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Enhancement by interferon of membrane HLA antigens in patients with combined immunodeficiency with defective HLA expression

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SUMMARY

Since interferon is known to enhance HLA A-B expression on lymphocytes from normal donors, we have tested the hypothesis that interferon could reverse the defective membrane expression of HLA antigens observed in some patients with combined immunodeficiency. Leucocytes from four patients with this syndrome, after overnight incubation with preparations of interferon, showed a clear enhancement in the percentage of cells bearing HLA A-B-C and β_2 microglobulin (but not HLA-DR) antigens as detected by membrane immunofluorescence. Functional HLA-A and B antigens also appeared on patients' T cell blasts treated with interferon, as shown by the ability of these blasts to be destroyed by specific cytotoxic T lymphocytes. Both α and β human interferons were effective. These effects were shown to be mediated by interferon (but not contaminants in our preparations) by the use of specific antiserum to interferon. It is likely that interferon acts on HLA synthesis, since *in vitro* addition of drugs known to inhibit nucleic acid or protein synthesis completely abolished the enhancing effect of interferon on membrane HLA expression. Interferons can therefore modulate leucocyte HLA expression and synthesis in patients with defective expression of these antigens, a finding which suggests that interferon treatment might be beneficial in this condition.

INTRODUCTION

Rare patients are characterized by the association of a special type of combined immunodeficiency and a profoundly defective expression of membrane HLA antigens on leucocytes (Schuurman *et al.*, 1979; Touraine *et al.*, 1978). We have observed a series of eight patients with this syndrome and confirmed the immunological deficiency of both antibody production and T cell-mediated function (Griscelli *et al.*, 1980). This autosomal recessive condition is lethal through severe infections due to viruses, *Pneumocystis carinii* and *P. moniliasis*. In all patients of our series, peripheral blood leucocytes were shown to poorly express HLA A, B, C and Dr antigens and the associated protein, β_2 microglobulin (β_2m) (Griscelli *et al.*, 1980).

Among many other biological effects, interferons are able to enhance the expression of H_2 (but not Ia) antigens on the membrane of mouse normal lymphoid cells and tumours, both *in vitro* (Lindahl, Leary & Gresser, 1973, 1974) and *in vivo* (Lindhal *et al.*, 1976). *In vitro* studies also showed that human leucocyte interferon is able to enhance the expression of HLA, A, B and β_2m (but not HLA-DR) antigens on human lymphoblastoid cell lines and peripheral blood leucocytes from normal donors (Fellous *et al.*, 1979). It was thus of interest to know whether interferon could

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enhance the expression of HLA, A, B or DR antigens in a disease where expression of these antigens is abnormal. In such patients, an important although variable proportion of leucocytes do not express HLA, A, B and DR antigens as detected by immunofluorescence. We have taken advantage of this situation to evaluate in a semi-quantitative way the enhancement of HLA antigen expression by counting the number of cells showing detectable HLA antigens after incubation in control culture medium or medium containing either α or β interferon.

MATERIALS AND METHODS

Patients. Three of the five patients studies in the present study have been previously described (AC, DB, RA) (Griscelli *et al.*, 1980). The clinical and biological aspects of the disease of patient OA, (the brother of patient RA) and of patient NH are similar to those of the previously published patients.

Interferons. Human β interferon (IFN) induced by viral infection of fibroblasts was from Rega Institute, Leuwen, Belgium. Human α IFN was produced by Sendai virus infection of leucocytes (Inst. Pasteur, Paris, France). The rabbit antiserum to human β IFN was a kind gift from Dr Billau (Rega institute) and had a neutralizing titre of 50,000 against 10 iu of β IFN.

Interferon assay. The titre of interferon was the reciprocal of the last dilution protecting 50% monolayers of human embryo fibroblasts against the cytopathic effects of vesicular stomatitis virus. The titre of antiserum to interferon was calculated by finding the last dilution inhibiting by 50% the anti-viral effect of 10 iu of the relevant interferon species.

