Characterization of Human Interferon-Gamma-Producing Leukocytes with Monoclonal Antileukocyte Antibodies

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Abstract. Monoclonal antibodies with specificities for subsets of human leukocytes have been used for the characterization of interferon (IFN) γ-producing cells. The production of IFN γ was demonstrated to be a function of OKT3* T lymphocytes. The capacity to secrete IFN γ was not restricted to the OKT4* or the OKT8* T-cell subset. BA-1* B lymphocytes and Leu7* natural killer cells did not contribute to the production of IFN γ. Ia*, OKM1* monocytes served an auxiliary function in the production of IFN γ. The requirement for accessory monocytes, however, was not absolute, because monocyte-free preparations of long-term cultured IL2-dependent T lymphocytes retained the capacity to secrete IFN γ.

Introduction

Interferons (IFN) are a heterogenous group of glycoproteins produced by animal cells in response to viral and nonviral stimuli [28]. On the basis of antigenic, biological, and physicochemical properties, human IFNs have been grouped into three major classes: IFN α (leukocyte type), IFN β (fibroblast type), and IFN γ (immune type). IFN α and IFN β have been purified to homogeneity and their molecular structure has been studied extensively [8, 19]. Much less information is available concerning the structure and properties of IFN γ. In vitro studies with crude preparations suggest that IFN γ primarily acts as an immunoregulatory agent [12] and that IFN γ exerts a significantly more potent antiproliferative effect on malignant cells than IFN α or IFN β [5]. The potential usefulness of IFN γ in the treatment of malignant disease has increased interest in the large-scale production, purification, and characterization of this type of IFN. Improved yields of IFN γ from leukocyte cultures would be greatly facilitated by the identification of the IFN-γ-producing leukocyte subset. Recently, a variety of murine monoclonal antibodies with specificities for subsets of human leukocytes have provided a more definitive method

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for their identification and functional characterization. OKT3 monoclonal antibody reacts with 100% of peripheral blood T lymphocytes [23]. OKT4 reacts with a subset that comprises 65% of peripheral T cells and shows inducer/helper and some cytotoxic activity [25]. OKT8 monoclonal antibody is reactive with 35% of peripheral T lymphocytes that demonstrate cytotoxic-suppressor activity [26]. BA-1 reacts with peripheral blood B lymphocytes [3]. OKIa and anti-Ia monoclonal antibody react with B lymphocytes, monocytes, and activated T lymphocytes [11, 24]. The antigen defined by OKM1 antibody is present on monocytes and granulocytes [6]. Leu 7 monoclonal antibody reacts with a differentiation antigen selectively expressed on leucocytes with natural killer activity [2]. We have used these monoclonal antibodies for the specific depletion of leucocyte subsets by complement-mediated lysis and for the identification of leucocyte subpopulations capable of producing IFN-γ. The results demonstrate that the production of IFN-γ is a function of OKT3⁺ T lymphocytes and is not restricted to either OKT4⁺ or OKT8⁺ T-cell subsets. Ia⁺, OKM1⁺ monocytes serve an auxiliary function in the production of IFN-γ.

Materials and Methods

Leukocyte Preparation. Mononuclear cells were separated from heparinized human peripheral blood by sedimentation on Ficoll-Isopaque gradients. Cells harvested from the interface were washed and resuspended in RPMI 1640 (Flow Laboratories, Bonn, FRG) tissue culture medium supplemented with 10% heat-inactivated fetal calf serum (FCS) and antibiotics. Adherent cells were depleted by incubating unfractionated leucocytes for 1 h in plastic tissue-culture flasks.

Leukocyte Fractionation. Monoclonal antibodies OKT3, OKT4, OKT8, OKIa, and OKM1 were purchased from Ortho Pharmaceutical Corporation, Raritan, NJ, USA. BA-1 monoclonal antibody was obtained from Hybritech Europe, Brussels, Belgium, anti-Ia monoclonal antibody from New England Nuclear Chemicals, Dreieich, FRG, and Anti-Leu 7 monoclonal antibody from Becton Dickinson Laboratory Systems, Rödermark, FRG. For leucocyte fractionation, plastic nonadherent cells were sus- pended at a concentration of 6 x 10⁶/ml in culture medium containing OKT3 at a final dilution of 1:50, OKT4 and OKT8 at a final dilution of 1:25, and BA-1, OK Ia, anti-Ia, OKM 1 or Anti-Leu 7 at a final dilution of 1:10. Cells to be treated with complement alone were incubated in culture medium without monoclonal antibodies. After 1-h incubation on ice, nontoxic rabbit complement (Mediapharm, Aschaffenburg, FRG) was added at a final dilution of 1:2, and the cells were incubated for 1 h at 37°C. The cells were layered on Ficoll-Isopaque gradients and centrifuged to deplete dead cells. Cells recovered from the interface were washed twice with culture medium before testing for IFN-γ production. The efficacy of leucocyte treatment with monoclonal antibodies and complement was assessed by enumerating cells reactive with the lysing monoclonal antibody using indirect immunofluorescence techniques described by Reinherz et al. [23]. Following complement-mediated depletion procedures, cells reactive with the lysing monoclonal antibody were always reduced to below 2%.