PHYSIOLOGY

Immunomodulating Effects of α-Interferon in *Pseudomonas aeruginosa* Infection

L. A. Ganova, N. Ya. Spivak, and V. A. Znamenskii*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 8, pp. 124-127, August, 1996
Original article submitted May 25, 1995

It is shown that *Pseudomonas aeruginosa* infection is accompanied in mice by impaired functional activity of lymphocytes and by greatly increased production of the mediators interleukin-1 and tumor necrosis factor, which prevents the development of adequate cell-mediated immune reactions and inhibits effector functions of macrophages and killer cells. After a single injection of α-interferon, the functional maturation of lymphocytes, particularly T cells, is rapidly restored, as well as the production of the indicated regulatory mediators, so that the deficit of effector cells is corrected.

Key Words: *interferon, effector cells, cytokines*

One of the most important properties of α-interferon (IFN) is pronounced immunomodulating activity [4]. The protective effect of IFN in bacterial infections is largely due to its ability to restitute effector functions of phagocytes and killers [10], but the mechanisms through which it exerts its immunomodulating action on these cells have not been elucidated.

In addition to phagocytizing and killing bacteria, macrophages produce major regulatory mediators such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) that initiate cellular immune reactions [1].

The development of *Pseudomonas aeruginosa* infection is characterized by substantial inhibition of the cellular immunity [7], involving impaired maturation of T and B lymphocytes which produce and release lymphokines mediating cellular reactions of immunity [1]. It was therefore interesting to find out how the relationship between the effector functions of macrophages and killer cells, on the one hand, and the proliferative activity of lymphocytes and cytokine (IL-1 and TNF) levels, on the other, may be influenced by IFN in animals with *P. aeruginosa* infection.

Department of Interferon and Immunomodulators, Institute of Microbiology and Virology, Ukrainian Academy of Sciences; *Institute for Advanced Medical Studies, Ministry of Health of Ukraine, Kiev

MATERIALS AND METHODS

C57Bl/6 mice weighing 18-20 g were used. Test mice were infected intraperitoneally at the LD_{50} level (1×10^7 cells per mouse) with a 24-hour culture of the exotoxin A-producing *P. aeruginosa* museum strain 103. Murine type I IFN, obtained as previously described [3], was injected intraperitoneally in a dose of 500 U/mouse within 24 h of the infection. Intact mice injected with this IFN in the same dose served as controls. At defined intervals, at least 6 mice from each group were decapitated and the immunological parameters of interest were recorded.

Peritoneal exudate macrophages, splenocytes, and thymocytes were obtained from the mice by the conventional method [2].

The phagocytic activity of macrophages was evaluated using the *E. coli* test culture 0111:B4 [3], by determining the number of phagocytes per 100 cells and the number of bacteria engulfed by a phagocyte.

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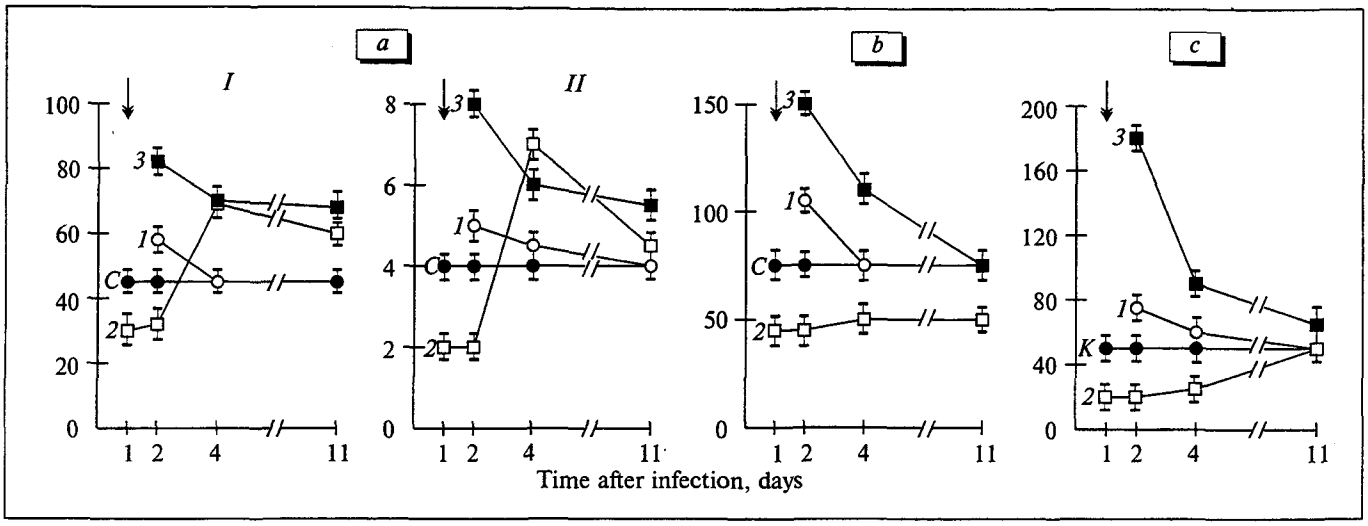