Incubation of leucocytes. Peripheral blood leucocytes (PBL) were isolated on a Ficoll-Hypaque gradient, then washed twice in Hank's buffer solution. PBL were incubated for 1–18 h at 37°C at 1×10^6/ml in RPMI 1640 culture medium (Gibco, Glasgow, Scotland) containing 5% fetal calf serum (Microbio Ass, Bethesda, Maryland) and glutamine (0·3 mg/ml Inst. Mérieux, Lyon, France). IFN was added at a final concentration of 10,000 iu/ml for α IFN, and of 2,000 iu/ml for β IFN. For neutralization, the antiserum was added in antibody excess to the β IFN preparation just before addition to the cells. After incubation, cell viability as judged by trypan blue exclusion was close to 100 % in all cultures.

Drugs inhibiting nucleic acids or protein synthesis. In some experiments, actinomycin, a drug which inhibitis nucleic acid synthesis or cycloheximide, known to suppress protein synthesis, was added respectively at doses of 0·25 μg/ml and 25 μg/ml to the incubation medium.

Immunofluorescence studies. Leucocytes were incubated with specific antisera for 30 min at 0°C then washed twice in Hank's buffer solution supplemented with NaN_3 (0·1%). Monoclonal anti-common HLA A, B and C antibodies (Sera Lab, Sussex, England) were used at a final concentration of 1:50 (Barnstable *et al.*, 1978). Monoclonal anti-β_2 m antibodies (Sera Lab) were used at a final concentration of 1:50 (Trucco, Stocker & Capellini, 1978). A hetero antiserum anti-β_2m raised in rabbits (Nordic Lab, Tilburg, The Netherlands), was also used and compared with monoclonal antibodies. Monoclonal anticommon DR antigen antibodies (Ortho-Pharm, Raritan, New Jersey, USA) were used at a final concentration of 1:5.

All immunofluorescence studies were indirect using rhodamin labelled goat anti-mouse immunoglobulins (Nordic Lab) or fluorescein labelled sheep anti-rabbit immunoglobulins prepared in our lab. In all tests, the control, i.e. goat or sheep antiserum added to cells without antibodies, was negative. Immunofluorescence preparations were examined with a Leitz Orthoplan microscope with an Opak-Fluor vertical illuminator (E. Leitz- Wetzlar, Germany) equipped for selective visualization of fluorescein or rhodamine.

Cell-mediated cytotoxicity. Cytotoxic T cells were induced in a mixed leucocyte reaction. In view of the poor stimulatory effect of the patients' leucocytes, we used haplo-identical stimulator cells from the patients' fathers to generate cytotoxic T cells specific for one of the patient's HLA haplotypes. Five million irradiated (2,000 rad) PBL from the father were added to an equal number of PBL from a normal unrelated donor and incubated for 6 days. The target cells were PHA-induced blasts from either patient or another donor unrelated to either the child or the father. Both sources of target cells were incubated for the last 18 h of culture with a preparation of α IFN

(10,000 iu/ml) or control medium. Specific cytotoxic indexes were calculated after a 4 h cytotoxic assay, using ^{51}Cr-marked target blast cells.

RESULTS

As shown in Fig. 1, the number of leucocytes showing positive fluorescence after incubation with anti-HLA-A, B and C antigens or β2m was profoundly diminished as compared to control leucocytes. Whereas less than 20% cells from any patient expressed detectable β2m, all normal individuals showed between 95% and 100% positive cells. No difference in percentage of positive cells was observed between leucocytes tested, before or after a 18 h incubation in culture medium.

