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Phenotype of human α-interferon producing leucocytes identified by monoclonal antibodies

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SUMMARY

Monoclonal antibodies with specificities for subsets of human leucocytes have been used for the characterization of α-interferon (α-IFN) producing cells. The production of α-IFN was demonstrated to be a function of Ia^+ leucocytes. $OKT3^+$ T lymphocytes, $BA\text{-}1^+$ B lymphocytes and Leu 7^+ natural killer (NK) cells did not contribute to the production of α-IFN. $OKM1^+$ monocytes were essential for the production of α-IFN in response to bacterial products or leukaemia cells, but were not required for the synthesis of virus- or poly I:C-induced α-IFN. The results indicate that α-IFN producing cells represent a heterogenous population of cells of the myeloid lineage.

INTRODUCTION

Interferons (IFN) are a heterogenous group of glycoproteins produced by animal cells in response to viral and non-viral stimuli (Stewart, 1979). Antigenic, biological and physicochemical properties allow the distinction of three major classes of human IFN: α-IFN (leucocyte), β-IFN (fibroblast) and γ-IFN (immune) (Stewart, 1979). α-IFN and β-IFN have been purified to homogeneity and analysis of gene sequences has permitted the deduction of complete amino acid sequences (Derynck *et al.*, 1980; Nagata, Mantei & Weissman, 1980).

In contrast to the advanced knowledge of the molecular structure, considerable uncertainty still exists on the cellular source of α-IFN in human peripheral blood. In view of the controversial results of previous studies on the producer cells of α-IFN (Peter *et al.*, 1980; Roberts *et al.*,1979; Stanwick, Campbell & Nahmias, 1981; Timonen *et al.*, 1980; Trinchieri *et al.*, 1978; Sugiyama & Epstein, 1978; Yamaguchi *et al.*, 1977), we felt it appropriate to re-evaluate this question by the utilization of refined techniques for the functional characterization of human leucocytes.

The recent introduction of a series of murine monoclonal antibodies with specificities for subpopulations of human leucocytes has provided more definitive methods for their identification and characterization. OKT3 monoclonal antibody reacts with 100% of peripheral blood T lymphocytes (Reinherz *et al.*, 1979a). BA-1 antibody reacts with peripheral blood B lymphocytes (Abramson, Kersey & LeBien, 1981). OKIa and anti-Ia monoclonal antibody react with B lymphocytes, monocytes and activated T lymphocytes (Hansen, Martin & Nowinski, 1980; Reinherz *et al.*, 1979b). The antigen defined by OKM1 monoclonal antibody is present on monocytes and granulocytes (Breard *et al.*, 1980). Leu7 monoclonal antibody reacts with a differentiation antigen selectively expressed on leucocytes with NK activity (Abo & Balch, 1981). We have used these monoclonal antibodies for the depletion of leucocyte subsets by complement-mediated lysis and for the identification of leucocyte subpopulations capable of producing α-IFN. The results demonstrate that the production of α-IFN is a function of Ia^+ leucocytes.

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MATERIALS AND METHODS

Leucocyte 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, Germany) 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.

Leucocyte fractionation. Monoclonal antibodies OKT3, OKIa and OKM1 were purchased from Ortho Pharmaceutical Corporation, Raritan, New Jersey, USA. BA-1 monoclonal antibody was obtained from Hybritech Europe, Brussels, Belgium; anti-Ia monoclonal antibody was obtained from New England Nuclear Chemicals, Dreieich, Germany and anti-Leu7 monoclonal antibody was obtained from Becton Dickinson Laboratory Systems, Rödermark, Germany. For leucocyte fractionation, plastic non-adherent cells were suspended at a concentration of 6×10^6/ml in culture medium containing OKT3 at a final dilution of 1:50 or BA-1, OKIa, anti-Ia OKM1 or anti-Leu7 at a final dilution of 1:10. Cells to be treated with complement alone were incubated in culture medium without monoclonal antibodies. After a 1 h incubation on ice, non-toxic rabbit complement (Cedarlane Laboratories, Hornby, Canada) 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 α-IFN 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 as described by Reinherz *et al.* (1979b). Following complement-mediated depletion procedures, cells reactive with the lysing monoclonal antibody were always reduced to below 2%.

