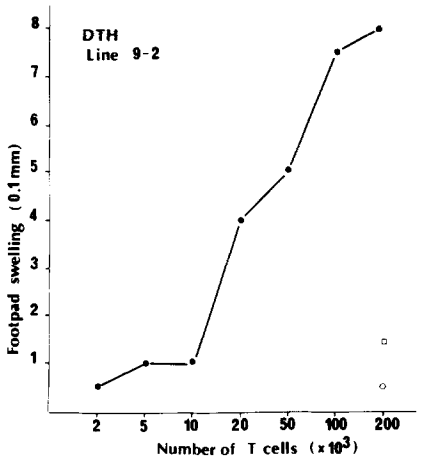

FIG. 5. Dose dependence of local DTH transfer. Graded numbers of cells were injected subcutaneously into one hind footpad of syngeneic recipient mice, together with soluble listerial antigen. Representative data of line 9-2; similar data were obtained with other lines. T cells alone (□); antigen alone (○). Means of three experiments; SD < 20%.

Discussion

Protective immunity to facultative intracellular bacteria and DTH to their antigens are mediated by specific T lymphocytes. The latter attract mononuclear phagocytes to the site of antigen deposition, cause them to form granulomas if the antigenic stimulus persists long enough, and activate macrophages for enhanced bacteriocidal capacity (reviewed in 1).

With the discovery of T cell subsets identifiable by Lyt differentiation antigens (reviewed in 24) and the realization that distinct T cell subsets mediate different T cell functions, the old argument originally raised by Koch (25), of whether or not antibacterial protection and DTH have a common basis was revived. In particular, it became possible to ask whether T cells belonging to the same, or to different, subpopulations mediate these two modalities. This question could not be settled until specific T cell clones were available to be tested for their capacity to mediate various T cell functions.

TABLE IV

Systemic (Intravenous) Transfer of Protection against L. monocytogenes and DTH to Listerial Antigens by Listeria-specific T Cell Lines

T cell line	Cell number injected	Log_{10} protection in spleen*	DTH (0.1 mm)‡
9-6	2×10^4	0.13	1.0
9-6	2×10^6	1.06	6.4
—	None	—	0.8
9-16	5×10^4	0.24	2.0
9-16	5×10^6	1.87	ND§
—	None	—	1.0

* Syngeneic recipient mice were infected with 8×10^4 viable *L. monocytogenes* 2 h before intravenous cell transfer. Means of four experiments; SD < 15%. For details see Materials and Methods.

‡ Syngeneic recipient mice were challenged for DTH with listerial antigen immediately before intravenous cell transfer. Means of four experiments; SD < 15%. For details see Materials and Methods.

§ Not done.

TABLE V

H-2 Restriction of Adoptive Protection against L. monocytogenes (Line 9-3)

Recipient mouse	H-2 complex					Log_{10} protection in spleen*
	K	I-A	I-E	I-C	D	
C57Bl/6	b	b	b	b	b	1.34
B10.A(4R)	k	k	b	b	b	0.17
B10.A(5R)	b	b	k	d	d	1.04
B10.MBR	b	k	k	k	q	0.18

* Recipient mice of different haplotype were infected with 9×10^4 viable *L. monocytogenes* 2 h before intravenous transfer of 3×10^6 T cells. Means of four experiments; SD < 10%. For details see Materials and Methods.

TABLE VI

Proliferative Responses of and Interleukin Induction by Recloned Listeria-specific T Cells

T cell line	Proliferative response* ([^{3}H]TdR uptake [cpm/2×10^4 T cells])	Interleukin activity* ([^{3}H]TdR uptake [cpm/10^6 thymocytes])
9-2	36,700	22,200
9-2-1	27,400	16,500
9-2-2	38,300	17,400
9-2-3	22,900	22,800
9-2-4	40,100	12,700
9-2-5	22,800	19,400
9-2-6	47,800	13,400
—	1,800	960

* In the absence of either accessory cells or antigen, proliferation of T cells was <2,000 cpm and interleukin activity induced by these T cells <1,000 cpm. Means of three experiments; SD < 15%. For details see Materials and Methods.

TABLE VII

Adoptive Protection against L. monocytogenes and DTH to Listerial Antigens by Recloned Listeria-specific T Cells (Subline 9-2-3)

Cell number injected	Log_{10} protection in spleen*	DTH (0.1 mm)‡
2×10^4	0.06	5.3
2×10^6	1.11	ND§

* Syngeneic recipient mice were infected with 7×10^4 viable *L. monocytogenes* 2 h before intravenous cell transfer. Means of four experiments; SD < 15%. For details see Materials and Methods.