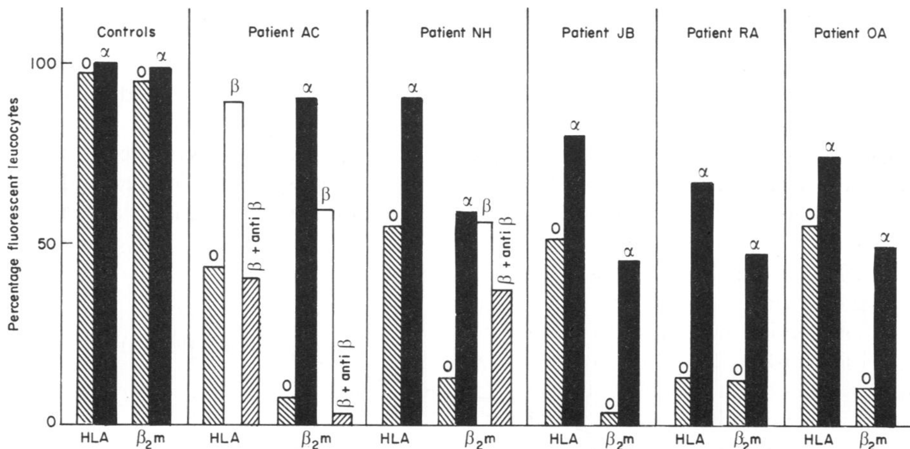

Fig. 1. Enhanced expression of HLA A-B antigens and β2m by interferons. Results are expressed as percentage of HLA A-B-C or β2m bearing leucocytes, incubated alone (▧) or with a IFN (10,000 iu/ml) (■) or β IFN (2,000 iu/ml) (□) or β IFN plus anti-β IFN antiserum (▨). Percentages of HLA-Dr antigen bearing leucocytes (not shown in this figure) were not modified by IFN treatment.

Table 1. Membrane expression of β2m on patients AC and NH and control leucocytes

	Percent of fluorescent positive leucocytes		
	Patients		
Incubation with	AC	NH	Control
Control medium	54	16	97
α IFN	92	58	98
α IFN + actinomycin	45	23	95
α IFN + cycloheximide	51	26	96
Actinomycin	50	22	95
Cycloheximide	45	27	97

Results are expressed as percentage of β2m bearing leucocytes, as detected by immunofluorescence studies. The cells were incubated for 18 h with medium (RPMI + 5% fetal calf serum) alone or supplemented with actinomycin (0·25 μg/l), or cycloheximide (25 μg/ml) with or without αIFN (10,000 iu).

Percentage of HLA-DR antigen bearing leucocytes (not shown in this table) were not modified by these experimental conditions.

Incubation of patients' leucocytes in presence of α IFN resulted in a significant enhancement of both β2m and common HLA A and B antigens. This effect was observed after a 18 h incubation, but not after a 1, 2 or 4 h incubation. For β2m, the enhancement was observed with both monoclonal antibodies and the hetero-antiserum. In contrast to the striking enhancement of HLA A, B and β2m expression, common DR antigens (which were found on only a small proportion of monocytes and B lymphocytes in the five patients) were not affected by incubation with interferon. Two species of interferon were equally effective in enhancing β2m expression, as shown in patients AC and NH. This effect was truly due to interferon, and not to contaminants in our preparation of β IFN, since no or only a weak enhancement was seen when an antiserum to IFN was added in the medium at a dose which completely neutralizes the anti-viral effect. Incubation with antiserum alone or with soluble immune complexes (immunoglobulins–anti-immunoglobulins) did not modify membrane expression of HLA antigens in either patients or controls. Addition of actinomycin or cycloheximide to the incubation medium containing α IFN for 18 h completely abolished the enhancement of β2m expression observed in the patient's leucocyte population incubated with α IFN alone. This procedure did not modify the β2m expression of the control population. Incubation with drugs alone (without IFN) had no effect on the HLA membrane expression in either patients or controls (Table 1).

In a different set of experiments, blast cells (instead of resting leucocytes) were used as targets of interferon. Fig. 2 shows that incubation of PHA-induced blast cells with α IFN for the last 18 h of a 6 day culture also resulted in an increased percentage of β2m bearing cells. In parallel, the ability of

