CONCISE REPORT

Gamma (Immune) Interferon Production by Leukocytes From a Patient With a T<sub>G</sub> Cell Proliferative Disease


We report a patient with a disease characterized by proliferation of T cells with Fc receptors for IgG (T<sub>G</sub>). However, unlike lymphoid cells from normal individuals or from patients with other lymphoid malignancies, the patient's lymphocytes spontaneously produced gamma interferon (IFN-γ) in vitro. The peripheral lymphocytes consisted of 95% T<sub>G</sub> cells, which exhibited the morpological characteristics of T-cell chronic lymphocytic leukemia (CLL) and were normal on cytochemical and chromosome analysis. The majority of T<sub>G</sub> cells were OKT3<sup>+</sup>, OKT8<sup>+</sup>, and OKT4<sup>+</sup>, 3A1<sup>+</sup>. These cells failed to express suppressor cell activity and displayed depressed levels of natural killer activity, but mediated antibody-dependent cell-mediated cytotoxicity. The spontaneous production of IFN-γ by human peripheral lymphoid cells as demonstrated in this study may serve as a probe for studying the relationship between IFN-γ and the proliferation of human T-cell subsets.

There are at least three general types of human interferon (IFN), referred to as alpha (α) (leukocyte), beta (β) (fibroblast), and gamma (γ) (immune) IFN. IFN-γ is thought to be produced by lymphocytes (probably T cells) in response to specific antigens, antigen–antibody complexes, mitogens, and anti-leukocyte antibodies. Because it is difficult to obtain sufficient quantities of human IFN-γ, this substance has not been extensively studied. Nevertheless, among the various types of interferons, the IFN-γ has been reported to be the most effective in enhancing antibody production and under certain circumstances, can potentiate the biologic actions of the other IFNs.

We report here a patient with a disease characterized by proliferation of T cells with Fc receptors for IgG (T<sub>G</sub>), whose lymphocytes spontaneously produced IFN-γ in vitro, a phenomenon that has not previously been recognized.

MATERIALS AND METHODS

Case Report

A 48-yr-old man was well until May 1978, when he developed arthralgias of both proximal and distal arm and leg joints unilaterally. Rheumatoid factor (RF) and low levels (1:20) of antinuclear antibody (ANA) were detected in serum. In August 1979, he first noticed exercise fatigue and malaise. Evaluation revealed a lymphocytosis of 4130 cells/cu mm. In February 1980, his hemoglobin was 13.8 g/dl, white blood count was 11,400 cells/cu mm, and lymphocyte count was 10,260 cells/cu mm. At this time, 92% of the lymphocytes were large and possessed numerous large azurophilic granules that stained intensely for β-glucuronidase and acid phosphatase. Physical examination in February 1980 demonstrated no abnormalities with normal liver and spleen size and no skin lesions. Serum aspartate aminotransferase (SGPT) and lactase dehydrogenase were moderately elevated, and a liver biopsy demonstrated sinusoidal infiltration with atypical lymphocytes. Bone marrow biopsy demonstrated infiltration with lymphoid aggregates and normal myeloid maturation. The diagnosis of chronic lymphocytic leukemia (CLL) was made. In August 1980, the patient was transferred to the National Institutes of Health (NIH).

Cell Surface Markers

Peripheral mononuclear cells were obtained from Ficoll-Hypaque separated heparinized blood. T cells were defined by their ability to form rosettes with sheep erythrocytes (E). B cells were enumerated by the presence of surface immunoglobulin (Ig) detected by direct immunofluorescence and intracellular Ig detected by indirect immunofluorescence. The presence of IgG and IgM Fc receptors were determined by rosetting with IgG- or IgM-sensitized bovine erythrocytes. Monocytes were identified on the basis of morphologically, nonspecific esterase staining, and the ability to ingest IgG-coated bovine erythrocytes.

Monoclonal Reagents

3A1 antibody defines a T-cell subset consisting of 85% E-rosetting positive peripheral lymphocytes. The OK reagents (Ortho Diagnostics, Raritan, N.J.) were used and included OKT4 (anti-inducer T cell), OKT3 (anti-T-cell), OKT8 (antissuppressor/cytotoxic T cell), and OKT6 (antithymocyte). D66 antibody defines 100% of thymic cells and 90% of peripheral T cells (gift of Dr. Alain Bernard). Antibody D1/12 binds to the nonpolymorphic Ia-like antigen (gift of Dr. A. Moretta), antibody 3F10 binds to HLA surface antigen, and antibody 4F2 binds to an antigen found on monocytes and a subset of activated lymphocytes.

