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INTERFERON ALPHA-INDUCED MODULATION OF LEUKOCYTE CELL SURFACE ANTIGENS: IMMUNOCYTOFLUOROMETRIC STUDY WITH HUMAN LEUKAEMIA/LYMPHOMA CELL LINES

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Summary. – Recombinant interferon alpha enhanced the MHC class I antigen density on human leukaemia/lymphoma cell lines REH, U-937 and HL-60, as measured by immunocytofluorometry using specific monoclonal antibodies. A similar effect was induced (as demonstrated in REH cells), also by human leukocyte interferon-alpha. The latter, however, caused no major alterations in the expression of leukocyte common antigen (ICA; CD45) and transferrin receptor (CD71) in the cell lines examined. In REH cells, there was no interferon-induced alteration of CD10 antigen (CALLA), which in this cell line is markedly down-regulated by 12-0-tetradecanoyl-phorbol-13-acetate (TPA). A decrease of CD4 antigen density on the cell membrane was induced by interferon-alpha in monoblastoid U-937 cells. No induction of MHC class I and II antigens by interferon-alpha was found in K-562 cell subline.

Key words: MHC antigens; leukocyte antigens; interferon-alpha; monoclonal antibodies; leukaemia cell lines; immunocytofluorometry

Introduction

Interferon (IFN) induces anti-viral, anti-proliferative, and immunomodulatory effects such as enhanced expression of cell surface MHC antigens, stimulation of cytotoxic T-lymphocytes and natural killer (NK) cell activities (rev. by Clemens and McNurlan, 1985; Hokland *et al.,* 1988). IFN-gamma-induced membrane expression of MHC class I antigens was observed in human myeloid-(Sutherland *et al.*, 1985) and lymphoid leukaemia cell lines (Gerrard *et al.*, 1988), as well as in non-haematopoietic tumour (carcinoma, neuroblastoma) cell lines (Gross *et al.*, 1987). Recombinant interferon (rIFN)-gamma was recently shown to induce down-regulation of CD4 antigen (HIV receptor) expression on peripheral blood monocytes and myelomonocytic cell lines (Faltynek *et al.*, 1989). This finding can be of clinical relevance as the expres-

sion of CD4 antigen on monocyte cell surface correlates with the susceptibility to HIV infection (Dalgleish *et al.*, 1984; Asjo *et al.*, 1987). The effects of human recombinant and leukocyte IFN-alpha upon the membrane expression of MHC class I, II, and some further leukocyte differentiation antigens were less frequently studied (Hokland *et al.*, 1988; Halloran *et al.*, 1989). The action of IFN-alpha on the cell surface expression of some leukocyte surface antigens in neoplastic haematopoietic cell lines are described in this communication.

Materials and Methods

Cell lines. Human promyelocytic leukaemia cell line HL-60, monoblastoid cell line U-937, immature erythroid-myeloid leukaemia cell line K-562 and non-T, non-B ALL cell line REH were kept as stationary suspensions in RPMI-1640 medium supplemented with 10 % foetal calf serum and in humidified 5.0 % CO_2 atmosphere.

Monoclonal antibodies. Monoclonal antibodies directed to MHC class II antigens (DR; monoclonal antibody Bra30; DP: monoclonal antibody BraFB6) were described in previous reports

Table 1. Immunocytofluorometric measurements of leukocyte surface antigens expressed on human leukaemia cell line REH after induction by interferon-alpha

Antigen	% of immunofluorescence positive cells (mean fluorescence intensity)[c]		
Monoclonal antibody	REH cells	REH cells-rIFN[a]	REH cells-L IFN[b]
Transferrin receptor MEM-75	98 % (78)	97 % (72)	95 % (68)
MHC class I antigen Bra 23/9	95 % (1205)	99 % (1520)	99 % (1840)
MHC class II antigen (DR) Bra30	78 % (1477)	95 % (1504)	78 % (1604)
MHC class II antigen (DP) BraFB6	97 % (360)	99 % (358)	100 % (898)
CD45 antigen Bra55	100 % (335)	100 % (264)	99 % (276)
CD10 antigen DGH 10	76 % (104)	98 % (72)	77 % (112)