Fig. 1. Disordered effector functions of peritoneal macrophages and killer cells in *P. aeruginosa*-infected mice (*2*) and correction of these functions by interferon (*3*). Ordinate: *a*) phagocytic activity of cells (*I* = % of phagocytes per 100 cells; *II* = number of phagocyte-engulfed bacteria, rel.units); *b*) bactericidal activity of cells, optical density units×100; *c*) natural killer activity, %. Here and in Figs. 2 and 3: *C*) control (intact) mice; *1*) interferon-treated mice (500 U/mouse). Arrows mark the time of interferon injection.

The bactericidal activity of peritoneal macrophages was measured in the nitro blue tetrazolium test using 96-well plates (Linbro) [2]. To each well containing 50 μl 0.1% nitro blue tetrazolium, 2×10^6 cells in 100 μl RPMI-1640 medium were added, incubated at 37°C for 20 min, sedimented by centrifugation at 400*g* for 10 min, and then disintegrated with 200 μl of DMSO (Sigma) after removing the supernatant. Optical density of the reduced diformazan was measured on a multiscan (Dynatech) at λ=540 nm against a well containing DMSO with no cells.

Natural killer activity was determined spectrophotometrically [2]. As targets, murine L-929 fibroblasts were used, the effector:target ratio being 100:1.

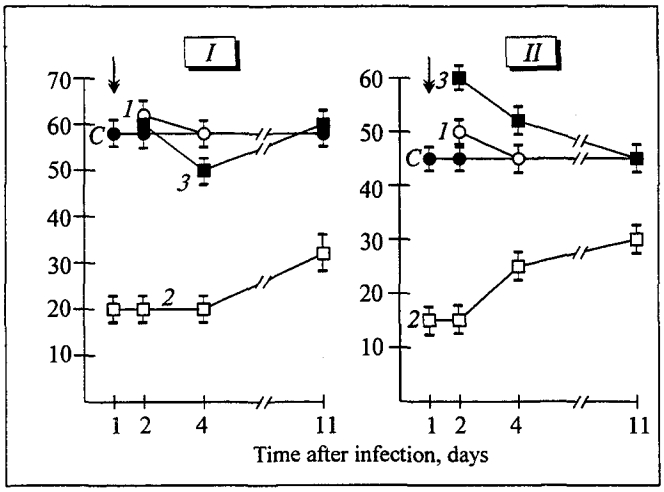

Fig. 2. Effects of interferon (*3*) on the blast transformation of splenocytes (*I*) in response to lipopolysaccharide and of thymocytes (*II*) in response to phytohemagglutinin in *P. aeruginosa*-infected mice (*2*). Ordinate: stimulation index, %.

The results were read on the multiscan at λ=490 nm, calculating the index of natural killer activity from the formula $(T_t-T_c)/T_c\times100\%$, where T_t and T_c are optical densities of the supernatants in the test and control wells, respectively.

The blast transformation reactions of splenocytes and thymocytes were evaluated by the rate of ^{3}H-thymidine incorporation into cellular DNA [2]. The cells were stimulated with *E. coli* 0111:B4 lipopolysaccharide or phytohemagglutinin (both from Sigma) added to final concentrations of 4 μg and 0.1 μg, respectively. The stimulation index was calculated from the formula $(a-b)/a\times100\%$, where *a* and *b* are readings in the stimulated and spontaneous samples, respectively.

IL-1 was assayed with comitogenic stimulation of ^{3}H-thymidine-labeled (final concentration=1 μCi) BALB/c thymocytes [3].

The biological activity of TNF was assessed by its cytotoxic action on the transplantable culture L-929 [3], and the results were read on the multiscan at λ=540 nm with calculation of the cytotoxicity index by the formula $(C-T)/C\times100\%$, where *C* and *T* are optical densities of wells containing cells treated with the cultivation medium (RPMI-1640 with 10% FCS) and with the test supernatant, respectively.