Interferon Assay

Mononuclear cell suspensions were resuspended to contain 2 × 10<sup>6</sup> cells/ml in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells were incubated at 37°C in an atmosphere containing 5% CO<sub>2</sub>. After varying times of incubation, the cell suspensions were centrifuged and the supernatant fluids were assayed for antiviral activity on human amnion (WISH) cells as previously described.
RESULTS

Analysis of Lymphocytes

The patient’s serum at the time of study showed positive RF (1:160) and ANA (1:20). Serum protein electrophoresis revealed a normal profile. Serum Ig levels were 304 mg IgM/dl (N: 1:37–204), 1164 mg IgG/dl (N: 1:710–1540), and 382 mg IgA/dl (N: 1:60–490). High levels of serum immune complexes (56% Clq binding) were observed.

The hematologic profile at the time of study showed a lymphocytosis and neutropenia (Table 1). Ninety-five percent of the lymphocytes were T cells, 88% of which expressed Fc receptors for IgG (TG). Since the majority of T cells formed E-rosettes only after an 18-hr incubation at 4°C, they are considered “low affinity” E-rosetting T cells.

These cells contained β-glucuronidase and acid phosphatase in an intense granular pattern but not terminal deoxynucleotidyl transferase (tdt). By chromosomal analysis, all cells were diploid. These TG cells were positive for HLA determinants, bone markers characteristic of some mature T cells (E-rosette receptors, IgG Fc receptors), and were negative for markers of B cells or monocytes (clg, slg, Ia, 4F2) (Table 2). Analysis of the T-cell surface antigen phenotype showed that the majority of the TG cells were OKT3, OKT4, 3A1 (Table 2). However, a small percent of these cells also expressed OKT4 or 3A1 antigens.

Patient T cells did not suppress PWM-induced immunoglobulin production when cocultured with normal allogeneic mononuclear cells. This is in contrast to the previously reported functional capability of normal TG cells. Normal T cells also can mediate NK and ADCC activity. NK activity by the patient’s T cells on K562 cells was significantly below normal levels. However, patient T cells did mediate ADCC activity against RL male-1 and Daudi cells.

Production of IFN by Cells in Culture

Mononuclear cells were collected from the patient 10 times during a 12-mo period and were cultured in vitro as described. At each of these times, the supernatant fluid collected from these cells after 1–7 day incubation contained 80–320 Units of antiviral activity (Table 3). Initial studies to propagate the cells showed that cells remained viable and released IFN for the first 3 wk in culture. After this time, cell viability decreased rapidly. In contrast, the supernatant fluid (at a 1:20 dilution) from similarly treated cells obtained from 20 normal individuals, 3 patients with systemic lupus erythematosus, 3 patients with chronic lymphocytic leukemia (CLL) (B cell), 2

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**Table 1. Hematologic Profile**

<table>
<thead>
<tr>
<th>White blood cells</th>
<th>Patient</th>
<th>Normal</th>
</tr>
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<tbody>
<tr>
<td>Neutrophils</td>
<td>810</td>
<td>3,000–5,800</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>12,410</td>
<td>1,500–3,000</td>
</tr>
<tr>
<td>Monocytes</td>
<td>270</td>
<td>285–500</td>
</tr>
<tr>
<td>Total</td>
<td>13,500</td>
<td>5,000–10,000</td>
</tr>
</tbody>
</table>

**Table 2. Cell Surface Antigen Phenotype of Normal and Patient T₉ Cells**

| Surface Marker | Percent T₉ Cells With Surface Antigens
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Non-T-cell Specific</td>
<td></td>
</tr>
<tr>
<td>4F2</td>
<td>0</td>
</tr>
<tr>
<td>1a</td>
<td>&lt;5</td>
</tr>
<tr>
<td>HLA</td>
<td>100</td>
</tr>
<tr>
<td>T-cell Specific</td>
<td></td>
</tr>
<tr>
<td>OKT3</td>
<td>&gt;95</td>
</tr>
<tr>
<td>OKT4</td>
<td>8</td>
</tr>
<tr>
<td>3A1</td>
<td>30</td>
</tr>
<tr>
<td>OKT8 (OKT5)</td>
<td>7</td>
</tr>
<tr>
<td>OKT6</td>
<td>0</td>
</tr>
</tbody>
</table>

*Purified suspensions of patient T₉ cells were obtained by rosetting procedures that enriched for low affinity E-rosetting cells. Patient T₉ suspensions contained 95% IgG FcR and E-rosette-positive large granular lymphocytes. Marker analysis was performed on a FACS-11 as described.

†Values for normal T₉ cells were taken from references 6 and 8 and from control studies on purified T₉ suspensions from 5 normal subjects.