[a, b, c] – as in Tab. 2

Table 2. Immunocytofluorometric measurements of leukocyte surface antigens expressed on monoblastoid U-937 cells after induction by IFN-alpha

Antigen	% of positive cells (fluorescence intensity)[c]	
Monoclonal antibody	U-937 cells	U-937 rIFN[a]
Transferrin receptor		
MEM-75	99 % (45)	99 % (53)
MHC class I antigen		
Bra 23/9	100 % (1750)	100 % (2819)
MHC class II antigen (DR)		
Bra 30	81 % (134)	80 % (128)
CD45 antigen		
Bra 55	100 % (506)	100 % (527)
CD4 antigen		
OKT4	100 % (165)	92 % (125)

[a] - recombinant interferon alpha, 10^3 U/ml, 48 hours
[b] - L IFN - leukocyte interferon alpha, 10^3 U/ml, 48 hours
[c] - relative units, determined by cytofluorometry

(Poláková *et al.*, 1985; Chorváth *et al.*, 1987; Hořejší *et al.*, 1988). Monoclonal antibody Bra23/9 against the MHC class I antigen (non-polymorphic determinant) was characterized previously (Plešková *et al.*, 1988). Monoclonal antibodies Bra55 and Bra11 directed to the leukocyte common antigen (LCA, CD45) were described elsewhere (Chorváth *et al.*, 1987; Sedlák *et al.*, 1989). Anti-transferrin receptor monoclonal antibody MEM-75 (Hořejší *et al.*, 1988) was provided by Dr. V. Hořejší, Institute of Molecular Genetics, Prague. CD10 monoclonal antibody (anti-CALLA) J5 was provided by Dr. B. Dörken (University of Heidelberg, F. R. G.) in the frame of the 4th International Workshop on leukocyte antigens (1988/89). Monoclonal antibody DGH.10-1-A9 (CD10) was provided by Prof. H. Grosse-Wilde (University of Essen, F. R. G.).

Interferons and interferon induction of cell lines. Human leukocyte interferon-alpha was prepared according to Fuchsberger and Borecký (1979); its specific activity was approximately 10^6 U/mg protein. Human rIFN-alpha$_{2a}$ was provided by the courtesy of Hoffmann-LaRoche. Induction was performed in 24-well plastic tissue culture cluster plates, at 5.10^5/ml cell density. No major differences in cell surface antigen expression between 24-or 48 hr interferon iductions were observed.

Immunofluorescence staining was performed as described previously (Chorváth *et al.*, 1987) and evaluated by immunocytofluorometry with the aid of FACStar (Becton-Dickinson) cytofluorometer equipped with a 5W argon laser at the excitation wavelength of 488 nm (Festin *et al.*, 1987). The software provided by the producer was utilized to determine the percentage of immunofluorescence positive cells and mean relative immunofluorescence intensities.

