Natural Killer Cell Activity From Hemophiliacs Exhibits Differential Responses to Various Forms of Interferon

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Patients with hemophilia are at risk for the development of acquired immunodeficiency syndrome (AIDS). Patients with AIDS have recurrent infections and/or malignancy and altered immune response, including decreased T lymphocyte counts, decreased T helper lymphocytes, defective T cell blastogenesis, hypergammaglobulinemia, defective natural killer (NK) activity and impaired response of NK to interferon-β (IFN-β). It is feasible that chronic antigen stimulation with subsequent release of interferon could be related to the impaired NK reactivity to IFN-β of patients with AIDS. Because hemophiliacs are subjected to chronic antigen stimulation secondary to the administration of foreign protein, the reactivity of NK cells from patients with hemophilia to IFN-α, IFN-β and IFN-γ was studied. Eight patients with hemophilia requiring high levels of clotting factor replacement were assessed. Three patients were antibody positive to HTLV-III. All had normal baseline NK cell function. In the first set of experiments, all patients responded normally to in vitro IFN-α by increasing NK activity, but four patients had significant failure and two had mild impairment in NK response to IFN-β. This latter observation was particularly evident at very low concentrations of IFN. In repeated experiments, seven of eight had impaired NK response to IFN-β and IFN-γ but normal response to IFN-α. Only one patient's NK cells responded better to IFN-γ. There was no obvious correlation of these findings to antibody status to HTLV-III. Chronic antigen stimulation and the modulation of interferon receptors are discussed as possible mechanisms that could produce these findings.

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Delayed hypersensitivity skin testing. The CMI-Multitest kit (Rhône-Poulenc, Montreal) was used. The quantitative and qualitative responses were recorded at 48 hours as recommended by the supplier.

RESULTS

All patients had white blood cell counts above 3.0 x 10^9/L, and only patient 7 had lymphopenia of <1 x 10^9 lymphocytes/L. Patient 7 also had a platelet count of 76 x 10^9/L; the platelet counts of the other patients were normal. Hypergammaglobulinemia was present in patients 2, 5, 6, and 8 in the IgG fraction. Patient 5 had an elevated IgA and patient 2 had an increase in his IgM level. The hypergammaglobulinemia was polyclonal in all cases as judged by protein electrophoresis. All patients had positive responses to at least one of the antigens tested by delayed hypersensitivity skin testing, using the CMI Multitest with a minimum recorded response of 3 mm of induration. Hence, none of the patients was completely anergic (data not shown).

Standard cell surface markers were assessed using monoclonal antibodies and a Becton Dickinson cytofluorograph (Table 1). All except patients 2 and 7 had an adequate percentage of T cells, and all except patients 5 and 7 had an adequate percentage of OKT4+ cells. Patient 5 had a decreased percentage of suppressor T cells. Patient 7 had decreased absolute helper and suppressor cell numbers. In patient 7, the helper (OKT4) to suppressor (OKT8) ratio was 0.6, for all others, it was >1.0. The percentage of Ig-positive B cells was normal and ranged from 5% to 11%. The percentage of Leu-7-positive cells ranged from 8% to 22% and was within normal limits. Hence, those patients with abnormalities in lymphocyte markers were those who had antibody to HTLV-III (patients 2, 5, and 7 from Table 1). The baseline level of NK cell activity was adequate (Fig 1). Most patients did not respond well to IFN-β, but did respond to IFN-α. IFN-β at 100 U/2 x 10^6 PBMCs in 0.6 mL of medium failed to induce (or induced to a lesser extent) increments in the percentage of lysis when compared with IFN-α. The NK activity of PBMCs from hemophilia patients averaged 60.6% (± 11.2%) when incubated with IFN-α and 50.4% (± 7.3%) when treated with IFN-β. The baseline NK average was 48.0% (± 8.8). Using a paired t test, there was a significant difference between the IFN-α-induced and IFN-β-induced responses (P < .001). The NK response of PBMCs from controls run simultaneously with the hemophilic PBMCs did not reveal any significant difference in response to IFN-α or IFN-β.

To assess this phenomenon further, the response of the NK cells to low concentrations of IFN-α and IFN-β was assessed in four separate experiments (Fig 2). The control PBMCs demonstrated the same dose–response curves to IFN-α as to IFN-β. However, the PBMCs of the hemophilia patients 1, 5, 6, and 8 were markedly less responsive to IFN-β than to IFN-α. Patients 2 and 3 demonstrated the same pattern, but to a lesser degree; only patient 4 responded better to IFN-β. Patient 8 was assessed on two separate occasions; the results were reproducible in that each time the PBMCs responded to IFN-α but essentially did not respond at all to IFN-β. Hence, the NK response to IFN-β was significantly depressed in four of eight patients and mildly depressed in 2 others.

All patients were reassessed again in terms of their responsiveness to all three types of IFN, α, β and γ. From Fig 3, it can be seen that control PBMCs tend to respond similarly to IFN-α, IFN-β and IFN-γ. However, patients with hemophilia responded normally to IFN-α but poorly to IFN-β and IFN-γ (patients 2, 3, 4, 5, 6, and 8). Patient 1 responded better to IFN-γ and demonstrated no real difference in response to IFN-α and IFN-β. Patient 7 did not show any significant defect in response to the three forms of IFN.

Therefore, most patients with hemophilia demonstrated a defective NK response to IFN-β and IFN-γ but normal response to IFN-α. Although some exceptions in reproducibility exist (eg, patients 1 and 4 in Figs 2 and 3), most patients have a consistently impaired NK response to IFN-β (Figs 1, 2, and 3).

DISCUSSION

These data indicate that most patients had essentially normal routine hematology and marker data. Minor aberra-
Fig 2. Four experiments comparing the natural killer (NK) response of PBMC from patients or controls to IFN-α ( --- ) or to IFN-β ( ---- ) are shown. IFN concentrations are expressed as U/2 x 10⁶ peripheral blood mononuclear cells in 0.6 mL. NK activity is expressed as lytic units per 10⁶ effector cells.

Fig 3. Six experiments comparing the natural killer (NK) response of peripheral blood mononuclear cells of patients and controls to IFN-α ( --- ), IFN-β ( ---- ), IFN-γ ( ..... ) are shown. NK activity is expressed as in Fig 2.
known if this abnormal IFN modulates cellular reactivity through the IFN-α receptor.

Therefore, there is evidence for an impaired NK augmentation secondary to IFN-β and IFN-γ which was common in patients with hemophilia and which did not correlate with the presence of antibody to HTLV-III. These data are consistent with the suggestion that the receptors for IFN-β and IFN-γ are correlated, if not identical, and the receptor for IFN-α is different. Down modulation of IFN receptors may be secondary to chronic antigen stimulation, presumably as a result of the continuous requirement for foreign protein related to coagulant therapy.

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REFERENCES

2. Popovic M, Sarngadharan MG, Read E, Gallo RC: Detection isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224:497, 1984
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