Induction of IFN production. Complement treated control cells or cells fractionated by treatment with monoclonal antibodies and complement were resuspended in RPMI-FCS at a concentration of $2{\cdot}5 \times 10^6$ cells/ml. Influenza A virus (A/X31; kindly provided by Dr J. Skehel, Mill Hill, England; 100 haemagglutinating units/ml), polyinosinic–polycytidilic acid (poly I:C, Boehringer, Mannheim, Germany; 100 μg/ml), *Corynebacterium parvum* (CP; Wellcome, Großburgwedel, Germany; 5 μg/ml) or $2{\cdot}5 \times 10^5$ heat (56°C, 45 min) treated Molt4 human leukaemia cells (Minowada, Ohnuma & Moore, 1972) were added to the cultures, which were then incubated at 37°C in a humidified 5% CO_2 atmosphere. Supernatants were harvested after 24 h and stored at −20°C until testing for antiviral activity.

IFN assay. Anti-viral activity in culture supernatants was determined by measuring the inhibition of the cytopathic effect (CPE) of Vesicular stomatitis virus (VSV) on human amnion (WISH) cells. Serial two-fold dilutions of culture supernatants were made in volumes of 0·05 ml in the wells of flat bottomed 96 well microtitre plates (Flow). WISH cells at a concentration of 2×10^4 in 0·05 ml minimal essential medium (MEM) (Flow) with 10% FCS were added and incubated at 37 C for 24 h in a humidified 5% CO_2 atmosphere. The resulting confluent monolayers were then drained by inversion of the microtitre plate over a sterile pad and 0·05 ml of a suspension of VSV in MEM-FCS were added to each well. Virus was allowed to adsorb at 37°C for 2 h, then the microtitre plates were drained by inversion and 0·1 ml of MEM-FCS were added to each well. The final evaluation of the CPE was done after a further incubation of the microtitre plates for 40 h at 37°C. Anti-viral units are expressed as the reciprocal of the highest dilution inhibiting 50% of the CPE and are equivalent to approximately one reference unit of the WHO human leucocyte reference interferon B 69/19.

IFN characterization. Anti-viral activity in culture supernatants was characterized as IFN by its sensitivity to the action of proteolytic enzymes and by its lack of virus specificity as demonstrated by the ability to protect WISH cells against challenge with both VSV or HSV 1. Classification of the anti-viral activity as α-IFN or γ-IFN was performed by examining its sensitivity to treatment at pH 2 or heat (56°C, 1 h), by determination of its host range, and by studying the effect of pre-incubation with antibody against α-IFN (kindly provided by Dr K. Cantell, Helsinki, Finland).

RESULTS

Preliminary experiments were performed to determine the physicochemical, biological and antigenic properties of anti-viral activity induced in cultured leucocytes. Anti-viral activity produced by leucocytes in response to influenza A/X31 virus, poly I:C, or Molt4 leukaemia cells was characterized as α-IFN. The anti-viral activity was insensitive to treatment at pH 2 or 56°C; it showed anti-viral effects on both homologous and heterologous bovine cells and the anti-viral activity was completely neutralized by incubation with antibody against human α-IFN. In contrast, anti-viral activity produced in response to stimulation with CP was demonstrated to be a mixture of α-IFN and γ-IFN. Approximately 80–90% of CP-induced anti-viral activity showed the characteristics of α-IFN, i.e. acid and heat stability, activity on heterologous cells, and neutralization by antibody against α-IFN. The remaining 10–20% of anti-viral activity shared several of the properties of γ-IFN, i.e. sensitivity to treatment at pH 2 and 56°C, restriction of the anti-viral activity to homologous cells, and lack of neutralization by incubation with antibody against α-IFN.