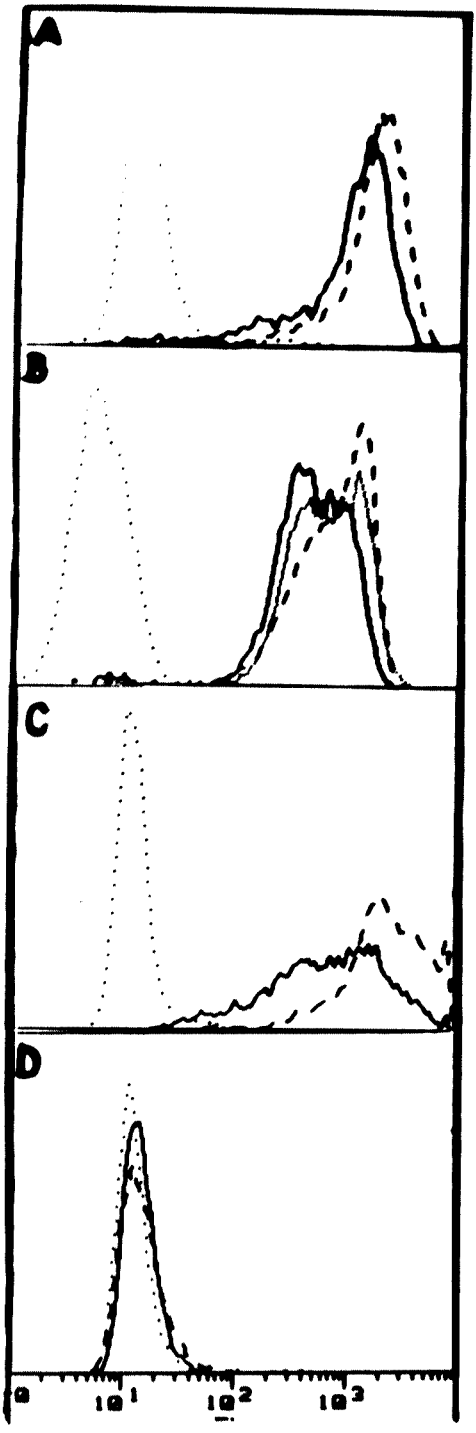

Fig. 1
Immunocytometric determination of the MHC class I antigen
Cell surface expression on U-937 cells (A), REH cells (B), HL-60 cells (C), and K-562 cells (D) before (full lines) and after (dashed lines) induction with rIFN-alpha, 10^3 U/ml, 24 hr. In 1B dotted line shows induction with rIFN, dashed line with rIFN combined with 1 μmol/l retinoic acid, for 48 hr. Spaced dotted line at the left side of histograms shows negative controls. Abscissa: fluorescence intensity (relative units); ordinate: number of cells.

Results

Both leukocyte interferon-alpha (L-IFN) and recombinant interferon-alpha$_{2a}$ (rIFN) exerted similar effects upon class I HLA antigen expression measured immunocytofotometrically in non-T, non-B ALL cell line REH, i. e. induced an increase of membrane antigen (immunofluorescence intensity) of MHC class I antigen and, to a lesser extent, of MHC class II (DP) antigen (Table 1, Fig. 1B, Fig. 2C). The up-regulation of class I HLA by rIFN on REH

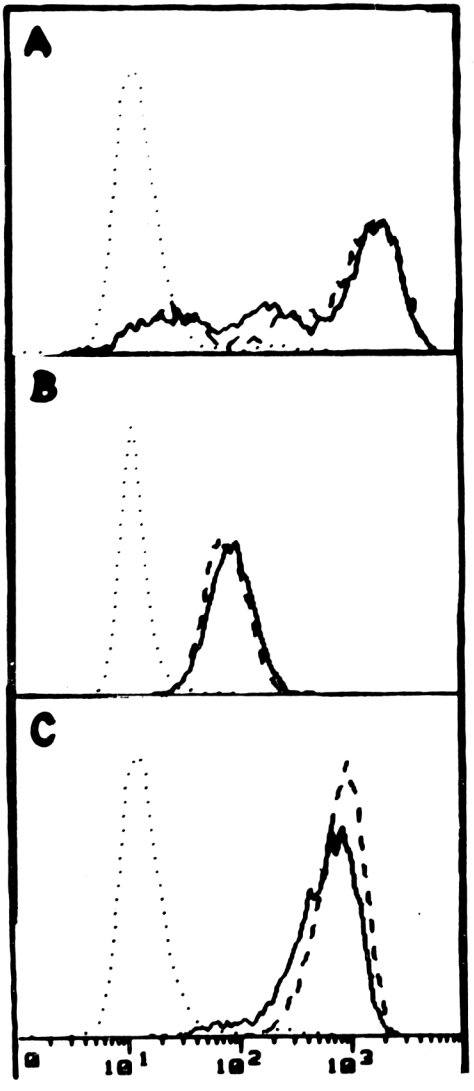

Fig. 2
Immunocytometric determination of MHC class II antigens
DR antigen = A, B; DP antigen = C on REH cells (A, C), HL-60 cells (B) before (full line) and after (dashed line) induction with rIFN-alpha (10^3 U/ml for 24 hr). Controls and further legend as Fig. 1

