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In vivo IL-1 potentiates both specific and non-specific arms of immune response to infection

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SUMMARY

Injection of 10^5 U interleukin-1 (IL-1) 4 hr before intravenous infection with *Listeria monocytogenes* hastens recovery of mice. This is accompanied not only by early stimulation of colony-forming cells in the spleen to levels higher than those in untreated, infected mice but also by accelerated activation of lymphokine-producing, specific T lymphocytes.

Interleukin-1 (IL-1) was discovered by virtue of its role *in vitro* of activating T lymphocytes via induction of IL-2 (Dinarello, 1984). More recently it has been shown to potentiate the production of macrophages and granulocytes from haemopoietic stem cells, either *in vivo* (Neta *et al.*, 1987; Morrissey *et al.*, 1988) or *in vitro* in synergy with other factors (Ikebuchi *et al.*, 1988; Zhou *et al.*, 1988). IL-1 alone does not stimulate haemopoietic cells, and this activity could be mediated by a number of pathways. Itself produced by macrophages and other cells under various conditions of stimulation, including infection (Cahill & Hopper, 1982; Petit *et al.*, 1988), IL-1 has been shown to induce the production *in vitro* of colony-stimulating factors (CSF) by a wide variety of cells (Segal *et al.*, 1987; Zucali *et al.*, 1986). *In vivo*, IL-1 removes serum inhibitors of CSF (T. R. Bradley and N. Williams, personal communication; C. Cheers and Y. F. Zhan, unpublished data). It also up-regulates CSF receptors on early progenitor cells, so that more cells respond to the CSF (Zhou *et al.*, 1988).

IL-1 given before infection enhances resistance of mice rendered neutropenic by cyclophosphamide (Van't Wout *et al.*, 1988; McIntyre *et al.*, 1989) or of normal mice (Ozaki *et al.*, 1987; Czuprinski *et al.*, 1988; Kurlander *et al.*, 1989). An effect on the haemopoietic system and phagocytic cells was demonstrated or implied in each of these studies but no assays were made of T-cell mediated immunity. Therefore, this study undertook a systematic examination of the response of mice to the facultative intracellular bacterium, *Listeria monocytogenes*, with and without IL-1 treatment. Natural resistance of mice to *L. monocytogenes* is critically dependent on numbers of colony-forming cells in the bone marrow and spleen, which provide a rapid inflammatory response of highly bactericidal cells in genetically resistant mouse strains (Young & Cheers, 1986; Wood *et al.*, 1986). Later in the course of infection, T lymphocytes are activated, secreting lymphokines which increase the bactericidal efficiency of tissue macrophages and attract further monocytes/macrophages to the site. Thus acquired cell-mediated immunity is dependent on continued haemopoiesis to supply these inflammatory cells (North, 1970; Campbell, 1986).

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C57Bl/10 mice, bred and maintained in the Dept. of Microbiology, University of Melbourne, were injected intravenously with 10^5 U (0·34 μg) recombinant human IL-1α (Hoffman-La Roche, Nutley, NJ), 4 hr before intravenous challenge with approximately 10^4 *L. monocytogenes* (Young & Cheers, 1986), and various assays were performed 2–7 days later. In order to obviate day to day variations in efficiency of the assay systems, five mice per treatment group were injected on each of Days −2, −3, −4, −5 and −7, then all mice were killed on the same day. The bacterial doses were checked retrospectively (Egan & Cheers, 1990).

Weighed fragments of spleen and liver were homogenized in 5 ml distilled water and dilutions were sampled onto horse blood agar for viable bacterial counts, as described previously (Egan & Cheers, 1990). Cell suspensions in Iscove's modified Dulbecco's medium with 60 μg/ml penicillin, 100 μg/ml streptomycin and 10% fetal calf serum were prepared from the remaining spleen fragments and from bone marrow (Egan & Cheers,1990).

The cell suspensions were assayed for colony-forming cells (CFC) using triplicate 1-ml cultures containing 5×10^4 viable bone marrow cells or 5×10^5 viable spleen cells and CSF supplied by 0·1 ml of an optimal dilution of pooled serum from lipopolysaccharide-injected mice (Young & Cheers, 1986). After 6 days incubation, colonies of more than 50 cells were counted under a dissecting microscope (× 200 magnification).

To measure T-cell activation, spleen cells were cultured at a density of 4×10^6 viable cells in the presence of 10^8 heat-killed *Listeria* organisms in 2 ml (Egan & Cheers, 1990). After 1 or 3 days at 37° in 5% CO_2 the supernatants were harvested. IL-2 was measured by its ability to support proliferation of the IL-2-

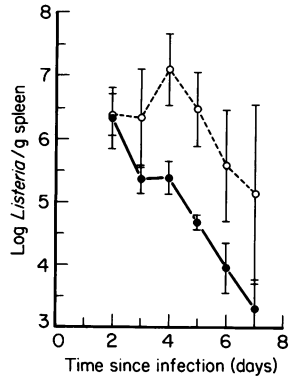

Figure 1. Effect of IL-1 on bacterial numbers in the spleens of *Listeria* infected mice. Mice were infected with 10^4 *Listeria* i.v. 4 hr after injection of 10^5 U IL-1 (●) or no IL-1 (○). Each point represents the mean and SD of five mice. Differences between treated and untreated groups were significant on Days 3–7 by Student's *t*-test. Day 3, $P<0.05$. Day 4, $P<0.001$. Days 5 and 6, $P<0.01$. Day 7, $P<0.05$.

