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Interferon-induced changes in the monocyte membrane: inhibition by retinol and retinoic acid

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Summary. The effects of human interferon-β on human peripheral blood monocyte function were examined *in vitro*. Interferon-β was shown to increase substantially the expression of both Fcγ receptors and HLA-DR antigens defined by monoclonal antibody. Retinol and retinoic acid were found to be antagonistic to these effects of interferon and physiological concentrations were sufficient to inhibit changes in the monocyte membrane induced by relatively high concentrations of interferon.

INTRODUCTION

In addition to direct anti-viral effects, interferons are known to influence both specific and non-specific components of the immune system (Gresser, 1980). T-lymphocyte cytotoxicity directed against histocompatibility antigens (Lindahl, Leary & Gresser, 1972), antibody-dependent K-cell activity (Herberman, Ortaldo & Bonnard, 1979) natural killer cell activity (Gidlund, Orn, Wigzell, Senik & Gresser, 1978) and macrophage-mediated killing of tumour cells (Schultz, Papamatheakis & Chirigos, 1977; Mantovani, Dean, Jerrels & Herberman, 1980) are all enhanced by interferon. It has been suggested that interferon may exert these effects through changes in the membranes of effector cells and possibly target cells (Fridman, Gresser, Bandu, Aguet & Neauport-Sautes, 1980). Interferon is known to increase Fcγ receptor expression by murine and human lymphocytes and lymphocyte-derived lines (Itoh, Inoue, Kataoka & Kunagai, 1980; Fridman *et al.*, 1980) and by murine macrophages (Hamburg, Manejars & Rabinovitch, 1978). Increased expression of lymphocyte H-2 (Lindahl *et al.*, 1974) and HLA-A and B antigens (Fellous, Kamoun, Gresser & Bono, 1979) also occurs in response to interferon.

Little is known about endogeneous factors which may regulate interferon activity *in vivo* and, in particular, little is known of the physiological signals which may be antagonistic or inhibitory towards interferon. A consideration of such factors is important both to a basic understanding of how interferon functions in host defence and to the question of how interferons may best be deployed, if at all, as anti-tumour drugs. Blalock & Gifford (1977) have shown that retinoids inhibit the induction and anti-viral activity (1975) of interferon. We have shown that retinoids, at low concentrations, influence the function of monocytes and macrophages (Rhodes & Oliver, 1980). In the present paper we describe substantial increases in the expression of monocyte Fcγ receptors and HLA-DR antigens in response to interferon *in vitro*. We show that these changes are inhibited by physiological amounts of retinol and retinoic acid.

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MATERIALS AND METHODS

Preparation and culture of monocytes

Normal human monocytes were obtained from defibrinated venous blood by Ficoll-Triosil gradient separation of mononuclear cells and subsequent adhesion to glass in RPMI 1640 medium containing 20° heat-inactivated foetal calf serum (FCS). After washing with Hanks's balanced salt solution (HBSS) adherent monolayers, in four-chamber tissue culture slides (Ames Co., Stoke Poges) were cultured in RPMI 1640 containing antibiotics and FCS at a concentration of 10%. After 24 hr of culture 98% of these cells were mononuclear and capable of ingesting optimally sensitized erythrocytes and were therefore, by definition, mononuclear phagocytes. Cultured monocytes, treated as described below, were washed three times before being assayed for Fcγ receptor, or HLA-DR antigen expression.

Interferons

Two sources of interferon-β (IFN-β) were employed: human foreskin fibroblast IFN with a specific activity of 2×10^6 reference units (r.u.) per mg protein (Collaborative Research, Waltham, Mass.) and human foreskin fibroblast IFN with a specific activity of $0{\cdot}8 \times 10^7$ r.u. per mg protein (Flow Laboratories, Irvine, Ayreshire). A mock IFN-β preparation with almost all IFN activity removed by a combination of physical and immunochemical column separations (90 r.u. per mg protein) was also obtained from Flow. IFN's were stored at −70°. The two IFNs produced comparable effects on monocytes.

Retinoids

Retinol and retinoic acid (all trans) were obtained from Sigma and stored for short periods at −20° protected from light. Before use the crystals were dissolved in ethanol and added immediately to RPMI 1640 containing FCS (10%). β-ionone was obtained from Aldrich Chemical Co. and stored at +4°. Before use it was dissolved in ethanol in a glass container. The concentration of ethanol in control and experimental monocyte cultures was 0·1%.