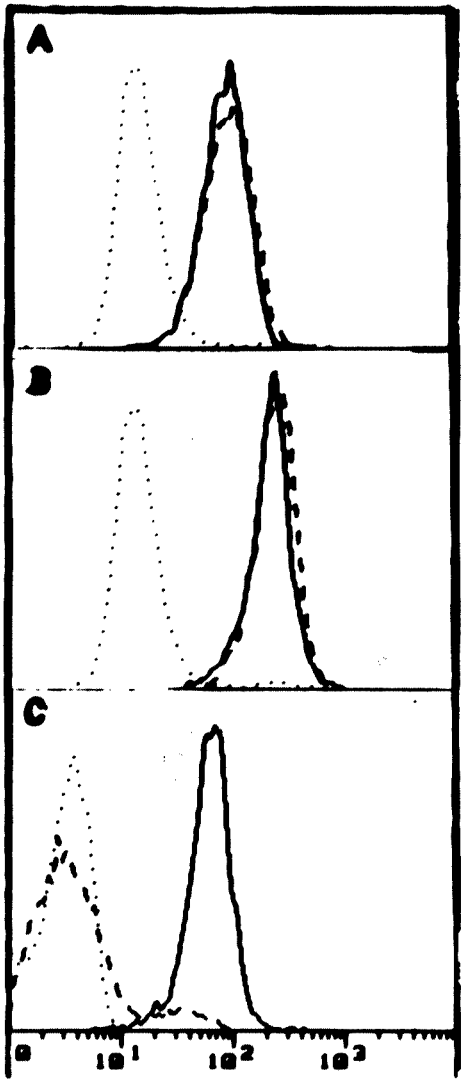

Fig. 3
Immunocytometric determination of CD10 antigen cell surface expression on REH cells
CD10 monoclonal antibody J5 (A) or DGH 10-1-A9 (B,C) before (full line) and after (dashed line) induction with rIFN-alpha (A), leukocyte IFN-alpha (B) and phorbol ester TPA (C) at 50 nmol/l concentration for 48 hr. Conditions, negative controls, and further legends as described in Fig. 1.

cells was slightly enhanced by retinoic acid (Fig. 1B), a substance known to modulate cell surface antigen expression (Gross *et al.*, 1987). No IFNα - induced alterations of three further leukocyte surface antigens, i. e. transferrin receptor (Table 1), leukocyte common antigen (Table 1, Fig 4A) and CD10 (CALLA) antigen (Table 1, Fig. 3A, 3B) were observed in REH cells. Both transferrin receptor (CD71) and CD10 antigen were markedly down-regulated on REH cells by 12-0-tetradecanoyl-phorbol-13-acetate (TPA), as shown in Figs. 3C, 5C.

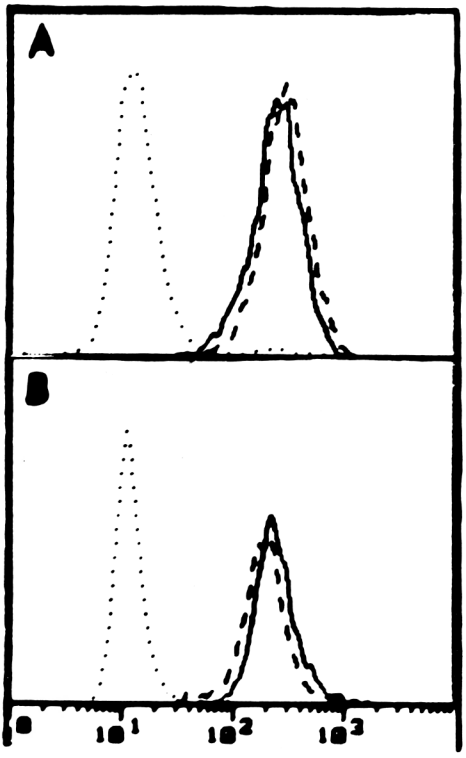

Fig. 4
Immunocytometric determination of CD45 antigen cell surface expression REH (A) and HL-60 cells (B) before (full line) and after (dashed line) induction with recombinant (A) or leukocyte IFN-alpha (B). Conditions and legends as to Fig. 1.

