INTERFERON STUDIES IN CHILDREN: A NEW ASSAY OF INTERFERON PRODUCTION AND ACTIVITY

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The interferon (IF) system consists of IF production on the one hand and the response to IF on the other. The purpose of this study is to try to evaluate both these aspects of the IF system by simultaneously assessing serum and cells from children and attempting to correlate the findings with their clinical status.

The parameters evaluated were: (1) *in vivo* serum IF levels; (2) *in vitro* production of types I and II IF by peripheral blood mononuclear cells (PBMC) following stimulation with poly-IC and PHA as well as with no extrinsic stimulation; (3) the ability of PBMC to promote viral replication, and (4) their sensitivity, *in vitro*, to the antiviral effect of exogenous human IF. We have assessed these parameters in healthy children and in those with viral, neoplastic, and immunodeficiency diseases.

Materials and Methods

Using micromethods, 2–5 ml of blood was sufficient for these studies which were done on 23 normal children, 17 children with viral illnesses, 12 with varied immunodeficiency diseases, and 9 with neoplasms.

The assay is based on the cytopathic effect (CPE) of vesicular stomatitis virus (VSV) on the human IF-sensitive fibroblast line MDBK, and its inhibition by IF. Ficoll-Hypaque separated PBMC are suspended in wells of microtiter test plates in tissue culture medium with and without phytohemagglutinin and poly-IC (for IF production) and with and without several concentrations of human leukocyte IF (for viral replication studies). After 24 hr of incubation, the cells that have been incubated with and without IF are infected with VSV while the IF-producing cells are left to incubate for an additional 24 hr. Twofold dilutions are then made of the supernatant tissue culture media of the PHA and poly-IC-stimulated as well as the nonstimulated cells. Serum that had previously been separated from the original blood sample is also serially diluted as well as a standard IF preparation. Fibroblasts are then seeded into the dilution-containing wells and into additional wells to be used for virus titration. The fibroblast monolayers are then infected with VSV and tenfold dilutions are made of the VSV-infected PBMC suspended in their supernatants on MDBK fibroblast monolayers. CPE is determined when control VSV-infected fibroblasts show confluent CPE.

Results

In normal healthy children blood serum IF was very low with a mean of <4 u/ml. Their PBMC responded well to stimulation with PHA and poly-IC with a mean IF value of 205u/ml. in each case. There was no IF production by

nonstimulated PBMC, and in 90% of these normal cases, PBMC promoted good viral replication with a mean $TCID_{50}$ of 10^4, and going as high as 10^7. Eighty percent of these latter were inhibited by 64u of exogenous IF, and 100 by 128u.

The findings in children with viral diseases were reversed. Here, 82% had serum IF values greater than 16u/ml. with a mean of 80u/ml. IF production by stimulated lymphocytes was less than in normals, particularly so with PHA, while viral growth on PBMC was markedly inhibited, occurring in only 1 of 17 cases.

In a mixed group of cases with various types of immunodeficiency including several cases of Down's syndrome, the trend seems to be intermediate between normal and those with viral diseases.

In the small group of neoplastic diseases, mainly leukemia and neuroblastoma, the serum IF levels and the PHA and poly-IC-stimulated PBMC response resembled those seen in viral infections. Of interest is that ⅓ of the neoplastic cases and immune deficiency patients showed spontaneous IF production by lymphocytes without stimulation. This could be a possible indication of some intrinsic viral or antigenic stimulant being present in lymphocytes in neoplasia and ID cases, and could hint at possible etiology. This finding is being further investigated.

Discussion

Overall, these preliminary studies are interesting because of the finding of an inverse relationship between *in vivo* plasma IF values, and the ability of PBMC to promote viral replication. The marked difference between normal children and those with viral infections is most striking.

In two patients treated with human leukocyte IF, one an immunosuppressed child with neuroblastoma and progressive herpes zoster infection, and the other a comatose pregnant woman with fulminant hepatitis B infection, the results of this IF assay system paralleled very closely the clinical condition of the patients, and the findings helped in the clinical management of the cases, and in particular was useful in determining when to terminate IF therapy.

The information obtained from our assay system in the diagnosis and prognosis of cases suspected of viral etiology, as well as in the assessment of cases which may benefit from IF therapy should prove of great benefit to the clinician.