Assays

The expression of HLA-DR antigens by cultured monocytes was determined by means of a rat monoclonal antibody recognizing a determinant in the non-polymorphic region of the HLA-DR structure (Brickell, McConnell, Milstein & Weight, 1981). Immunoglobulin, prepared by salt fractionation from ascitic fluid, was coupled to the surface of trypsin-treated sheep erythrocytes by means of chromic chloride (Coombs, Wilson, Eremin, Gurner, Haegert, Lawson, Bright & Munro, 1977) and used in a titration rosette assay. The expression of Fcγ receptors was determined by means of ox erythrocytes specifically sensitized with heat-inactivated rabbit anti-ox antiserum, in a titration rosette assay (Rhodes, 1977). Rosette formation in this system was specifically inhibited by heat-aggregated human IgG (absorbed with ox and sheep erythrocytes) in a dose-dependent manner whereas the reaction of the monoclonal reagent with the monocyte membrane remained unaffected (Fig. 1). This binding inhibition assay was performed with optimally sensitized erythrocytes (anti-sheep erythrocyte antiserum at 500 p.p.m. or monoclonal anti-HLA-DR antibody at 200 μg per ml). Monocytes were incubated for 30 min at 4° with absorbed heat-aggregated human IgG (63°, 20 min) and then washed three times with cold HBSS. Coated erythrocytes were added in serum-free HBSS and allowed to settle for 1 hr at 20°. The monolayers were then washed three times with HBSS, fixed with 1% glutaraldehyde and stained with citrate-buffered Giemsa. Cells with three or more attached erythrocytes were counted as rosettes and expressed as a percentage of the total monocyte population. In subsequent assays titration of the monoclonal reagent in the coupling procedure, using a suboptimal dose range, was performed in order to obtain a panel of erythrocytes bearing increasing amounts of monoclonal antibody. The same titration procedure was employed in the specific sensitization of erythrocytes by anti-ox erythrocyte antibody. Rosette formation by cultured monocytes took place as described above.

RESULTS

The data in Fig. 1 shows that the binding of specifically sensitized ox erythrocytes by monocytes was inhibited by preincubation of the monocytes with heat-aggregated human IgG in a dose-dependent manner. In contrast, the reaction of the monoclonal anti-HLA-DR antibody with the monocyte membrane remained unaffected. These data indicate that the Fcγ receptor and the DR structure are distinct in the monocyte membrane and that Fc binding does not contribute to the formation of rosettes in the monoclonal system. As an additional control, normal rat IgG coupled to trypsin-treated sheep erythrocytes

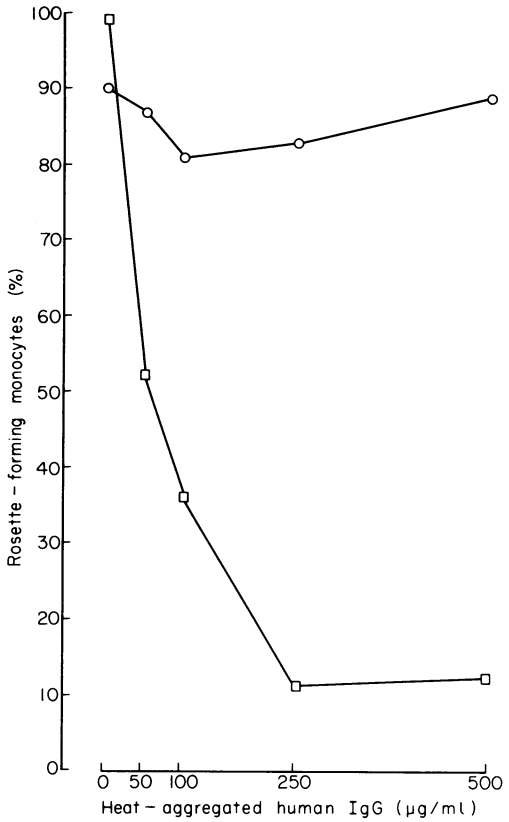

Figure 1. Monocyte rosette formation following incubation with heat-aggregated human IgG. Ox red blood cells (RBC) specifically sensitized with heat-inactivated rabbit anti-ox RBC antiserum at 500 p.p.m. (Fcγ rosettes, □). Sheep RBC coupled with rat monoclonal anti-HLA-DR antibody at 200 μg/ml. DR rosettes (○).

formed rosettes on less than 5% of cultured monocytes.

Titration of the monoclonal reagent in the coupling procedure yielded a panel of erythrocytes bearing increasing suboptimal amounts of anti-HLA-DR antibody. This panel was employed in the rosette assay and, in that region of the dose–response curve where the amount of monoclonal antibody on the erythrocyte became a limiting factor in rosette formation, the assay revealed heterogeneity among monocytes presumably, and not unexpectedly, reflecting differences in the density of HLA-DR determinants. More importantly, for present purposes, the titration assay provides a sensitive method for detecting changes in the expression of these antigens. The same procedure has been used (Rhodes, 1975a; Rhodes, 1977) and is used here, for detecting changes in Fc receptor activity. The initial part of the dose–response curve indicates monocytes with a high avidity for the reagent, whether through specific recognition of membrane determinants by the monoclonal antibody or through Fc binding of the polyclonal anti-erythrocyte antibody. The latter part of the curve includes monocytes with lower avidities. A shift to the left in the dose–response relation indicates increased binding of the reagent.