The data were statistically analyzed as described earlier [2].

RESULTS

The development of *P. aeruginosa* infection was associated with a substantial decline of the effector functions performed by phagocytes and killer cells

(Fig. 1). Particularly inhibited were the phagocytic and bactericidal activities of macrophages. Natural killer activity was also depressed over a prolonged period. The ingesting capacity of phagocytes and natural killer activity were restored on days 4 and 11 postinfection, respectively. The bactericidal activity of peritoneal exudate macrophages had risen slightly by day 4 but remained depressed throughout the 11-day observation period. A single IFN injection into infected mice resulted in rapid stimulation of the effector cells whose activity on days 4-11 was similar to that recorded for intact animals.

The formation of a trigger signal for effector cells is largely determined by proliferative and functional activities of lymphocytes [10]. As shown in Fig. 2, in infected mice the proliferative responses of B and T cells to the specific inducers were strongly inhibited. IFN injection into infected mice enhanced these responses, especially that of thymocytes. Since a proliferative response to mitogen involves production of interleukin-2 (IL-2) and expression of receptors for it, IFN appears capable of influencing not only phenotypic changes [6], but also the functional maturation of T cells.

We recorded IL-1 and TNF production upon cultivation of a spleen cell pool where the functional activity of macrophages is under the control of regulatory lymphocytes. During the development of *P. aeruginosa* infection, the production of these two mediators was greatly increased, especially on day 1 after infection (Fig. 3). Subsequently, their levels, particularly of TNF, decreased, and IL-1 production on day 4 was somewhat higher than that of TNF relative to its values in intact mice. For IFN-injected infected mice, alterations in the production of these mediators were similar but occurred earlier, on day 1 postinjection. On day 4, the further fall in IL-1 combined in this group with a greatly enhanced TNF production by splenocytes, followed by its decline to values recorded for intact mice.

Lipopolysaccharide is known to induce IL-1 and TNF production in the body [9]. A sharp rise in the levels of mediators initiating cellular immune reactions can probably block the reception of regulatory signals by immunocompetent cells with the result that the effector functions of these are suppressed. Production of the indicated monokines is restituted via selective regulation of cAMP and cGMP syntheses [9]. The IL-1 level is also lowered as a consequence of IgG binding to monocytes, which initiates synthesis of an antagonist for the IL-1 receptor [8]. Our results warrant the conclusion that IFN not only lowers the overall levels of IL-1 and TNF, but also

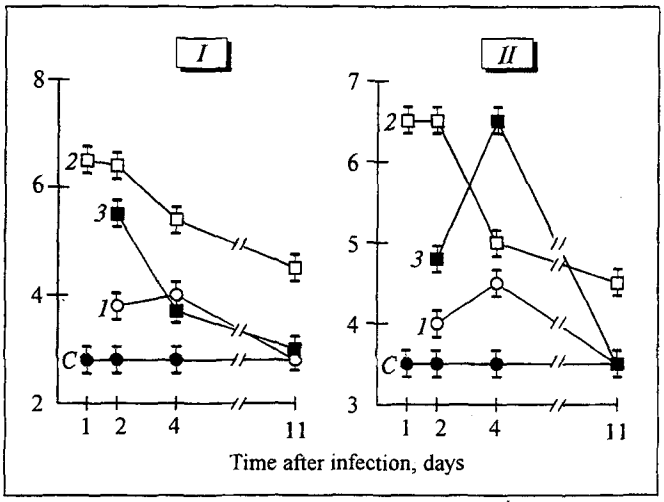

Fig. 3. Modulation of interleukin-1 (*I*) and tumor necrosis factor (*II*) production in *P. aeruginosa*-infected mice and correction of their production by interferon (*3*). Ordinate: level of cytokine production: *I*) rate of radiolabel incorporation into cellular DNA, cpm×10³; *II*) cytotoxicity index, ×100%.

elicits rapid correction of their production, which is probably a necessary condition for restoring immunoreactivity of the organism. The modulating action of IFN on the synthesis of these two cytokines can apparently be mediated by arachidonic acid metabolites [10] and the cyclic nucleotide system [5].

In summary, IFN produces immunomodulating effects on macrophages and killers, whose effector functions are inhibited in *P. aeruginosa* infection, by virtue of its ability to correct rapidly the functional maturation of lymphocytes, notably T cells, and the production of the monokines IL-1 and TNF which mediate the cellular immune response in its early phases.

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