CORRESPONDENCE

GAMMA INTERFERON AND APLASTIC ANEMIA

To the Editor:

Certain aspects of the published data on the role of γ-interferon (IFN-γ) in hematopoiesis in vitro and in bone marrow failure states were not emphasized in the article by Torok-Storb and co-workers.1

Beginning with the initial observation of Kagan and colleagues,2 many investigators have demonstrated that T cells from patients with aplastic anemia can suppress hematopoiesis in vitro.3-5 Bacigalupo and associates5 found that the supernatants of aplastic lymphocyte cultures were as effective as the cells themselves in suppression and that lectin-activated normal lymphocytes produced an inhibitory factor. These observations were important because they implicated a soluble inhibitory factor and suggested an experimental system that would avoid the allogeic effects and imprecision that plague co-culture experiments.

We reported that the inhibitory activity in these lymphocyte supernatants was IFN-γ; our studies have been confirmed by Murphy and colleagues,4 who found also that lymphotixin was produced and acted to enhance the activity of IFN-γ, a not unexpected result given the coordinated expression of lymphokines in T cells and the known synergy between factors in inhibiting cell proliferation. Interferon production by an activated lymphocyte with the same surface phenotype as is found in the circulation of aplastic patients (CD8+, Tact, HLA-DR+)5 has also been confirmed in other laboratories.10

In analysis of patient material, we11 reported multiple abnormalities of interferon production: (a) enhanced production of interferon by lectin-stimulated mononuclear cells; (b) spontaneous production of interferon, not seen in normal cultures; (c) improvement in colony formation by aplastic bone marrow in the presence of antibodies to interferon; and (d) elevated serum levels of interferon in about 30% of patients, as determined by bioassay of fresh material by a specialist in interferon who was blinded to the clinical nature of the samples.

Abnormalities of interferon production in aplastic patients have been confirmed by other laboratories12-15 (and E.D. Zanjani, personal communication); even the abstract cited in support by Torok-Storb and co-workers concluded that "there were IFN-γ abnormalities in some patients with aplastic anemia."15 The data presented in the article by Torok-Storb and co-workers are indeed also similar to those of Zoumbos and colleagues.11 First, more than one-third of patients' cells spontaneously produced interferon, in some cases to very high levels. Second, although Fig 2 (of Torok-Storb article) is plotted on a semilogarithmic scale that tends to compress the data lines, extraction of the interferon values at the plateaus and their statistical analysis shows a significant difference between lectin-stimulated interferon production by the four patients and five controls (P < .05 by Student's t test), again similar to the data of Zoumbos and colleagues and other investigators.

That supernatants derived from patient's cell cultures containing high concentrations of interferon did not inhibit in the study of Torok-Storb and co-workers is almost certainly due to the concomitant presence of growth factors (GM-CSF). Growth factors are antagonistic to interferon's actions in hematopoietic cell culture systems.14 More important, a major aim of our studies was to replace co-culture experiments with measurements of specific molecules and cell types. That interferon—recombinant IFN-γ—is inhibitory of hematopoietic cell proliferation has been reported by many laboratories16-20 and in human patients treated with interferon.21 Torok-Storb and co-workers22 have been unable to demonstrate recombinant interferon's inhibitory effect in their cell culture system.

Finally, legitimate debate exists on the presence of interferon in serum. Our data11,15 were generated using the virus inhibition assay, which has recently been shown to detect lymphotixin as well as interferon.22 The synergy between these factors probably makes the bioassay more sensitive but less specific than the radioimmunoassay. Bioassays are not intrinsically less valuable than radioimmunoassays, however. Radioimmunoassays with monoclonal antibodies rely on detection of single or restricted numbers of epitopes, which can differ between natural and recombinant molecules24 and may be lacking on molecules produced in pathologic states. In addition, molecules with very high specific activity, such as interferon, may be easier to detect with biologic than chemical techniques. Nevertheless, preliminary data obtained by Dr Leonidas Plataniotis in our laboratory (Fig 1), however, suggest that some patients with aplastic anemia will have circulating IFN-γ in their sera.

Ample laboratory data and clinical observations continue to make immune mediation of bone marrow failure an attractive hypothesis. It seems likely to me that hematopoietic depression will be effected by inhibitory molecules and that these abnormalities of the immune system reflect a viral pathogenesis, for which there is increasing evidence. The immunologic and hematopoietic systems are enormously complicated, however, and extrapolation from in vitro experiments to pathogenic mechanisms is an increasingly daunting task. Whatever interpretation eventually proves correct, it should be clear that, despite the negative title and tone, many of the differences described in the article by Torok-Storb and co-workers and in our own results, as well as those of other investigators, are more apparent than substantial.

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Fig 1. γ-Interferon (IFN-γ) as measured by radioimmunoassay in American and Chinese serum samples. Data were obtained without knowledge of the clinical history. Linearity of the assay was assessed with the internal standards provided by Centocor (Malvern, PA) but values were assigned by comparison with a natural IFN-γ reference standard (NIAID Gg23-901-S30).

REFERENCES


RESPONSE

To the Editor:

In response to Dr Young’s letter, I would like to reiterate three points. First, we reported that γ-interferon (INF-γ) could not be detected in serum samples obtained from 50 patients with aplastic anemia (AA). We thought this information should be published because it differed dramatically from that of Zoumbos and colleagues, who detected significant levels (>10 U/mL) in 41% or 10 of 24 patients studied. Dr Young’s data, published above, now indicate that 17% or 11 of 63 patients had serum levels >10 U/mL, which differs significantly from his previous observations.

Second, in their original publication, Zoumbos and colleagues stated that “Virtually every patient with aplastic anemia reported in this study showed abnormal interferon production in vitro.” Their data indicated dramatic differences in the kinetics of INF production. Patient peripheral blood mononuclear cells (PBMCs) produced >1,000 U/mL of INF-γ 24 hours after lectin stimulation, and levels continued to increase over seven days. In contrast, normal PBMC reached peak levels of <1,000 U/mL at day 5 and decreased to baseline levels by day 7. Our data differed dramatically from these observations. Both normal and AA PBMCs reached plateau levels of INF-γ three days post-lectin stimulation, and in both groups INF-γ levels were maintained over seven days. Clearly, in our hands the kinetics of INF-γ production did not differ between the two groups.

Third, we too believe it is noteworthy that we cannot demonstrate inhibition of BFU-E with INF-γ. This continues to be the case. Indeed, using enriched populations of precursors, we often see INF-γ augmenting BFU-E growth. Comparable results using MY 10-selected precursors have recently been reported by Strauss and associates. These observations cannot be explained merely by the presence of GM-CSF in our cultures. Because growth of hematopoietic precursors in vitro requires growth factors, either added from exogenous sources or produced in situ, it is safe to assume that all
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