The data in Fig. 2 show that the expression of Fcγ receptors by purified human monocytes subsequently cultured for 24 hr in the presence of IFN-β is substantially increased over that of control cultures containing no interferon or mock interferon. Concentrations as low as 10^2 r.u. per ml induced significant increases in receptor activity but the effect of IFN at 10^3 r.u. per ml was nevertheless completely inhibited by retinoic acid at 10^{-6} M, and almost completely by retinol at 10^{-6} M (Fig. 2). The β-ionone portion of the

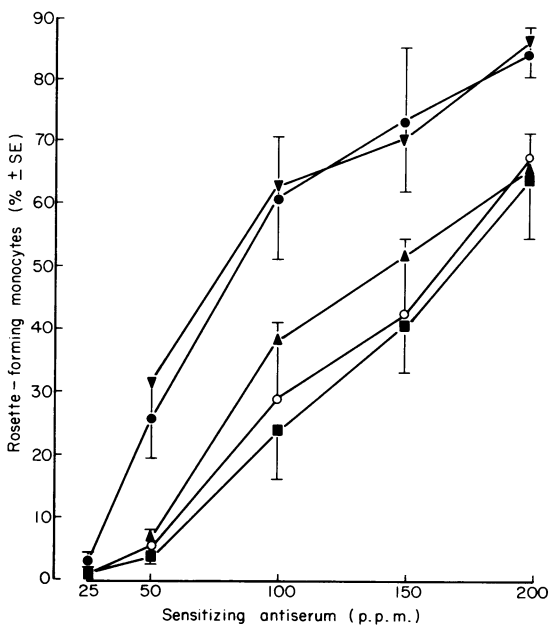

Figure 2. Monocyte Fcγ rosette formation as a function of the dose of anti-RBC antiserum. Monocytes were cultured for 24 hr in RPMI 1640 containing heat-inactivated foetal calf serum at 10% under the following conditions: no addition or mock IFN (○), IFNβ at 10^3 r.u./ml (●), IFNβ at 10^3 r.u./ml plus retinoic acid at 10^{-6} M (■). IFNβ at 10^3 r.u./ml plus retinol at 10^{-6} M (▲). IFNβ at 10^3 r.u./ml plus β ionone at 10^{-6} M (▼).

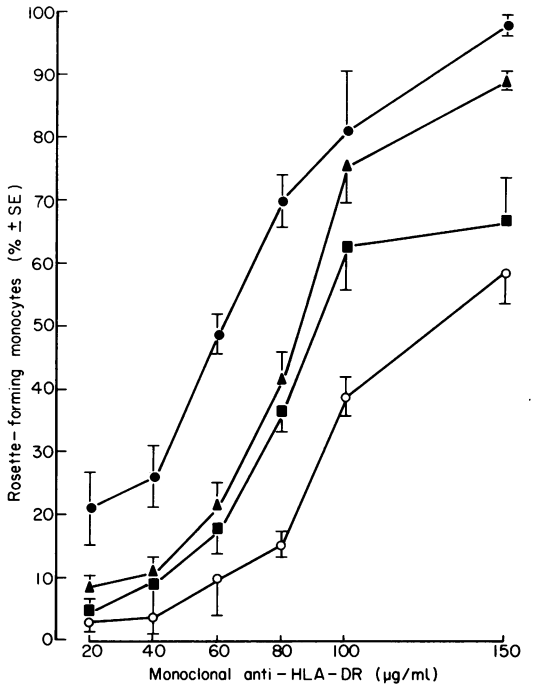

Figure 3. Monocyte DR rosette formation as a function of the dose of rat monoclonal anti-HLA-DR antibody. Monocytes were cultured for 24 hr in RPMI 1640 containing FCS at 10% under the following conditions: no addition or mock IFN (○), IFNβ at 10^3 r.u./ml (●), IFNβ at 10^3 r.u./ml plus retinoic acid at 10^{-6} M (■), IFNβ at 10^3 r.u./ml plus retinoic acid at 10^{-7} M (▲). Each point is the mean ± SE of four or more independent experiments.

retinol molecule was also tested in this system. As shown in Fig. 2, β-ionone at 10^{-6} M did not inhibit the effect of IFN on monocyte Fcγ receptors. (Phytol and arachidonic acid, both of which share certain structural characteristics with retinol and retinoic acid, were also found to be without effect.)

