Human Polymorphonuclear Neutrophil Functions Are Unaffected by Human Interferon-α2

BARRY FARR, JACK M. GWALTNEY, JR., FREDERICK G. HAYDEN, AND GERALD L. MANDELL

Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Received 31 May 1983/Accepted 27 August 1983

Leukocyte-derived interferons have been reported to enhance the functions of polymorphonuclear neutrophils. This study reveals no effect of the highly purified recombinant DNA-produced human interferon-α2 on the oxidative, chemotactic, adherent, phagocytic, and bactericidal activities of human polymorphonuclear neutrophils in vitro.

Studies with leukocyte-derived interferon (IFN) have reported stimulatory effects on neutrophil function, including stimulation of nitroblue tetrazolium (NBT) dye reduction (16) and of phagocytosis (1, 14). Because the purity of leukocyte-derived preparations was sometimes less than 1%, we chose to study the effects of a highly purified recombinant DNA-produced human IFN on the function of human neutrophils in vitro.

Human IFN-α2 (HuIFN-α2) (specific activity, 108 IU/mg of protein) was provided by the Schering Corp., Bloomfield, N.J. The lyophilized powder was reconstituted in normal saline, frozen in multiple aliquots at −70°C, and thawed immediately before use. Antiviral activity in an appropriate titer was confirmed by bioassay against encephalomyocarditis virus in an independent laboratory (19).

Natural killer cell activity, measured by 51Cr release from target K-562 cells and studied as a second control to show HuIFN-α2 activity (2, 4, 7), was enhanced by an average of 350% in cells of two donors after 12 h of preincubation with HuIFN-α2 at a concentration of 200 IU/ml.

Blood was obtained from healthy volunteers without prior history of IFN therapy and processed as required for the different assays described below. Polymorphonuclear neutrophils (PMN) were incubated with HuIFN-α2 at concentrations ranging from 10 to 106 IU/ml for 20 min at 37°C and then compared with control PMN from the same donor incubated with Hanks balanced salt solution (HBSS). Because a prior study (14) with leukocyte-derived IFN had shown enhancement of phagocytosis by PMN only after a longer (3-h) preincubation with the IFN preparation, phagocytic and bactericidal assays were performed after a 20-min preincubation and after a 3-h preincubation with HuIFN-α2.

Phagocytic and bactericidal assays with Staphylococcus aureus 502A were performed as previously described (11). Initial PMN-to-bacteria ratios were ca. 1:1. Total, supernatant (cell-free), and sediment (cell-associated) bacterial counts in the samples were measured by differential centrifugation, serial dilution, and quantitative culture.

NBT dye reduction was studied by the method described previously by Matula et al. (13) and utilized by Pak in studies with leukocyte-derived IFN (16).

Chemiluminescence was measured continuously in a Chemglow Photometer at 37°C (Aminco, Silver Spring, Md.) as previously described (11).

Granulocyte adherence was studied in nylon fiber columns, through which whole heparinized blood was filtered at 37°C after prior incubation with HuIFN-α2 (10 to 104 IU/ml), HBSS, or endotoxin (0.005 μg/ml) for 20 min at 37°C (12).

Migration to chemotactic stimuli was measured in agarose plates (15) after prior incubation with HuIFN-α2 (10 to 105 IU/ml) or HBSS. The chemotactic response of normal PMN to HuIFN-α2 in concentrations of 102 to 106 IU/ml was also assessed.

Statistical analysis was performed with the paired t test for each set of experiments.

In the phagocytic and bactericidal assay, bacteria were removed from the supernatant with equal efficacy (>99% removal by 1 h) by PMN preincubated for 20 min with HBSS or HuIFN-α2 (10 to 104 IU/ml). The number of cell-associated bacteria was similar in the two groups at each time point, and total bacterial counts declined by ca. 99% with both control and HuIFN-α2-treated PMN. Paired T testing showed no significant differences at any time point. Similar results were obtained with a 3-h preincubation with 103 or 104 IU of HuIFN-α2 per ml.
PMN incubated with HBSS or HuIFN-α2 (10 to 10^4 IU/ml) exhibited ca. 5% NBT response, whereas endotoxin-treated cells showed a 72% NBT response.

PMN showed normal chemiluminescence with opsonized zymosan, f-methionine-phenylalanine and phorbol myristate acetate. Chemiluminescence was not stimulated by HuIFN-α2 (10 to 10^4 IU/ml), nor did prior (20-min) preincubation with these concentrations of HuIFN-α2 affect chemiluminescent response to opsonized zymosan.

Adherence of PMN to nylon fibers was 30.8 ± 5.7%. Adherence was not significantly altered by prior incubation with HuIFN-α2 (10 to 10^4 IU/ml), but was increased to 47.3 ± 2.2% in PMN exposed to endotoxin (P = 0.03, paired t test).

Control PMN exhibited normal chemotactic responses to f-methionine-phenylalanine (10^-4 M), which were not significantly altered by prior incubation with HuIFN-α2 (10 to 10^5 IU/ml). Control PMN exhibited no chemotaxis to HuIFN-α2 (10^2 to 10^5 IU/ml).

These negative results differ from previously published studies showing stimulatory effects in vitro on PMN by leukocyte-derived IFN preparations at the same IFN concentrations used in this study. Most of the previous studies showed effects after 0- to 30-min preincubations with the leukocyte-derived preparations, although one study required 3 h of preincubation for enhancement of phagocytosis (14). Our study with HuIFN-α2 showed no effect on five PMN functions after a 20-min preincubation, and no effect on phagocytosis or bactericidal activity after a 3-h preincubation as well. Other biological activities of this lot of HuIFN-α2 were confirmed by assay of antiviral activity and enhancement of natural killer cell activity.

As mentioned above, the leukocyte-derived IFN preparations used in previous studies varied widely in the degree of purification. Some preparations contained protein that was probably less than 1% IFN (1, 3, 5, 6, 8-10, 14, 16-18), so that some differences could be accounted for by impurities in the leukocyte-derived preparations. Some of these studies attempted to show specificity by abolition of the effect in the presence of antibody to the leukocyte-derived IFN preparations; but the inhibition of activity by polyclonal antibodies raised to an impure preparation does not necessarily prove that the activity was due to IFN, a minor constituent in the mixture. However, it should be noted that unlike the recombinant DNA-produced HuIFN-α2, multiple IFNs are present in the leukocyte-derived preparations, and it remains possible that some IFN different from HuIFN-α2 may be mediating these effects on PMN. In addition, our studies do not exclude the possibility of some indirect effect by HuIFN-α2 on PMN occurring in vivo.

(This work was presented in part at the Southern Section of the American Federation of Clinical Research, New Orleans, 27 January 1983.)

We thank R. D. Pearson and D. L. Smith for assistance in the natural killer cell assay.

Supported in part by Public Health Service grants A107046 and A129504 from the National Institutes of Health.

LITERATURE CITED


