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# Enhanced expression *in vivo* of HLA-ABC antigens and $\beta_2$-microglobulin on human lymphoid cells induced by human interferon-α in patients with lung cancer. Enhanced expression of class I major histocompatibility antigens prior to treatment

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## SUMMARY

The effect of cloned human interferon-α (IFN-α) on the expression of HLA-ABC antigens (HLA-ABC) and $\beta_2$-microglobulin ($\beta_2$m) on human peripheral lymphoid cells *in vivo* was studied by cytofluorometry using monoclonal antibodies and fluorescein-labelled rabbit anti-mouse immunoglobulin. A significant increase in the mean fluorescence intensity of HLA-ABC (median 59%, $P < 0.001$) and $\beta_2$m (median 57%, $P < 0.001$) on small lymphoid cells was observed 24 h after initiation of IFN-α treatment ($50 \times 10^6$ units IFN-α/m$^2$ three times a week). The enhanced expression of these antigens *in vivo* was found in 11 of 12 examined patients with primary bronchial carcinoma. A concomitant increase in serum $\beta_2$m (median 90%, $P < 0.001$) was found in all patients. In contrast the amount of cell-associated HLA-ABC and $\beta_2$m remained unchanged ($P > 0.1$ and $P > 0.5$, respectively) by day-to-day analysis of an untreated healthy control group. An increased expression of both HLA-ABC (mean 55%, $P < 0.0005$) and $\beta_2$m (mean 23%, $P < 0.01$) was also observed prior to treatment in the lung cancer patients when compared to a group of age matched healthy individuals. Treatment with IFN-α caused a significant redistribution of mononuclear cells resulting in both absolute and relative lymphopenia. Pre-treatment lymphocyte counts were $1.09 \times 10^9$/l (range 0.49–1.73), post-treatment counts were $0.55 \times 10^9$/l (range 0.39–1.06).

**Keywords** human interferon-α HLA-ABC antigens $\beta_2$-microglobulin lung cancer

## INTRODUCTION

Interferon (IFN) is known to induce a variety of biological effects on lymphocytes including induction of (2′–5′) oligo (A) synthetase (Knight *et al.*, 1980; Wallach, Fellous & Revel, 1982), inhibition of DNA synthesis in stimulated lymphocytes (Lindahl-Magnusson, Leary & Gresser, 1972), and augmentation of natural killer cell activity (Gidlung *et al.*, 1978; Hebermann, Ortaldo & Bonnard 1979).

IFN enhances the expression of H-2 (class I histocompatibility antigens) on murine

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lymphocytes both *in vitro* and *in vivo* (Lindahl, Leary & Gresser, 1974; Lindahl *et al.*, 1976), and enhanced expression of HLA-ABC antigens (HLA-ABC) (class I antigens) and $\beta_2$-microglobulin ($\beta_2$m) on human lymphoid cells have been demonstrated *in vitro* (Heron, Hokland & Berg, 1978). IFN does not seem to change the expression of HLA-DR (class II antigens) (Heron, Hokland & Berg, 1978). It has been demonstrated recently, that recombinant IFN-$\gamma$, but not crude or recombinant IFN-$\alpha$, is able to increase HLA-DR synthesis and expression on peripheral blood monocytes *in vitro* (Basham & Merigan, 1983).

The aim was to study the effect of a preparation of cloned IFN-$\alpha$ on, (a) the expression of class I histocompatibility antigens (HLA-ABC and the associated light chain $\beta_2$m) on human lymphoid cells *in vivo*, (b) the influence on serum $\beta_2$m concentrations and (c) changes in distribution of peripheral mononuclear cells during a phase II clinical trial elucidating the anti-tumour effect of IFN-$\alpha$.

## MATERIALS AND METHODS

*Patients and healthy individuals.* Twelve patients with primary lung cancer were included in the study, six with previously untreated squamous cell carcinoma (WHO I) and six with small cell carcinoma (WHO II) who were resistant to conventional therapy (Sobin, 1979). The group included one woman and 11 men with a median age of 61·5 years (range 51–67 years). None of the patients received surgery, chemotherapy or radiotherapy in a 4 week period before treatment. The number of blood leucocytes varied from 6·2 to $13{\cdot}4 \times 10^9$ cells/l at start of treatment. Each patient received $50 \times 10^6$ units IFN-$\alpha$/m$^2$ by intramuscular injection three times a week. Informed consent was obtained in all cases. An untreated control group of 10 healthy individuals seven women and three men, median age 30·5 years (range 27–37 years), from the laboratory were chosen for determination of analytical and day-to-day variation. An age matched group of 10 healthy persons, three women and seven men, median age 63·5 years (range 54–72 years), were chosen for comparison of HLA-ABC and $\beta_2$m expression in the patients prior to treatment with IFN-$\alpha$.

