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RECOMBINANT GAMMA INTERFERON ENHANCES NATURAL KILLER CELL ACTIVITY SIMILAR TO NATURAL GAMMA INTERFERON

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SUMMARY: The enhancement of human natural killing activity by recombinant human gamma interferon (IFNγ) and natural human IFNγ were similar over a wide concentration range. Enhancement of natural killing activity by both interferons was neutralizable by antibody to natural IFNγ, as well as by antibody to a synthetic peptide representing the first 20 N-terminal amino acids of IFNγ based on cDNA structure. The findings with recombinant IFNγ provide conclusive evidence that IFNγ is responsible for the enhanced natural killing activity seen in IFNγ preparations.

Interferons (IFN) have been shown to augment natural killing (NK) activity after in vivo and in vitro administration (1-4). Our studies in vitro have shown that partially purified IFNγ was 100 times more active per unit of antiviral activity in the enhancement of NK activity than either IFNα or IFNβ against mouse L cell targets (5). Preparations of IFN contain other types of biological activity that may be important and contribute to the cytotoxicity (6,7). Therefore, we determined whether preparations of recombinant IFNγ enhanced NK activity to the same extent as natural IFNγ. In the present study, we also show the inhibition of IFNγ enhancement of NK activity with antibody to natural IFNγ, as well as with antibodies to a synthetic peptide comprising the 20 N-terminal amino acids of human IFNγ as determined from the cDNA sequence (8). The results provide conclusive evidence that IFNγ is responsible for the enhanced NK activity associated with IFNγ preparations.

Abbreviations used are:
IFNγ, gamma interferon; NK, natural killing; CPE, cytopathogenic effect; FBS, fetal bovine serum.

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MATERIALS AND METHODS

Interferon (IFN) assay. Human IFN was quantitated in human amnion WISH cells using a previously described cytopathogenic effect (CPE) inhibition microassay (9). Sindbis virus was used as challenge at an input multiplicity of 50. Titers (units/ml) are expressed as the reciprocal of the IFN dilution that inhibits 50% of the CPE. Since there is no reference standard for IFNγ, an internal laboratory standard was used (10).

Leukocyte preparation. Leukocytes were prepared from human peripheral blood by the Ficoll-Hypaque gradient separation method (11). Plastic adherent cells were removed by incubating 5 ml of a 5×10^6/ml leukocyte suspension in plastic tissue culture dishes for 1 h at 37C. All enriched leukocyte populations were suspended at a final concentration of 10×10^6 cells/ml in Eagle minimal essential medium (EMEM) supplemented with 2% fetal bovine serum (FBS). For IFNγ treatment, 0.3 ml of leukocyte suspension (10×10^6 cells/ml) was added to dilutions of the IFNγ or IFNγ neutralized by specific antibody and incubated for 18 h at 37C. The cells were washed twice by centrifugation, suspended in EMEM and added to target cells as described below. Control lymphocytes were incubated in EMEM without IFN, and otherwise treated identically with the test lymphocytes. Human IFNγ, $10^{5.2}$ units per mg protein, was prepared according to previously described methods (10). Recombinant human IFNγ was produced by transfection of monkey cells with the cDNA and kindly provided by Genentech Inc. (12). The methods of production and use of the specific antisera used in these studies has been previously described (13).

Assay of NK activity. Mouse L cells were propagated in 96 well microtiter plates (Falcon Plastics, Oxnard, Calif.) in EMEM with antibiotics and 2% FBS and prelabeled with sodium chromate (^{51}Cr) at a concentration of 10 μCi/2×10^5 cells/4 h at 37C. After incubation, the target cells were washed three times with EMEM and human lymphocytes added at an effector to target cell ratio of 10:1. Specific ^{51}Cr release was determined from triplicate cultures after incubation at 37C for 18 h. The percent specific ^{51}Cr-release was calculated as R=[E-S/M-S] x 100 where E is cpm in the experimental well, S is spontaneous release and M is the maximal release in the presence of 1% saponin.

RESULTS AND DISCUSSION

Various concentrations of recombinant IFNγ and partially purified IFNγ were compared for their relative abilities to enhance NK activity of human peripheral lymphocytes against mouse L cell targets. The enhancement of killing was similar for the two IFNs over a wide concentration range (Figure 1). Thus, recombinant IFNγ was capable of enhancing killing in a manner similar to that of natural IFNγ. We next determined whether enhanced killing activity by recombinant IFNγ could be neutralized by antibody to natural IFNγ and by antibody to a synthetic peptide representing the first 20 N-terminal amino acids of

