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Interleukin 2 Induction in Lyt 1^+23^- T Cells from *Listeria monocytogenes*-Immune Mice

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Peritoneal exudate T lymphocytes from mice experimentally infected with the intracellular bacterium *Listeria monocytogenes* secreted high interleukin 2 activities after interaction with syngeneic normal macrophage presenting listerial antigen in vitro. *L. monocytogenes*-immune cells secreting IL 2 were radioresistant and bore the phenotype Thy 1^+ Lyt 1^+23^-.

The T-cell system of mice can be divided into distinct subpopulations discernible by their Lyt phenotype (1, 8). For adequate immune responses to occur, different Lyt T-cell subpopulations have to interact with each other and with accessory cells (H. Cantor and R. K. Gershon, Fed. Proc. 38:2058–2064, 1979). Recently, it has been shown that different stimuli can activate T cells to secrete interleukin 2 (IL 2) (9, 13), and evidence has been accumulated that Lyt 1^+ helper T-cell-derived IL 2 fulfills an important mediator function in the generation or differentiation of antigen-primed Lyt 23^+ cytotoxic T cells (CTLs) (3, 12, 14, 15).

Cell interactions in acquired antibacterial immunity are much less well understood (2). However, it has been shown that Lyt 1^+ and Lyt 123^+ T cells participate in the immune response to the intracellular bacterium *Listeria monocytogenes* (2a, 5, 6; S. H. E. Kaufmann, H. Hahn, and M. M. Simon, Scand. J. Immunol., in press).

In the present study, we show that *L. monocytogenes*-immune Lyt 1^+ T cells induced in vivo are potent secretors of IL 2 upon restimulation with macrophage-associated listerial antigen in vitro, indicating a role for IL 2 in acquired immunity against intracellular bacteria.

C57BL/6 mice were infected with approximately 4×10^3 to 6×10^3 live *L. monocytogenes* organisms (strain EGD) (5, 7). After 7 days, peritoneal exudates were induced by intraperitoneal injection of 1.5 ml of 10% protease peptone, and peritoneal exudate cells (PECs) were collected after another 3 days. Peritoneal exudate T lymphocytes (PETLs) were enriched by two passages of cells over nylon wool columns (4). As a source of normal macrophage, PECs from nonimmunized syngeneic mice were harvested 3 days after intraperitoneal injection of 1.5 ml of 10% protease peptone. PECs (10^6/ml) from nonimmunized mice were incubated in macrotiter plates at 37°C under 5% CO_2 for 2 h, and nonadherent cells were removed by extensive washing. Adherent PECs were cocultured with 5×10^7 heat-killed *L. monocytogenes* organisms for another 2 h, and then the supernatants were discarded. Different numbers of *L. monocytogenes*-immune PETLs were added to the antigen-pulsed PECs, and the cells were cultured in a total volume of 1 ml of RPMI 1640 medium containing 5% fetal calf serum, L-glutamine, penicillin, and streptomycin. After 24 h, supernatants were collected and tested for IL 2 activity by their ability to substitute for the requirements of helper T cells during the induction of Lyt 123^+ thymocytes to become alloreactive CTLs, as described previously (14).

The release of IL 2 activity required the presence of *L. monocytogenes*-immune T cells, normal syngeneic macrophage, and listerial antigen (Table 1). The amount of IL 2 activity depended upon the number of immune PETLs present in the culture; 10^5 PETLs was sufficient. Furthermore, IL 2 secretion appeared to be rather insensitive to X-irradiation (at 2,000 roentgens), although at low concentrations and at low effector-to-target cell ratios, some differences became manifest (Table 1).

To determine the approximate molecular weight of IL 2, supernatants were passed over a Sephadex G-75 column (Fig. 1). In accordance

TABLE 1. IL 2 activity after in vitro interactions between *Listeria*-immune PETLs and normal macrophage in the presence of heat-killed *L. monocytogenes*

Group no.	No. of PETLs in primary culture[a]	% Specific lysis of ^{51}Cr-labeled P 815 with following % (vol/vol) of supernatant assayed:											
		50			25			12.5			2.5		
		10:1[b]	2:1	0.4:1	10:1	2:1	0.4:1	10:1	2:1	0.4:1	10:1	2:1	0.4:1
1	10^4	11	0	0	2	0	0	0	0	0	0	0	0
2	10^5	73	23	8	49	9	0	20	1	0	0	0	0
3	10^6	75	72	52	75	61	26	77	33	12	67	16	8
4	10^6 (2,000 R)[c]	76	45	15	80	55	16	67	15	1	36	5	0
5	10^6 (no macrophage in culture)	11	0	0	8	0	0	0	0	0	0	0	0
6	10^6 (normal PETLs)	0	0	0	0	0	0	0	0	0	0	0	0

[a] Except as noted, *Listeria*-immune PETLs were cocultured with syngeneic macrophages and 5×10^7 heat-killed *L. monocytogenes* organisms for 24 h. Supernatants were tested for IL 2 activity by their ability to substitute for the requirements of helper T cells during the induction of Lyt 123^+ thymocytes to become alloreactive CTLs, as described previously (14). In the absence of either antigen or *Listeria*-immune PETLs, the percent specific lysis of target cells was 0.

[b] Ratio of effector to target cells.