The expression of HLA-DR antigens by cultured monocytes was strikingly increased in the presence of IFN-β compared with that of control cultures containing no IFN or mock IFN (Fig. 3). Once again the effect of IFN at 10^3 r.u. per ml was substantially, though not completely, inhibited by retinoic acid at concentrations of 10^{-6} M and 10^{-7} M (Fig. 3).

DISCUSSION

Our observations on increased monocyte Fc receptor expression in response to human fibroblast interferon (predominantly IFN-β, *Nature*, **287**, 110) are in agreement with the observations of others on lymphoid cells (Fridman *et al.*, 1980; Itoh *et al.*, 1980) and macrophages (Hamburg *et al.*, 1978). As suggested by Fridman *et al.* (1980) such a change may underlie increased cytotoxicity reactions where these depend on Fc receptor activity.

The striking increase in the expression of monocyte HLA-DR antigens in response to IFN-β described here is of interest because T lymphocytes seem to recognize foreign determinants in association with products of the DR (Bergholtz & Thorsby, 1977) or rodent Ia (Paul, Shevach, Thomas, Pickeral & Rosenthal, 1977) regions in the macrophage membrane. It is the polymorphism of these products which may account for genetic differences in immune responsiveness (Rosenthal, Barcinski & Blake, 1977). The expression of these antigens is clearly a dynamic property of monocyte and macrophage membranes. Scher, Beller & Unanue (1980) have described a T-cell product which induces Ia positive macrophage populations *in vivo*. T lymphocyte-derived IFN could play a role in this kind of regulation. Leucocyte-derived IFN does not, however, increase DR antigen expression by lymphoid cells, although HLA-A and B antigen expression is enhanced (Fellous *et al.*, 1979).

The mechanism by which IFN induces changes in the monocyte membrane is unknown but altered ratios of intracellular cyclic nucleotides may be instrumental since IFN increases cGMP levels (Tovey, Rochette-Egly & Castagna, 1979) and the opposite effect, in the form of increased cAMP levels, is known to inhibit macrophage Fc receptor expression (Rhodes, 1975b; Muschel, Rosen, Rosen & Bloom, 1977).

Little is known of the endogeneous factors which may be inhibitory or antagonistic towards IFN activity and the clinical trials of IFN in cancer have proceeded against this background. The system in which we have sought to test IFN-β and its inhibitors is directly relevant to human cancer in that we already know that macrophage Fcγ receptor expression is depressed in the vicinity of primary lung carcinomas even though systemic activation of monocytes occurs, in this respect, in these patients (Rhodes, Plowman, Bishop & Lipscomb, 1981). This depression of receptor expression is mediated *in vitro* by a tumour-derived signal (Rhodes *et al.*, 1981; Rhodes, Bishop & Benfield, 1979). Evidence that retinoids constitute an inhibitory signal in the regulation of IFN activity comes from the work of Blalock and Gifford who showed that retinol and retinoic acid inihibited both

the induction (1977) and anti-viral action (1975) of IFN. The present study shows that retinol and retinoic acid inhibit IFN-induced changes in the monocyte membrane. Moreover, the retinoids were effective at concentrations in the physiological range, considerably below those employed by Blalock and Gifford. The effects of retinoids on monocyte function in the absence of exogenous IFN (Rhodes & Oliver, 1980) may thus be due to inhibition of endogenous monocyte IFN.

The inhibition of IFN by retinoids might well be considered a potentially pro-tumour effect (Gresser & Tovey, 1978) and not, therefore, consistent with the anti-tumour role ascribed to retinoids (Peto, Doll, Buckley & Sporn, 1981). However, this theoretical inconsistency can perhaps be resolved in the following way. Human carcinoma tissue contains binding proteins for retinoic acid which are *not* present in surrounding normal tissue (Chytil & Ong, 1978). Since these human retinoid-binding proteins are a pathological feature in mature tissue, exclusively associated with tumour cells, it seems reasonable to suggest that they may divert endogenous retinoids from the pathway involved in maintaining normal differentiation. Retinoids sequestered by such binding proteins may then be available as local inhibitors of IFN-dependent anti-tumour host defence. We find that a human tumour-derived signal is antagonistic to the effects of IFN on monocytes (Rhodes & Bishop, unpublished observations) and the possibility that this is a tumour-associated retinoid is currently under investigation. The involvement of IFN in host tumour relations may, however, be more complicated than is assumed in the foregoing model. For example, IFN may render target cells more resistant to natural killer cells (Welsh, Karre, Hansson, Kunkel & Kiessling, 1981) and in these conditions retinoids, inhibitory towards IFN, might render target cells more susceptible to natural killing.

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