As demonstrated in Table 1, depletion of $OKT3^+$ T lymphocytes by antibody-mediated complement-dependent lysis resulted in a marked enrichment for α-IFN producing cells. Enhancement of α-IFN yields varied from two- to eight-fold. Depletion of $BA\text{-}1^+$ B lymphocytes had no significant effect on the production of α-IFN (Table 2). Lysis of Ia^+ cells with two different monoclonal antibodies reactive with the HLA-DR framework markedly reduced or even abrogated α-IFN production (Table 3). Elimination of leucocytes expressing the monocyte marker antigen

Table 1. α-IFN production of human leucocytes depleted of a subset reactive with a monoclonal anti-T lymphocyte antibody

Expt No.	Leucocyte fraction	Recovered cells (%)	Yield of IFN (iu/ml) following induction with			
			A/X31	Poly I:C	CP	Molt4
1	Unfractionated	100	800	640	320	640
	$OKT3^-$	29	2,400	5,120	640	1,280
2	Unfractionated	100	1,600	1,280	160	320
	$OKT3^-$	27	4,800	1,920	320	640
3	Unfractionated	100	1,600	2,560	640	320
	$OKT3^-$	30	12,800	5,120	2,560	1,280

Table 2. α-IFN production of human leucocytes depleted of a subset reactive with a monoclonal anti-B lymphocyte antibody

Expt No.	Leucocyte fraction	Recovered cells (%)	Yield of IFN (iu/ml) following induction with			
			A/X31	Poly I:C	CP	Molt4
1	Unfractionated	100	3,200	640	nd	320
	$BA\text{-}1^-$	87	3,200	640	nd	320
2	Unfractionated	100	3,200	320	320	640
	$BA\text{-}1^-$	90	4,800	320	320	320
3	Unfractionated	100	800	960	640	320
	$BA\text{-}1^-$	93	800	640	640	240

nd = not done.

Table 3. α-IFN production of human leucocytes depleted of subsets reactive with monoclonal anti-Ia antibodies

Expt No.	Leucocyte fraction	Recovered cells (%)	Yield of IFN (iu/ml) following induction with			
			A/X31	Poly I:C	CP	Molt4
1	Unfractionated	100	6,400	320	nd	1,280
	Ia^-	72	< 100	20	nd	< 10
2	Unfractionated	100	9,600	240	320	640
	Ia^-	82	800	10	< 10	< 10
3	Unfractionated	100	1,600	960	160	160
	Ia^-	87	< 100	80	< 10	< 10
4	Unfractionated	100	3,200	640	160	640
	Ia^-	82	200	80	< 10	< 10
	$OKIa^-$	78	400	160	< 10	40
5	Unfractionated	100	3,200	1,280	320	640
	$OKIa^-$	79	400	160	< 10	80
6	Unfractionated	100	6,400	2,560	640	640
	$OKIa^-$	92	800	160	20	40

nd = not done.

Table 4. α-IFN production of human leucocytes depleted of a subset reactive with a monoclonal anti-monocyte antibody

Expt No.	Leucocyte fraction	Recovered cells (%)	Yield of IFN (iu/ml) following induction of			
			A/X31	Poly I:C	CP	Molt4
1	Unfractionated	100	6,400	2,560	640	640
	$OKM1^-$	82	3,200	640	160	240
2	unfractionated	100	3,200	1,280	320	640
	$OKM1^-$	74	1,600	1,280	20	80
3	Unfractionated	100	800	640	480	960
	$OKM1^-$	86	600	640	40	120
4	Unfractionated	100	6,400	nd	640	320
	$OKM1^-$	80	6,400	nd	160	160
?	Unfractionated	100	1,600	320	320	160
	$OKM1^-$	78	2,400	320	80	80

nd = not done.

Table 5. α-IFN production of human leucocytes depleted of a subset reactive with a monoclonal antibody against natural killer cells

Expt No.	Leucocyte fraction	Recovered cells (%)	Yield of IFN (iu/ml) following induction with			
			A/X31	Poly I:C	CP	Molt4
1	Unfractionated	100	3,200	2,560	1,280	2,560
	$Leu7^-$	65	3,200	1,280	320	640
2	Unfractionated	100	1,600	320	640	640
	$Leu7^-$	59	1,600	320	640	320
3	Unfractionated	100	3,200	640	640	160
	$Leu7^-$	63	2,400	640	480	160

OKM1 resulted in diminished yields of α-IFN in response to induction with CP or Molt4 leukaemia cells, but had only moderate or no effects on the production of virus- or poly I:C-induced α-IFN (Table 4). Depletion of Leu7$^+$ cells with NK activity did not cause consistent changes in the ability to secrete α-IFN (Table 5).