*IFN.* Human recombinant leucocyte A IFN (RO 22-8181) from *E. coli* bacteria was kindly provided by Hoffmann-La Roche, Basle, Switzerland. The IFN preparations were purified to at least 96% homogeneity and were free of detectable endotoxin.

*Monoclonal antibodies.* Monoclonal antibodies defining HLA-ABC antigens (W6/32, Sera-Lab. Ltd, Sussex, UK), and $\beta_2$m (Becton-Dickinson) were used.

*Sample collection.* On day zero 50 ml venous blood was drawn into a similar amount of RPMI 1640 culture medium (GIBCO, Biocult), containing penicillin ($0{\cdot}5 \times 10^6$ iu/l), streptomycin (333 mg/l) and heparin ($10^4$ iu/l). A serum sample was also obtained immediately before injection of IFN-$\alpha$. Mononuclear cells were isolated immediately and processed for quantitative immunofluorescence within a few hours after collection. The serum samples were stored at $-20$°C until analysed. Twenty-four hours later the procedure was repeated.

*Isolation of mononuclear cells.* The mononuclear cells were isolated by gradient centrifugation on Lymphoprep (Nygaard and Co., Oslo, Norway) at 1,200*g* for 30 min (Böyum, 1968). The isolated cells were washed three times in RPMI medium and then quantified in a haemocytometer chamber.

*Preparation of cells for quantitative immunofluorescence.* $10^6$ cells in 100 $\mu$l RPMI 1640 containing 10% vol./vol. heat-inactivated fetal calf serum (FCS) (GIBCO, Bio-Cult) and sodium azide (20 mM) were incubated with 100 $\mu$l monoclonal antibody in saturating amounts as determined by titration. After 30 min at 4°C the cells were washed three times in RPMI and then incubated with FITC-labelled F(ab)$_2$ fragments of rabbit anti-mouse immunoglobulin (DAKO, Copenhagen, Denmark) in saturating amounts for 30 min at 4°C, washed once in RPMI 1640 and twice in isotonic phosphate buffer pH 7·4 with 10% (vol./vol.) FCS (Forni, 1979). Finally the cells were resuspended in 1% (wt/vol.) paraformaldehyde (Merck) for fixation at 4° (Lanier & Warner, 1981).

*Quantative immunofluorescence by flow cytometry.* Between 50,000–70,000 fixed cells were analysed with a fluorescence activated cell sorter (FACS IV, Becton-Dickinson). Gates for forward angle light scattering were chosen to include only the lymphocyte fraction of the mononuclear cells.

A batch of glutaraldehyde fixed chicken erythrocytes were used for calibration of the FACS IV. The mean fluorescence intensity of specifically fluorescent lymphocytes was calculated in arbitrary units (au) using the mean fluorescence intensity of fixed chicken erythrocytes as a reference value (Loken & Stall, 1982; Loken, Stout & Herzenberg, 1979). The mean fluorescence intensity of the cells was calculated by use of the formular $\Sigma ic/\Sigma c$ in which $i$ is the channel number and $c$ the number of cells counted in the channel. Mononuclear cells treated with an irrelevant monoclonal antibody (mouse anti-mouse H-2K) (Becton-Dickinson) and fluorescein labelled $F(ab)_2$ rabbit anti-mouse immunoglobulin (DAKO) served as controls. The threshold for fluorescence detection was chosen so that maximally 0·5% of control cells became 'fluorescent'.

*Analytical and biological variation of mean fluorescence intensity.* The analytical variation was determined by double determination after parallel processing of two samples from 10 healthy individuals. The procedure included separate isolation of the mononuclear cells, separate labelling of cells with antibodies and separate estimation of fluorescence on FACS IV. The biological day-to-day variation of cell associated HLA-ABC and $\beta_2$m was also measured in 10 healthy individuals. The results from these measurements and from the 12 patients treated with IFN-$\alpha$ were compared for each individual using Student's paired $t$-test.

*Estimation of serum $\beta_2$m by radioimmunoassay.* $\beta_2$m was estimated in serum samples by radioimmunoassay (Plesner, Nørgaard-Pedersen & Boenish, 1975). The coefficient of variation on duplicates was 9%.

*Absolute number of leucocytes and leucocyte subsets.* Leucocytes were estimated by standard procedures on Hemalog 8 (Technicon). Differential counts were obtained by a combination of light scatter measurements and automated cytochemistry on Hemalog D (Technicon).

## RESULTS

A significant increase in the mean fluorescence intensity of HLA-ABC (median 59%, $P < 0{\cdot}001$) and $\beta_2$m (median 57%, $P > 0{\cdot}001$) on small lymphoid cells was observed 24 h after initiation of IFN-$\alpha$ treatment. The enhanced expression of these antigens *in vivo* was found in 11 of 12 patients examined (Table 1). In contrast the amount of cell associated HLA-ABC and $\beta_2$m remained unchanged ($P > 0{\cdot}1$ and $P > 0{\cdot}5$, respectively) by day-to-day analysis of the healthy control group (Table 2).