In monoblastoid U-937 cells rIFN-alpha induced an increase in antigen density (immunofluorescence intensity) of MHC class I antigen (Table 2, Fig. 1A). No major rIFN-induced alterations in MHC class II (DR) and leukocyte common (CD45) antigens were found in these cells (Table 2). Cell surface expression of CD4 antigen (HIV receptor) which is dramatically down-regulated on U-937 cells by TPA (Fig. 6B), decreased, though, to a lesser extent, also after induction by rIFN-alpha (Table 2, Fig. 6A).

A slight down-regulation of transferrin receptor was induced by rIFN-alpha also in human promyelocytic leukaemia cell line HL-60 (Table 3, Fig. 5A), where also a slight increase in membrane expression of MHC class I (Table 3, Fig. 1C), but no major alterations of MHC class II (Table 3, Fig. 2B) and CD45 (Table 3, Fig. 4B) antigens were found.

Finally in the immature erythroid-myeloid leukaemia K-562 cells which are unable to synthesize constitutively MHC class I and class II antigens, no induction of these antigens occurred due to the action of rIFN-alpha (Table 4, Fig. 1D). The percentage of transferrin receptor positive cells was unchanged, but

Table 3. Immunocytofluorometric measurements of leukocyte surface antigens expressed on promyelocytic HL-60 human leukaemic cells induced by interferon-alpha

Antigen	%positive cells (fluorescence intensity)[c]	
Monoclonal antibody	HL-60	HL-60, rIFN[a]
Transferrin receptor		
MEM-75	98 % (735)	100 % (404)
MHC class I antigen		
Bra 23/9	53 % (721)	52 % (822)
MHC class II antigen (DR)		
Bra 30	89 % (100)	96 % (92)
CD45 antigen		
Bra 55	97 % (228)	87 % (172)

[a] - recombinant interferon alpha, 10^3 U/ml, 48 hours
[c] - relative fluorescence units

Table 4. Immunocytofluorometric measurements of leukocyte surface antigens expressed on human leukaemia cell line K-562 induced by interferon-alpha

Antigen	% positive cells (fluorescence intensity)[c]	
Monoclonal antibody	K-562 cells	K-562 cells, rIFN[a]
Transferrin receptor		
MEM-75	99 % (285)	99 % (326)
MHC class I antigen		
Bra 23/9	5 % (155)	6 % (51)
MHC class II antigen (DR)		
Bra 30	5 % (118)	4 % (38)
MHC class II antigen (DP)		
Bra FB6	5 % (57)	3 % (126)

[a] - recombinant interferon 10^3 U/ml, 48 hours
[c] - relative fluorescence units

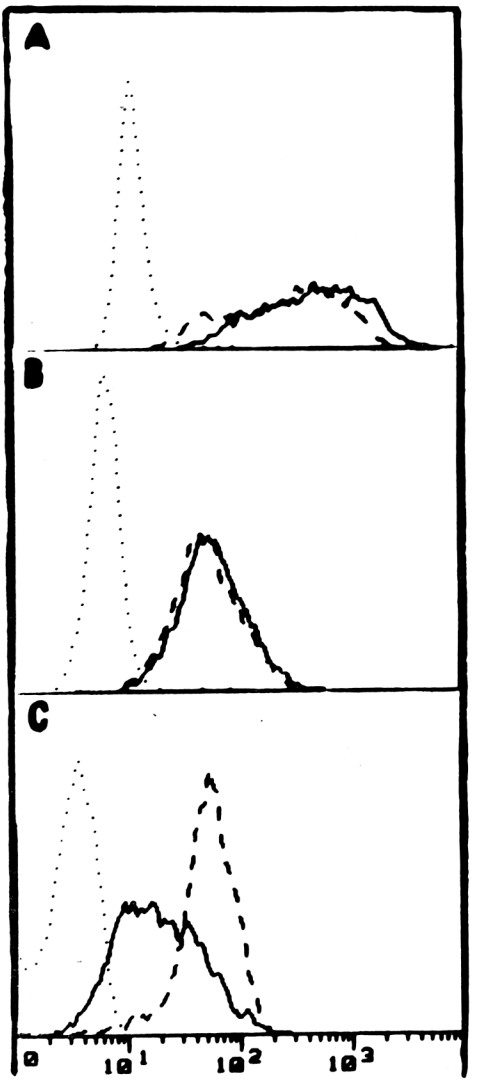

Fig. 5
Immunocytometric determination of the expression of transferrin receptor HL-60 (A) and U-937 (B, C) cells, before (full line) and after induction (dashed line) with rIFN-alpha (A, B - 10^3 U/ml rIFN, for 48 hr). 5C = U-937 cells before (dashed line) and after induction with 50 nmol/l TPA for 48 hr (full line).

a slight increase of immunofluorescence intensity with anti-transferrin receptor monoclonal antibody was observed on K-562 after rIFN induction (Table 4).

