Defective Gamma-Interferon Production in Peripheral Blood Leukocytes of Patients with Acute Tuberculosis

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Production of interferon (IFN)-gamma by peripheral blood leukocytes (PBL) was examined in cultures of unseparated fresh whole blood exposed to phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM). The yield of IFN-gamma was measured by a newly developed immunoradiometric assay. Nine of 14 patients with acute pulmonary tuberculosis (TB) showed a depressed IFN-gamma response to Con A and/or PWM. Only four of these TB patients also showed a depressed IFN-gamma response to PHA. Stimulation of the patients' PBL cultures with PHA in the presence of exogenous interleukin 2 (IL 2) produced normal IFN-gamma yields in all but the most severely depressed patients. PBL cultures of TB patients with defective IFN-gamma production in response to mitogenic lectins also produced less IFN-gamma after stimulation with tuberculin PPD. Although some patients showed a moderate degree of lymphopenia, their OKT4/T8 lymphocyte ratios were mostly normal or close to normal, with the notable exception of one TB patient who has been diagnosed to have the acquired immune deficiency syndrome (AIDS).

KEY WORDS: Interleukin 2; T-cell mitogens; tuberculin; helper/suppressor T cells; acquired immune deficiency syndrome (AIDS).

INTRODUCTION

Interferon (IFN)-gamma plays an important role in the modulation of several events during the generation of immune responses, including the activation of macrophages for microbicidal (1) and tumoricidal (2, 3) activities; the induction of MHC antigens, especially the class II MHC antigens including Ia antigen (4); the induction of Fc receptor expression (5, 6); and the stimulation of B-cell differentiation (7). These and other immunomodulatory actions of IFN-gamma (reviewed in Refs. 8 and 9) may be impaired during primary or acquired immunological disorders. One reason for such an impairment can be a decreased production of IFN-gamma as a consequence of a defect in the cell populations producing it or some other functional abnormality.

Evaluation of interferon (IFN)-gamma production in peripheral blood leukocytes (PBL) from patients with immune disorders and/or infectious diseases may help to clarify the pathophysiological mechanisms involved in the disease process. In this study, we have examined IFN-gamma production in PBL cultures from patients with acute pulmonary tuberculosis (TB). We have employed a simple method of evaluation of IFN-gamma production in cultures of the patients' unseparated whole blood. IFN-gamma yield from the cultures was measured with the aid of a recently developed sandwich immunoradiometric assay (10) affording greater sensitivity, accuracy, and specificity than conventional bioassays. About two-thirds of the TB patients examined showed a marked decrease in IFN-gamma production in response to at least one of the lectins employed. The addition of interleukin 2 (IL 2) to the cultures largely restored defective IFN-gamma production in a majority of the patients. The functional consequences of defective IFN-gamma production in patients with severe acute pulmonary TB remain to be determined.
MATERIALS AND METHODS

Subjects. Fourteen patients hospitalized with the diagnosis of acute pulmonary TB were studied. The presumptive diagnosis of TB was based on the demonstration of acid-fast bacilli in the sputum and/or caseating granulomata on tissue biopsy and was subsequently confirmed by a positive culture for Mycobacterium tuberculosis. The disease stage ranged from pleural effusion with no other pulmonary involvement ("effusion"), to pulmonary involvement without evidence of cavitation ("moderate"), to evidence of cavitation ("advanced"). Extrapulmonary involvement, if any, was confirmed by lymph node biopsy. Blood for the determination of IFN-gamma production was taken before the initiation of antibiotic therapy, except in patient 1, whose blood was drawn 4 days after the initiation of therapy, and patients 7 and 8, who had been treated for 34 and 44 days, respectively, at the time their blood was first examined for IFN-gamma production but were not responding to therapy (see Table II). In some patients another determination of IFN-gamma production was made after the onset of therapy.

Twenty-one healthy hospital employees served as control subjects for the determination of the normal ranges of IFN-gamma production in response to mitogenic lectins.

Mitogens. Phytohemagglutinin (PHA) was prepared in the laboratory of Dr. Joel D. Oppenheim at NYU Medical Center. Concanavalin A (Con A) was purchased from Pharmacia, Piscataway, NJ, and purified pokeweed mitogen (PWM) from E.-Y. Laboratories, San Mateo, CA. Unless indicated otherwise, PHA was added to cultures of peripheral blood leukocytes (PBL) at a final concentration of 2 ~g/ml, Con A at 5 ~g/ml, and PWM at 1 ~g/ml. The cell suspension was then distributed in 17 x 100-mm plastic tissue culture tubes (1 ml/tube) and mitogen, PPD, or IL 2 was added as indicated. The tubes were loosely capped and incubated at 37°C in a CO2 incubator. At the end of incubation supernatants from the cultures were harvested and the IFN-gamma present was quantitated in an immunoradiometric assay as described below. All IFN-gamma assays were done in duplicate.

Radioimmune Assay (RIA) of IFN-Gamma. A recently developed solid-phase sandwich RIA (10), employing two murine monoclonal antibodies, has been used for the quantitation of IFN-gamma. RIA kits (IMRX IFN-gamma RIA), containing antibody-coated polystyrene beads and a 125I-labeled solution of tracer antibody, have been manufactured and supplied by Centocor, Malvern, PA. This assay is specific (unlike most bioassays, the RIA does not detect IFN-alpha or IFN-beta) and sensitive (the lower limit of sensitivity is 0.1-0.2 unit/ml). IFN-gamma titers are expressed in reference units based on the value of NIH standard Gg 023-901-530 (National Institute of Allergy and Infectious Diseases, Bethesda, MD).

RESULTS

IFN-Gamma Production in Response to PHA, Con A, and PWM in PBL Cultures from Healthy Donors. The availability of a sensitive and specific immunoradiometric assay for IFN-gamma (10) enabled us to develop a standardized procedure for the evaluation of IFN-gamma production in PBL cultures stimulated with mitogenic lectins or anti-