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Increased expression of human monocyte HLA-DR antigens and Fcγ receptors in response to human interferon *in vivo*

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SUMMARY

The expression of class II MHC encoded antigens (HLA-DR) and Fcγ receptors by peripheral blood monocytes from untreated patients with small cell carcinoma of the bronchus was compared with that of normal donors. Fcγ receptor expression was found to be elevated in these patients in comparison with normal. In contrast HLA-DR antigen expression by patients' monocytes was somewhat depressed in comparison with normal. Continuous intravenous infusion of a total of 400 megaunits/m^2 of human lymphoblastoid interferon-α (IFN-α) over a 5 day period markedly increased both monocyte HLA-DR antigen expression and Fcγ receptor expression in comparison with that of untreated patients and normal donors. The initial increases declined somewhat but were still evident after 3 weeks of intermittent intramuscular IFN-α therapy.

Keywords monocytes macrophages Ia antigens interferon lung cancer

INTRODUCTION

Studies made *in vitro* have established that interferons increase the expression of both MHC encoded antigens (Lindahl, Leary & Gresser, 1974; Fellous *et al.*, 1979) and Fc receptors (Fridman *et al.*, 1980; Itoh 1980) at the surface of lymphoid cells. Such changes also occur *in vivo* in a murine system (Lindahl *et al.*, 1976). Studies of human monocytes *in vitro* have shown that interferon (IFN) increases the expression of Fc receptors and class II MHC antigens (HLA-DR) (Rhodes & Stokes, 1982). These membrane molecules appear to be important in a variety of immunological functions and IFN may exert immunoregulatory effects by inducing quantitative or qualitative changes in their expression. A phase II clinical trial of human IFN-α (Namalwa lymphoblastoid IFN, Wellcome Biotechnology Ltd.) in small cell carcinoma of the bronchus (Jones *et al.*, 1983) enabled us to ask: (1) whether the expression of class II MHC antigens and Fcγ receptors by peripheral blood monocytes is altered in this group of patients in comparison with normal. (2) Whether IFN induces the same effects on cancer patients monocytes *in vivo* as on normal monocytes *in vitro*. (3) Whether IFN-induced changes persist during treatment.

MATERIALS AND METHODS

Patients. Ten patients (seven male and three female) with proven small cell carcinoma of the lung and previously untreated, were studied. Seven patients had disease localized to the chest and

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three had disseminated disease. Their mean age was 61 years (range 55–66 years). Their general medical condition was good.

IFN treatment. Treatment was given using human lymphoblastoid IFN (Wellferon, Wellcome Biotechnology Limited). This highly purified IFN is a mixture of at least five molecular subspecies encoded by a family of structural genes (Allen & Fantes, 1980) and produced by the Burkitt's lymphoma derived cell line Namalava. Its specific activity varies from $81–213 \times 10^6$ u/mg of protein. The IFN, stabilized with human serum albumin, was administered by continuous intravenous infusion (clockwork pump) during the first 5 days of treatment (for details see Jones *et al.*, 1983). On days 1 and 2, 50 megaunits/m^2 were administered daily; on days 3–5 inclusive, 100 megaunits/m^2 were administered daily. Following the infusion, the patients received 3 megaunits/m^2 by intramuscular injection three times a week, commencing on day 8, to a total of 10 injections.

Monocyte preparation. Venous blood was taken before the commencement of treatment and immediately following discontinuation of the infusion. A further sample was taken just prior to the sixth intramuscular injection (3 weeks after the initiation of IFN therapy). Human monocytes were obtained from defibrinated venous blood by Ficoll-Triosil gradient separation of mononuclear cells and subsequent differential adhesion to glass in RPMI 1640 medium containing 20% heat-inactivated fetal calf serum (FCS). After washing with Hank's balanced salt solution adherent monolayers were subjected to the assays described below.