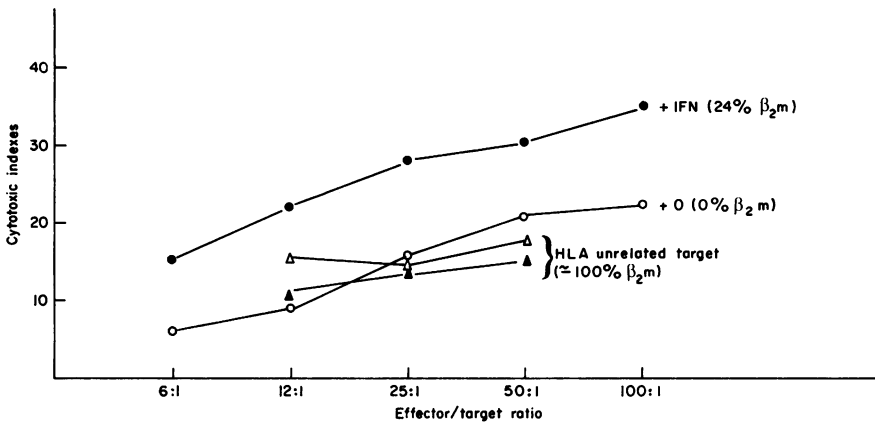

Fig. 2. Enhanced specific lysis of interferon treated target leucocytes from patient AC by allogeneic cytotoxic T cells. Cytotoxic indexes were obtained in a 4 h assay using patient's PHA-induced target cells, incubated in medium alone (○——○) or with α IFN (10,000 iu/ml) (●——●). PHA-induced blast cells from an HLA unrelated donor were also incubated in medium alone (△——△) or with α IFN (▲——▲) and tested as control target cells. In brackets, percentage of β2m bearing blast cells at time of the cytotoxic assay.

these interferon treated blasts to be destroyed by specific cytotoxic T cells increased. In fact, untreated blasts which did not express detectable β2m at all were not destroyed significantly more than unrelated target cells. In contrast, a positive specific cytotoxicity correlated with clear detectability (24%) of β2m on patient's blast cells. In another patient (RA), however, we observed a parallel failure of interferon to enhance β2m expression on blast cells and to increase target cell recognition by specific cytotoxic T cells. This apparent resistance to IFN was not consistent in this patient since her resting leucocytes did show enhanced HLA expression in another experiment (see Fig. 1).

DISCUSSION

Our results, obtained in patients affected with combined immunodeficiency with defective expression of HLA antigens, are in agreement with results obtained in normal experimental animals

(Lindahl *et al.*, 1973, 1974, 1976) and in normal individuals (Fellous *et al.*, 1979) and indicate that expression of HLA-A and -B antigens,. but not DR, is regulated by IFN (Fellous *et al.*, 1979; Vignaux & Gresser, 1977). Previous studies have used allo-antiserum absorption techniques, allowing a quantitative evaluation of the number of HLA molecules per cell (100 % of normal leucocytes being positive). Although our immunofluorescence technique can hardly detect quantitative differences of HLA antigens per cell, it permits us to visualize the appearance of detectable antigens on pathological cells, thus allowing the detection of an increased percentage of positive cells. Modulation of a genetically determined HLA defect by IFN molecules favours the hypothesis that the abnormal expression of HLA in this syndrome is not due to a lack of genetic information for the fabrication of HLA A and B antigens, but rather to an abnormal regulation of synthesis. Our observation that drugs able to inhibit nucleic acid or protein synthesis block the enhancing effect of interferon on the membrane expression of HLA antigens strongly suggest that defective expression is secondary to defective synthesis rather than membrane insertion of these antigens in this syndrome. In our laboratory, preliminary experiments using biosynthetic techniques also indicate that HLA-A and -B polypeptide synthesis is impaired in the leucocytes of four patients. Altogether, these observations shed new light on the pathogenesis of defective HLA-A and -B expression in this syndrome.

The frequency and severity of viral infections in patients with deficient HLA expression is striking. It is well known that T cells recognize viral infected target cells through the concomitant recognition of HLA-A and -B, but not DR antigens (Blanden, 1970; Koszinowski & Ertl, 1975; Michael *et al.*, 1977; Zinkernagel & Doherty, 1975). Thus, enhancing HLA-A and -B antigens expression on HLA deficient patients' leucocytes may increase their T cell-mediated anti-viral host defence. Indeed, IFN was shown in one of our patients to enhance the ability of target cells to be recognized by specific cytotoxic T cells, although this was an allogeneic and not an anti-viral cytotoxic assay. Preliminary observations (to be reported elsewhere) after treatment by intramuscular injections of human α IFN in two patients, however, have not suggested any clear correlation between enhanced expression of HLA-A and -B antigens and obvious clinical benefit. However, our observation that membrane HLA-A and -B antigens and β_2m are better expressed on leucocytes from the two patients treated *in vivo* confirms our *in vitro* results and encourages further work on the regulation of membrane antigen expression and function in patients treated with interferons as immunoregulatory mediators.

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