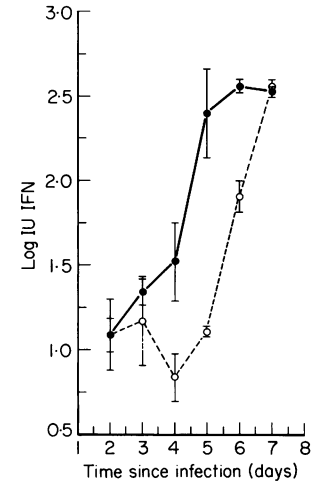

Figure 2. Effect of IL-1 on the *in vitro* production of IFN-γ by spleen cells from *Listeria*-infected mice. Spleen cell suspensions were prepared from mice infected with 10^4 *Listeria* i.v. 4 hr after injection of 10^5 U IL-1 (●) or no IL-1 (○). Each point represents the mean and SD of triplicate cultures. No IFN-γ was produced by cells from uninfected mice, either injected with IL-1 or not, or by any of the cells cultured without antigen.

Table 1. Effect of IL-1 on spleen CFC and bone marrow CFC of normal and infected mice

Day since infection	CFC/5 × 10^5 spleen cells		CFC/5 × 10^4 bone marrow cells	
	+IL-1	−IL-1	+IL-1	−IL-1
0	—	20±5	—	65±6
2	17±4	13±4	103±9	86±5
3	50±1	19±6	108±8	98±10
4	69±14	43±8	86±4	114±10
5	141±6	63±8	79±8	51±4
6	129±9	78±7	64±3	52±8
7	137±4	146±1	47±5	41±2

Mice were infected i.v. with 10^4 *Listeria monocytogenes*. Half were injected i.v. with 10^5 U IL-1 4 hr before infection. All data represent the mean of triplicate culture ± SD.

dependent cell line of CTLL (Gillis *et al.*, 1978). Uptake of [^{3}H]thymidine by the cells at 18–24 hr was taken as a measure of the relative amount of IL-2 present. Interferon (IFN) in the supernatants was assayed by its ability to protect mouse L cells from lysis with Semliki Forest virus (Egan & Cheers, 1990). IFN titres were expressed as International Units (IU) by comparison with an international reference standard (National Institute of Health, Bethesda, MD, catalogue number Gg 02-901-533). The identity of the IFN was checked by neutralization with a monoclonal antibody prepared from a rat–mouse hybridoma, R4-6A2 (ATCC, Rockville, MD) (Havell, 1986).

Figure 1 shows the protective effect of IL-1 on *Listeria*-infected mice. Protection was first evident 3 days after infection. This correlated with an early increase in CFC in the spleen of IL-1-treated mice, evident by Day 3 (Table 1). The IL-1-treated mice maintained a clear advantage over untreated mice until the seventh day. On the other hand, there was little or no effect of IL-1 on CFC in the bone marrow (Table 1), which appears to be less sensitive to outside influences than the spleen (Metcalf, 1982). This depression of bacterial growth and enhancement of spleen CFC numbers has been observed in five consecutive experiments.

T-cell activation in the infected mice was measured *in vitro* by the production of two lymphokines, IFN-γ and IL-2, and the results presented are typical of three experiments performed. Interferon in the supernatants of 3-day cultures is shown in Fig. 2. IFN-γ-producing T cells were first clearly demonstrated 5 days after infection in IL-1-treated mice and even at 6 days cells from the IL-1-treated mice produced more IFN-γ than untreated mice. By 7 days post-infection, each group showed similar levels of IFN-γ production.

Because activated T cells bear receptors for IL-2, this lymphokine is rapidly absorbed during *in vitro* culture (Miller & Orme, 1989). However, it was detectable in 1-day cultures of spleen cells from IL-1-treated mice infected 5 days earlier (Table 2). Again IL-1-treated mice showed a clear advantage over the untreated until 7 days post-infection.

It is thus clear that IL-1 treatment of mice potentiates both the non-specific and the specific arms of the immune response to this facultative intracellular bacterium. From the chronology of the results, it seems likely that the effect on CFC in the spleen contributes to the early arrest of bacterial numbers in that organ. Nevertheless, it is possible that T-cell activation *in vivo* is effective earlier than apparent from the *in vitro* assay, which may be insufficiently sensitive to detect small early changes. Although the effect of IL-1 treatment on T-cell activation appears later than its effect on CFC, anything that potentiates the expansion of the T-cell population can only aid in recovery from infection, and contribute to immunological memory to protect against future infection. It is not yet clear whether the effect of IL-1 accelerating T-cell activation is a direct one on antigen presentation and T-cell activation or is secondary to the

Table 2. Effect of IL-1 treatment on IL-2 production by spleen cells from *Listeria* infected mice*

Time since infection (days)	IL-2 activity (c.p.m. ± SD)†	
	Untreated	IL-1 treated
0	80 ± 10	—
2	74 ± 23	74 ± 34
3	76 ± 59	59 ± 3
4	52 ± 18	59 ± 15
5	46 ± 9	1084 ± 314
6	515 ± 137	5560 ± 296
7	10,502 ± 2186	10,144 ± 1816

*Mice were infected i.v. with 10^4 *L. monocytogenes*. Half were injected i.v. with 10^5 U IL-1 4 hr before infection.

†IL-2 activity measured by uptake of [^{3}H]thymidine by 3×10^3 CTLL cells. Mean ± standard deviation of triplicate cultures. Background proliferation of CTLL cells in the absence of IL-2 has been subtracted. No IL-2 was produced by cells from uninfected mice either injected with IL-2 or not, or by any of the cells cultured without antigen.

lighter bacterial load in the treated mice. However, it is generally agreed that lower bacterial numbers decrease immunizing efficiency (Berche, Gaillard & Sansonetti, 1987), suggesting that IL-1 is acting directly.

These and other questions as to the mode of action of IL-1 are currently under investigation. Whatever the answers, these experiments are extremely encouraging to those contemplating the use of IL-1 in therapy of immune deficiencies of all sorts.

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