[c] PETLs were irradiated with 2,000 roentgens before the primary culture with a Philips RT 200.

with the known molecular weight of IL 2, the helper activity resided in the 30,000-to-35,000 molecular weight fraction; no activity was demonstrable in the 18,000 molecular weight fraction.

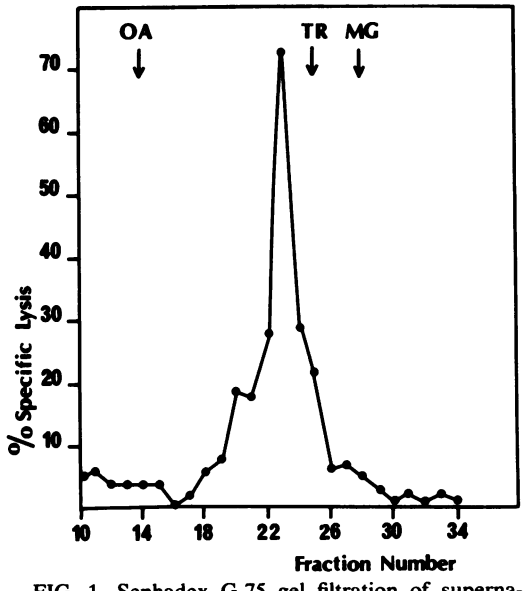

FIG. 1. Sephadex G-75 gel filtration of supernatants of *Listeria*-specific cultures. A total of 10^6 *Listeria*-immune PETLs were cocultured with 10^6 syngeneic macrophages and 5×10^7 heat-killed *L. monocytogenes* organisms. After 24 h, supernatants were collected, and 10 ml of pooled supernatant was passed over a Sephadex G-75 column. Fractions were assayed for IL 2 activity at 50% (vol/vol) by their ability to substitute for the requirements of helper T cells during the induction of Lyt 123^+ thymocytes to become alloreactive CTLs as described previously (14). Lysis of ^{51}Cr-labeled P 815 tumor cells was tested at a 10:1 ratio of effector to target cells. OA, Ovalbumin (45 kilodaltons); TR trypsin (24 kdaltons); MG, myoglobulin (17 kdaltons).

Next, the Lyt phenotype of the IL 2-secreting T-cell subset was determined. PETLs (3×10^7/ml) from *L. monocytogenes*-immune mice were treated at room temperature with anti-Lyt antiserum for 30 min, washed, and incubated in selected rabbit complement as described previously (5). Anti-Lyt antiserum had been prepared according to the method of Shen et al. (10) as described previously (11). Treatment of PETLs was repeated once. After treatments, 10^6 unselected PETLs or corresponding numbers of selected PETLs were cocultured with 10^6 *L. monocytogenes*-pulsed macrophage. After 24 h, supernatants were collected and tested for IL 2 activity by their ability to induce clonal expansion of alloreactive CTLs as described previously (14). *L. monocytogenes*-immune T cells responsible for IL 2 secretion bore the phenotype Thy 1^+ Lyt 1^+23^- (Table 2).

Our experiments demonstrate that *L. monocytogenes*-immune Lyt 1^+ T cells are potent secretors of IL 2 after interactions with syngeneic macrophage presenting listerial antigens in vitro. Earlier studies have shown that proteins mitogenic for thymocytes are produced in cocultures of *L. monocytogenes*-immune Lyt 1^+ T cells with macrophage and listerial antigen (16; Kaufmann et al., in press). These mitogenic activities mainly resided in the molecular weight range around 18,000 and presumably are a macrophage product (IL 1) (Kaufmann et al., in press). Thus, *L. monocytogenes*-immune Lyt 1^+ T cells are involved in secretion of IL 1 and IL 2, indicating that both interleukins might exert mediator functions in the generation of the immune response to intracellular bacteria.

TABLE 2. IL 2 secretion by *Listeria*-immune Lyt 1^+23^- T cells

Group no.	Primary culture[a]		% Specific lysis of ^{51}Cr-labeled P 815 with following % (vol/vol) of supernatant assayed:								
	Treatment of PETLs	T cell subset	50			25			5		
			10:1[b]	5:1	0.4:1	10:1	5:1	0.4:1	10:1	5:1	0.4:1
1	None	Unselected	70	34	11	63	44	13	37	9	1
2	C	Unselected	63	40	14	60	12	14	56	19	4
3	Anti-Thy 1.2 + C	None	0	1	0	0	1	1	3	0	2
4	Anti-Lyt 1.2 + C	Lyt 1^-23^+	0	0	0	0	2	3	0	0	0
5	Anti-Lyt 2.2, 3.2 + C	Lyt 1^+23^-	67	35	18	69	49	20	51	10	2
6	Mixture of groups 4 + 5 (1:1)	Lyt 1^+23^- + Lyt 1^-23^+	68	48	14	72	47	19	60	23	3
7	10^5 untreated PETLs	Unselected	66	34	5	41	13	6	26	3	1

[a] Equivalents of 10^6 *Listeria*-immune PETLs were cocultured with 10^6 syngeneic macrophage and 5×10^7 heat-killed *L. monocytogenes* organisms for 24 h. Supernatants were tested for IL 2 activity by their ability to induce clonal expansion of alloreactive CTLs, as described previously (14). C, Complement.

[b] Ratio of effector to target cells.

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