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**Table 3. IFN Produced by Leukocytes In Vitro From a Patient With T₉ Proliferative Disease**

<table>
<thead>
<tr>
<th>Lymphocytes Collected* (Months After Admission)</th>
<th>IFN (Units)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80–160</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
</tr>
<tr>
<td>4</td>
<td>240</td>
</tr>
<tr>
<td>5</td>
<td>80–240</td>
</tr>
<tr>
<td>6</td>
<td>160–320</td>
</tr>
<tr>
<td>11</td>
<td>80–160</td>
</tr>
<tr>
<td>12</td>
<td>80–160</td>
</tr>
</tbody>
</table>

*Leukocytes collected and tested 10 times during a 12-mo period.
†The antiviral activity, expressed in IFN units, was calculated as the reciprocal of the highest dilution of fluid that reduced the number of vesicular stomatitis virus plaques by 50% in human amnion (WISH) cells.
patients with acute lymphocytic leukemia (ALL) (one non-T non-B cell, and one T cell), and 2 patients with Sézary’s syndrome did not contain antiviral activity. On no occasion was antiviral activity demonstrated in the serum (tested at a 1:16 dilution) of the patient.

Antiviral activity in the supernatant fluids was characteristic of IFN. The fluids inhibited replication of vesicular stomatitis virus on human but not on mouse cells. Moreover, the samples were not toxic for cells and the antiviral activity was not lost after dialysis at pH 7.2, but was destroyed by trypsin. IFN from the patient’s cells was inhibited by pH 2.0 treatment and was not affected by anti-α or anti-β IFN antisera. We conclude that the leukocytes produced a γ-IFN.

We next attempted to identify the cell(s) producing IFN-γ. Suspensions consisting of 90% TG cells produce IFN. When these cells are separated into glass-adherent and glass-nonadherent populations, only the nonadherent cells produce IFN. Incubation of these cells with the cytotoxic monoclonal anti-T-cell antibody (D66) and complement resulted in the loss of IFN production. These studies suggest that cells bearing T-cell antigens either produce IFN themselves or are involved in the production of IFN.

DISCUSSION

We describe a patient with a proliferation of TG cells, neutropenia, and a pathologic diagnosis of T-cell CLL. Recently, there have appeared reports of patients with a similar disease with or without recurrent infections. Functional studies and phenotypic characterization of the TG cells in certain of the previous studies and in the present study revealed that they were OKT8⁺, OKT4⁻, lacked suppressor and NK activity, but expressed ADCC activity. However, of particular note in our patient is the fact that unlike normal lymphoid cells, these cells spontaneously produced immune (γ) IFN.

It is unclear at present whether this is merely a proliferation of TG cells caused by disordered immunoregulatory mechanisms or whether this is truly a neoplastic proliferation of TG cells. It is conceivable that IFN-γ, which has been shown to trigger proliferation and/or differentiation of TG cells, is the underlying stimulus for the proliferation of TG cells in our patient. Certain studies have suggested that a deficiency in IFN production may be a predisposing factor in metastatic neoplasia. Conversely, we report here a T-cell proliferation associated with in vitro IFN-γ production.

In earlier studies, we showed that both α- and γ-IFN are present in the sera of patients with systemic lupus erythematosus and related autoimmune diseases, but the detection in vitro of de novo produced IFN was not demonstrated (J. Hooks, unpublished data). A possible disorder observed in our patient and also noted in other reported cases is the presence of an autoimmune disorder (i.e., rheumatoid arthritis with circulating immune complexes). Despite the finding of the production of IFN-γ in vitro, IFN could not be demonstrated in the serum of our patient. There are a number of possible explanations for this finding. It is entirely possible that low levels of IFN (<16 U) released in vivo are not readily detected in our assay system. Alternatively, IFN released in vivo may be utilized or complexed, culture conditions may selectively trigger the cells to produce IFN, or IFN production or detection is blocked by serum inhibitors. Since the patient’s serum did not reduce the ability of lymphocytes from normal individuals to produce both α- and γ-IFN (data not shown), it is unlikely that serum inhibitors are present.

IFN has multiple regulatory actions on lymphoid cells and immune responses. We report a patient whose cells spontaneously produce γ-IFN in vitro. Although the precise role of IFN in the pathogenesis and/or expression of this hematologic disorder is uncertain at present, these studies point to the possibility that IFN might play a regulatory role in immunologic disorders and/or lymphoid malignancies.

ACKNOWLEDGMENT

We thank Lawrence Clark and Bruce Suit for technical assistance and Dr. Donald Karcher for assistance in reviewing pathology data.

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