‡ Recloned T cells were injected subcutaneously into one hind footpad of syngeneic recipient mice together with soluble listerial antigen in a total volume of 0.05 ml. In the absence of either T cells or antigen, DTH was <1.0. Means of four experiments; SD < 15%; For details see materials and Methods.

§ Not done.

In this paper, we describe the in vitro cloning and propagation of continuous T cell lines specific for the intracellular bacterium *L. monocytogenes*. These T cell lines are restricted by the H-2IA locus of the MHC. They are not only active in vitro but also in vivo, conferring antibacterial protection and DTH upon an immunologically unprimed host. Also, others have gathered data showing that in vitro propagated T cells specifically mediate protection to live *L. monocytogenes*.[3]

In all likelihood, our T cell lines are in fact clones of *Listeria*-specific T cells. Cloning in double-layer soft agar, however, is not unproblematic, because colonies that develop in the agar could have been derived from associations of macrophages, antigen, and several T cells (16, 26, 27). Thus, T cell lines established from such agar colonies could be the progeny of more than one T cell. However, recloned sublines exerted biological activities in vitro and in vivo at a degree comparable to the original line. It is therefore most likely that the original T cell line and other *Listeria*-specific T cell lines used in the present study all are clones derived from single antigen-specific H-2-restricted T cells. Thus, the notion originally proposed by Koch (25) and violently disputed by Rich (28) has been decided in favor of an identical cellular mediator of both DTH and antibacterial protection.

Analysis of the cellular immune response protecting mice from infections by intracellular bacteria, therefore, has been simplified and concentrated on the action of specific T cell lines in vitro and in vivo. At the same time, our results show that *Listeria*-specific T cell lines can induce different biological functions.

The notion of one regulatory T cell population inducing a variety of immune effector functions is furthermore supported by several reports that indicate that cloned T cells with helper activity for B cells do so not only in vitro but also in vivo (29), can kill target cells in vitro (30), induce secretion of different mediators (14, 20), and confer DTH (30, 31). As a common feature, these functions are all restricted by the I region of the H-2 locus of the MHC of the mouse.

Numbers of cloned *Listeria*-specific T cells in our experiments required for proliferative responses, induction of interleukin secretion, and local DTH transfer were at

[3] Kearns, R. J., and E. C. DeFreitas. The in vitro propagation of antigen-specific T lymphocytes which adoptively transfer resistance to *Listeria*. Manuscript submitted for publication.

least 50–100 times lower than those required in experiments using heterogeneous T cell populations from *Listeria*-immune mice (7, 8, 32).[2] Thus, it appears that interleukin secretion at the single cell level remained unaffected by the in vitro propagation. On the other hand, comparatively high cell numbers had to be used for successful systemic transfer of protection and DTH, possibly because the capacity to migrate into inflammatory foci had been altered as a result of in vitro propagation. Alternatively, *Listeria*-specific T cells used for transfer are part of a regulatory cell circuit and, for optimal responses to occur, have to interact with other specific T cells belonging to a different subset. Involvement of more than one T cell subset with *Listera* specificity in vivo indeed is suggested by our recent observations (*a*) that Lyt-1,2,3 T cells are crucially involved in adoptive transfer of DTH and protection in murine listeriosis (7), and (*b*) that Lyt-1 T cells alone can specifically interact with antigen-pulsed macrophages in vitro (8, 32).[2] This requirement of regulatory cell interactions could then be overcome by the using of high numbers of *Listeria*-specific T cells as indicated by this report.

Establishment of continuously growing T cell lines active in antibacterial immunity will facilitate direct studies of influences of immune cells, factors, pharmacological agents, and adjuvants on the T cell population conferring protection as well as studies on influences of protective T cells on the immune system. New rational therapeutic approaches to infections with intracellular bacteria will undoubtedly emerge from such studies with the perspective of adoptively immunizing patients with intracellular infections by means of autologous homogeneous T cell lines propagated in vitro or monoclonal factors derived therefrom.

Summary

Peritoneal exudate T lymphocytes from mice immunized with live *Listeria monocytogenes* were cloned in double-layer soft agar containing heat-killed *L. monocytogenes* (lower layer) and syngeneic accessory cells (upper layer). Colony-derived T cells were propagated in vitro in the presence of listerial antigen, syngeneic accessory cells, and T cell growth factor. In vitro proliferation, interleukin secretion, and bystander help for B cells of six such T cell lines and several sublines derived from them were found to be antigen dependent and restricted by the H-2IA locus of the major histocompatibility complex. In vivo, these T cell lines conferred delayed-type hypersensitivity to listerial antigen and protection to live *L. monocytogenes*. It is concluded that different biological functions of acquired antibacterial immunity can be mediated by a single T cell population.

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