DISCUSSION

The present results suggest that the production of α-IFN is a function of Ia$^+$ leucocytes. Ia antigens have been demonstrated to be expressed on adherent or non-adherent monocytes, B lymphocytes and activated T lymphocytes (Reinherz *et al.*, 1979a). The possible involvement of T lymphocytes in the production of α-IFN was ruled out by the observation that removal of OKT3$^+$ T lymphocytes resulted in enhanced yields of α-IFN. We further could not detect any substantial production of α-IFN by stimulation of interleukin 2-dependent long term cultured T lymphocytes (which showed expression of Ia antigens) with viruses, synthetic polynucleotides or tumour cells (unpublished results). In contrast to the general agreement that α-IFN is produced by non-T cells (Peter *et al.*, 1980; Roberts *et al.*, 1979; Stanwick *et al.*, 1981; Timonen *et al.*, 1980; Trinchieri *et al.*, 1978; Yamaguchi *et al.*, 1977), contradictory results have been published regarding the contribution of other lymphocyte or monocyte subpopulations. Thus, B lymphocytes (Yamaguchi *et al.*, 1977), null lymphocytes (Peter *et al.*, 1980), or macrophages (Roberts *et al.*, 1979; Stanwick *et al.*, 1981) have been implicated in the synthesis of IFN in response to viral or non-viral inducers. According to our results with the BA-1 monoclonal antibody which is reactive with all surface immunoglobulin expressing B lymphocytes in human peripheral blood (Abramson *et al.*, 1981), B cells apparently did not contribute to the production of α-IFN. Experiments with the OKM1 monoclonal antibody demonstrated that, depending on the type of inducer, different leucocyte populations may be preferentially triggered to secrete α-IFN. Thus, OKM1$^+$ monocytes were essential for obtaining optimal yields of IFN following induction with CP or leukaemia cells, but were not required for the synthesis of virus- or poly I:C-induced IFN. These results, however, do not exclude the possibility that—in analogy to their role in the production of α-IFN—OKM1$^+$ monocytes may function as accessory rather than as producer cells in the synthesis of mitogen- or tumour cell-induced α-IFN.

Previous studies have suggested that producer cells of α-IFN and NK cells are largely overlapping, if not identical populations (Peter *et al.*, 1980; Timonen *et al.*, 1980; Trinchieri *et al.*, 1978). This interpretation was based on the observation that leucocyte fractionation on density gradients established a direct relationship between cells with the typical morphology of large granular lumphocytes (LGL), NK activity and α-IFN production (Timonen *et al.*, 1980). In addition, bright cytoplasmic anti-α-IFN immunofluorescence was induced in cells with the characteristic LGL morphology by contact with tumour cells (Timonen *et al.*, 1980). Recently, the identification of human leucocytes with NK activity has been improved by the availability of a specific monoclonal antibody (Abo & Balch, 1981). Elimination of cells reactive with this antibody by complement-mediated lysis has been reported to result in marked decreases of NK activity (Abo & Balch, 1981). Depletion of Leu7$^+$ NK cells in our experiments, however, did not cause significant reductions in the yields of anti-viral activity and thus argues against a major role of NK cells in the production of α-IFN. These findings further indicate that the augmentation of the cytotoxic activity of NK cells by IFN (Herberman, 1981) may not be the consequence of positive self regulation but rather of cellular cooperation.

Based on our observations with four different viral or non-viral inducers, we propose that the production of α-IFN is a function of Ia$^+$, OKT3$^-$, BA-1$^-$, Leu7$^-$ leucocytes. The findings would be compatible with the interpretation that α-IFN producing cells represent a heterogenous population of cells of the myeloid lineage. Further elucidation of the interrelationship of leucocytes involved in non-specific immunoregulatory or effector functions is dependent on improved methods for the isolation of myelomonocytic cells at different developmental stages.

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