Discussion

Both leukocyte-and recombinant interferon-alpha increased the HLA class I

antigen cell surface density in human leukaemia lymphoma cell line REH. Similarly, recombinant IFN-alpha was shown to increase the MHC class I antigen density in U-937 cells. These findings confirm previous reports on the ability of both IFN gamma (Sutherland *et al.*, 1985; Gerrard *et al.*, 1988; Rosa and Fellous, 1988) and IFN alpha/beta to increase HLA class I expression on tumour cells (Hokland *et al.*, 1988; Halloran *et al.*, 1989). Such process might be of potential clinical relevance, as the induction and up-regulation of HLA class I antigens in human solid tumours stimulated the autologous cytotoxicity of peripheral blood lymphocytes against tumour cells *in vitro* (Vánky *et al.*, 1989).

No interferon-induced alterations were found in the expression of further leukocyte surface antigens, such as leukocyte common antigen (CD45) and transferrin receptor (CD71), which is dramatically down-regulated by the action of tumour promoting phorbol ester (TPA) in human neoplastic haematopoietic cell lines. There was no IFN alpha-induced modification in the membrane expression in common ALL (CD10, CALLA) antigen, which again

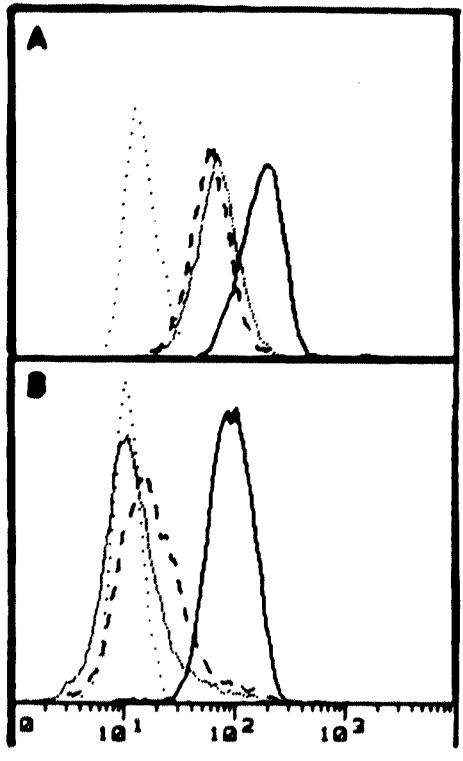

Fig. 6
Down-regulation of CD4 antigen U-937 cells (A, B) induced by rIFN-alpha, 10^3 U/ml, for 24 hr (A, dotted line); rIFN-alpha, 5.10^3 U/ml, for 24 hr (dashed line, A); TPA- 50 nmol/l for 24 hr (dashed line, B). Untreated cells (full line; A, B). Negative controls (without monoclonal antibody) prior to - (spaced dotted line) and after TPA induction (dotted line); see legend to Fig. 1.

is extensively down-regulated by TPA on REH cells (Srivastava *et al.*, 1984; Sakagami *et al.*, 1984).

The inability of IFN-alpha to induce the expression of MHC class I antigen in K-562 cells is in our experiments at variance with the data of Sutherland *et al.*, (1985); this may be explained due to the differences in K-562 sublines and interferon preparations examined in both studies. Finally, the IFN-alpha-induced decrease of cell surface expression of CD4 antigen, analogous to the IFN-gamma-induced effect on CD4 antigen expression in human peripheral blood monocytes and myelomonocytic cell lines described recently (Faltynek *et al.*, 1989) was observed in our study on U-937 cells. This effect upon HIV receptor (CD4 antigen) might play a role in modulating the susceptibility to HIV infection.

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