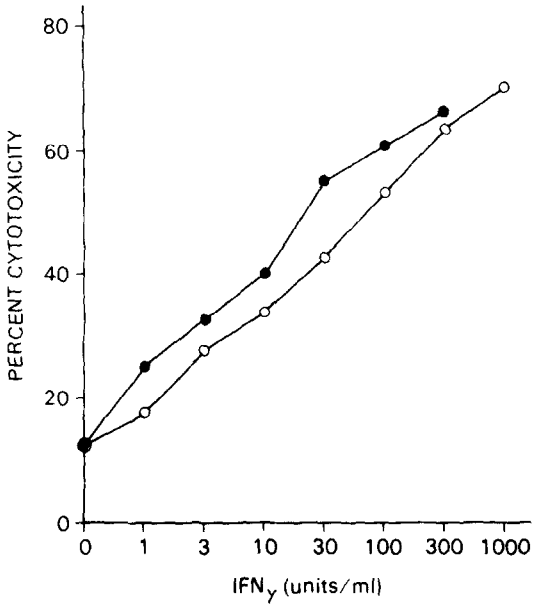

Figure 1. Effect of recombinant and natural IFNγ on natural killing activity of human lymphocytes. Human lymphocytes were treated with various concentrations of natural IFNγ (•--•) and recombinant IFNγ (o--o) for 16 h, washed and tested at a ratio of 10 lymphocytes to one mouse L cell in an 18 h ^{51}Cr release assay as described in the Materials and Methods.

IFNγ based on cDNA structure. The data show that both preparations of IFNγ were equally and completely neutralized with regards to enhancement of natural killing by antibody to the whole molecule or by antibody to the N-terminal sequence alone (Figure 2). The antiviral activity of the recombinant and natural IFNγs was also neutralized to the same extent by antibody to the whole molecule or to the peptide (data not shown). Thus, recombinant and natural IFNγ behaved the same in NK and antiviral activity and were neutralized to the same extent by specific antibodies.

The fact that recombinant IFNγ can enhance NK activity in a manner not unlike that of partially purified IFNγ is evidence that IFNγ is responsible for the enhanced killing associated with IFNγ preparations. We have recently produced antibodies in rabbits to a synthetic peptide encoded by the 5' end of human IFNγ cDNA that neutralized the antiviral activity of natural IFNγ (8). The fact that

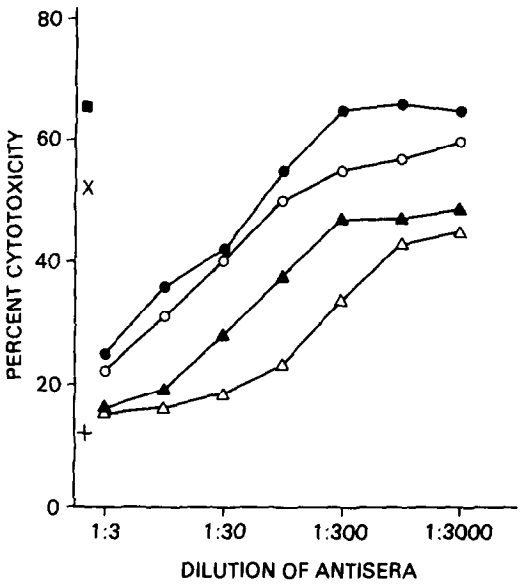

Figure 2. Neutralization of enhanced NK activity by recombinant and natural IFNγ with antibodies to natural IFNγ and to synthetic peptide. Ten units of recombinant IFNγ and 30 units of natural IFNγ, by back titration, were tested in neutralizations against various dilutions of antibodies to IFNγ and to synthetic peptide by incubation for 1 h at room temperature prior to testing the IFNγs for enhanced natural killing activity. One ml of each antiserum neutralized 5-10,000 units of human IFNγ activity. Key: ●, natural IFNγ + anti-IFNγ; 0, natural IFNγ + anti-peptide; ▲, recombinant IFNγ + anti-IFNγ; Δ, recombinant IFNγ + anti-peptide; ■, natural IFNγ alone; X, recombinant IFNγ alone; +, mock IFN alone or mock IFN plus antibody.

this antibody completely neutralized the ability of both natural and recombinant IFNγ to enhance killing is further evidence that IFNγ, or at least an antigenically and structurally related lymphokine, is responsible for the enhanced killing activity seen in IFNγ preparations. It also follows that non-IFNγ substances from monkey cells are not involved in NK or antiviral activities of the recombinant IFNγ preparation. Further, neutralization of both the antiviral and the NK activity of IFNγ by antibodies to the N-terminal part of the molecule suggests that the N-terminal end of the IFNγ protein plays an important role in the expression of its biological activity. Antibodies to other sequences of amino acids in the IFNγ molecule will further help determine the structure-function properties of IFNγ.

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