Assays. The expression of HLA-DR antigens by cultured monocytes was determined by means of a rat monoclonal antibody recognizing a determinant in the monomorphic region of the HLA-DR structure (Brickell *et al.*, 1981). Immunoglobulin prepared by salt fractionation of ascitic fluid was coupled to the surface of trypsin treated sheep erythrocytes by means of chromic chloride (Coombs *et al.*, 1977). Titration of the monoclonal reagent in the coupling procedure using a sub-optimal dose range yielded a panel of cells bearing increasing amounts of anti-HLA-DR antibody. These cells, in HBSS containing 10% FCS, were allowed to settle on to monocyte monolayers in Lab-tek chamber slides for 1 h at 20°C. The monolayers were then washed twice in HBSS containing 10% FCS, fixed with 1% glutaraldehyde, and stained with citrate buffered Giemsa. The percentage of monocytes forming rosettes was determined and expressed as a function of the dose of monoclonal antibody. Fcγ receptor expression was characterized in the same way using a panel of ox erythrocytes specifically sensitized with increasing sub-optimal amounts of rabbit anti-ox erythrocyte antibody. Rosette formation in the latter system is specifically inhibited by heat-aggregated human IgG in a dose-dependent manner whereas the recognition of membrane determinants by the monoclonal reagent remains unaffected (Rhodes & Stokes, 1982). The resultant dose–response curves provide a sensitive reflection of monocyte HLA-DR antigen expression and monocyte Fcγ receptor expression.

RESULTS

The expression of monocyte Fcγ receptors was found to be elevated in untreated patients with small cell carcinoma of the bronchus in comparison with that of normal donors (Fig. 1). This result was expected since an increase in monocyte Fcγ receptor activity is characteristic of cancer patients irrespective of the tumour histology or anatomical site (Rhodes, 1977; Ruco *et al.*, 1980). In contrast the expression of monocyte HLA-DR antigens recognized by the monoclonal antibody YE2.36 HLK was somewhat reduced compared with normal (Fig. 2). This differential change indicates that increased Fcγ receptor expression does not reflect a generalized increase in monocyte membrane activity.

At the end of the initial 5 day intravenous infusion of IFN a marked increase in the expression of Fcγ receptors by peripheral blood monocytes was observed (Fig. 1). Whether or not this represents an effect on newly recruited monocytes of bone marrow origin or on cells already in the blood compartment, or a mixture of both, it is clear that the monocytes of untreated patients although activated in this respect are not maximally activated and that IFN induces the same effect on cancer patient's monocytes *in vivo* as on normal monocytes *in vitro*.

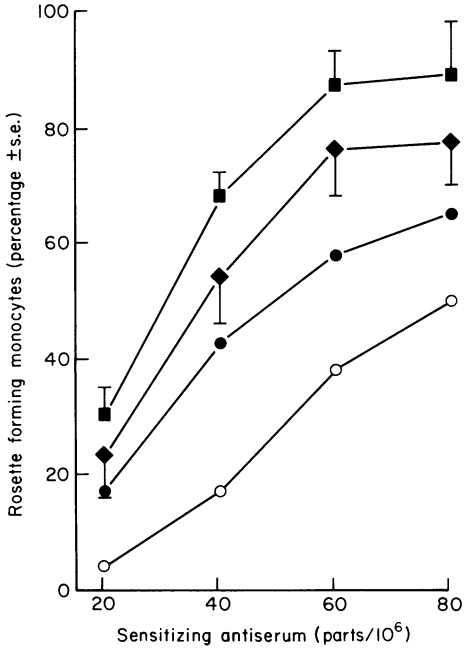

Fig. 1. Peripheral blood monocyte Fcγ receptor expression. Normal donors (○). Patients immediately prior to treatment (●). Patients after 5 days intravenous IFN (■). Patients after an additional five intramuscular injections of IFN (3 weeks after commencement of therapy) (♦). Cells from each patient were compared with those of a normal donor assayed simultaneously. The same normal donor was employed for comparison with a given patient at all three determinations (prior to treatment, 5 days into treatment, and 3 weeks into treatment).

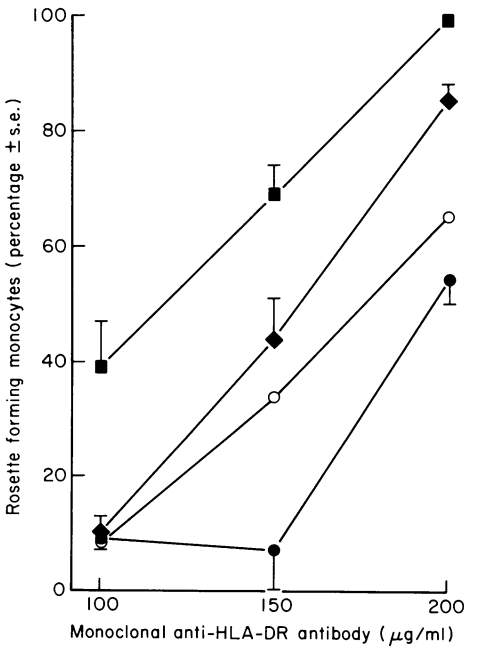

Fig. 2. Peripheral blood monocyte HLA-DR antigen expression. Normal donors (○). Patients immediately prior to treatment (●). Patients after 5 days intravenous IFN (■). Patients after an additional 5 intramuscular injections of IFN (3 weeks after commencement of therapy) (♦).

Assays for HLA-DR antigen expression performed after 5 days of intravenous infusion of IFN revealed that in spite of the depressed starting point in untreated patients DR antigen expression became elevated to well above normal levels in response to IFN, again reflecting the effects of IFN on normal monocytes *in vitro* (Fig. 2).

The third assay provided information on monocyte function after 3 weeks of IFN therapy. The data show that in spite of continued administration of IFN, Fcγ receptor expression declined from the initial increase seen at day 5, although remaining above that seen in untreated patients (Fig. 1). Monocyte HLA-DR antigen expression also declined after the initial increase at day 5 in spite of the continued administration of IFN (Fig. 2). After 3 weeks of treatment it was still above normal levels, however, and well above the depressed levels observed in untreated patients.

DISCUSSION

It is now well established that IFNs induce changes in the expression of MHC encoded antigens and Fcγ receptors at the surface of leucocytes exposed to IFN *in vitro* (Lindahl *et al.*, 1974; Fellous *et al.*, 1979; Fridman *et al.*, 1980; Itoh *et al.*, 1980; Rhodes & Stokes, 1982). Such changes also occur in normal animals treated with IFN or IFN inducers (Lindahl *et al.*, 1976; Hamburg, Manejias & Rabinovitch, 1978). These membrane molecules appear to be important in a variety of immunological functions and some of the immunoregulatory effects of IFN may be exerted through quantitative or qualitative changes in their expression. Here we investigate the changes induced by huIFN-α administered to patients with previously untreated small cell carcinoma of the bronchus. The functional status of the patients' peripheral blood monocytes was already altered prior to treatment. Fcγ receptor expression was elevated (a common characteristic of patients with solid tumours as shown by Rhodes 1977 and Ruco *et al.*, 1980) whereas HLA-DR (Ia) antigen expression was depressed. The depression of HLA-DR in these patients is interesting. Prostaglandins have recently been shown to be potent inhibitors of macrophage Ia antigen expression (Snyder, Beller & Unanue, 1982), and since plasma prostaglandin levels are generally elevated in patients with solid tumours (Goodwin, Husby & Williams, 1980) this provides one possible explanation.

Since both monocyte function and serum factors regulating monocyte function are perturbed in untreated cancer patients (Rhodes, Bishop & Benfield, 1979) it is interesting to observe that, in spite of this, monocytes in the peripheral blood of cancer patients are able to respond to IFN *in vivo* in the same way that normal monocytes respond *in vitro*. It is, however, somewhat suprising to see that the initial IFN-induced changes decline over a subsequent 2 week period despite continued, although intermittent, IFN administration. The clinical results of this trial of huIFN have been reported elsewhere (Jones *et al.*, 1983) but it is relevant to note here that no objective clinical benefit was observed in any patient.

A recent authoritative report on interferon therapy advises that clinical trials of IFN be accompanied by all laboratory investigations relevant to a biological response modifier (WHO Technical Report, 1982). The present paper describes one such investigation and other reports are beginning to appear. Lucero *et al.* (1982) found natural killer activity by peripheral blood lymphocytes of cancer patients enhanced by both leucocyte and fibroblast IFN, while the former also increased serum levels of β_2-microglobulin reflecting effects seen *in vitro*. Einhorn & Jarstrand (1982) observed depressed phagocytic activity in peripheral monocytes from cancer patients receiving long-term IFN therapy, the opposite of what they had observed *in vitro* over a shorter time course. Maluish *et al.* (1982) reported a consistent and sustained elevation of monocyte function in some patients undergoing IFN therapy.

One relatively neglected area in studies of IFN is the identification of endogenous signals that are antagonistic or inhibitory towards IFN (Rhodes & Stokes, 1982; Abb, Abb & Deinhardt, 1982; Baron *et al.*, 1981). Such signals may be 'utilized' by progressing tumours and may thus facilitate tumour resistance to anti-tumour host defence (Rhodes *et al.*, 1979; Matsubara, Suzuki & Ishida, 1980; Matsubara *et al.*, 1980). In this context agents that prevent or reverse inhibition of IFN action are of interest (Rhodes, 1983). Further controlled clinical trials of IFN in cancer are justified (WHO, 1982) and these should permit further investigation of the biological effects of interferon and the factors that regulate IFN *in vivo*.

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