The FACS IV histograms for quantitative immunofluorescence of HLA-ABC for an individual patient before and after initiation of IFN-$\alpha$ treatment is shown in Fig. 1. The increased mean fluorescence intensity is reflected by a right ward transposition of the peak of fluorescent cells (Fig. 1D, arrow compared with Fig. 1B). When the monocyte population as defined by light scatter measurements (Fig. 1A & C, right peak) was included in the measurements of fluorescence intensity, a higher mean fluorescence intensity prior to treatment and a more pronounced relative increase in the mean fluorescence intensity of HLA-ABC and $\beta_2$m was found after IFN-$\alpha$ treatment. With regard to HLA-ABC and $\beta_2$m expression on the single-cell level these results were considered less reliable, since they could at least in part be explained by a relative monocytosis (absolute lymphocytopenia) 24 h after administration of IFN-$\alpha$ (Table 3). This is reflected by a shift in the relative size of the lymphocyte and monocyte peaks by light scatter measurements with FACS IV, as shown (Fig. 1A compared with Fig. 1C). No increase in percentage of 'fluorescent' cells of the control samples was observed after treatment with IFN-$\alpha$.

The standard deviations obtained by estimation of HLA-ABC and $\beta_2$m of two identical samples from 10 healthy individuals was 0·43 au and 0·42 au, respectively (Table 2). The median for HLA-ABC was 4·75 au (range 3·68–6·26 au), and for $\beta_2$m 9·22 au (range 6·94–11·5 au).

Also the pre-treatment expression of HLA-ABC and $\beta_2$m was found to be higher in the lung cancer patients compared to an untreated healthy control group of age matched individuals (Table 4). In patients with small cell carcinoma the mean elevation of HLA-ABC was 71% ($P < 0{\cdot}0005$) and of $\beta_2$m 57% ($P < 0{\cdot}0005$) before initiation of IFN-$\alpha$ treatment when compared to an age matched control group of healthy individuals (Table 4).

The level of mean fluorescence intensity expressed in au was higher for $\beta_2$m than for HLA-ABC.

**Table 1.** Estimation of HLA-ABC antigens and $\beta_2$m on lymphocytes from patients treated with IFN-$\alpha$ by quantitative immunofluorescence on FACS IV and of serum $\beta_2$m by radioimmunoassay.

| | | Lymphocyte-associated HLA-ABC antigens and $\beta_2$m | | | | Serum $\beta_2$m | |
|---|---|---|---|---|---|---|---|
| Patient No. | Day | HLA-ABC (au) | HLA-ABC (Pct increase) | $\beta_2$m (au) | $\beta_2$m (Pct increase) | $\beta_2$m (nmol/l) | $\beta_2$m (Pct increase) |
| 1 | 0 | 8·6 | 69 | 14·61 | 67 | 316 | 42 |
| | 1 | 14·51 | | 24·35 | | 450 | |
| 2 | 0 | 7·47 | 27 | 11·46 | 37 | 440 | 84 |
| | 1 | 9·47 | | 15·67 | | 810 | |
| 3 | 0 | 9·46 | 39 | 14·55 | 40 | 190 | 121 |
| | 1 | 13·17 | | 20·30 | | 420 | |
| | 29 | | | | | 325 | |
| 4 | 0 | 9·51 | −12 | 13·95 | 3 | 240 | 150 |
| | 1 | 8·41 | | 14·34 | | 600 | |
| 5 | 0 | 7·09 | 42 | 11·83 | 59 | 210 | 95 |
| | 1 | 10·06 | | 18·81 | | 410 | |
| 6 | 0 | 8·31 | 62 | 12·92 | 58 | 200 | 128 |
| | 1 | 13·43 | | 20·40 | | 456 | |
| 7 | 0 | 6·0 | 133 | 8·54 | 138 | 335 | 82 |
| | 1 | 13·99 | | 20·33 | | 610 | |
| 8 | 0 | 7·71 | 36 | 10·98 | 51 | 360 | 63 |
| | 1 | 10·48 | | 16·55 | | 585 | |
| | 21 | | | | | 730 | |
| 9 | 0 | 6·52 | 66 | 10·52 | 55 | 300 | 51 |
| | 1 | 10·84 | | 16·28 | | 610 | |
| | 29 | | | | | 540 | |
| 10 | 0 | 7·84 | 93 | 11·49 | 85 | 190 | 195 |
| | 1 | 15·13 | | 21·24 | | 560 | |
| | 21 | | | | | 690 | |
| 11 | 0 | 6·41 | 56 | 9·91 | 53 | 206 | 12 |
| | 1 | 10·02 | | 15·15 | | 230 | |
| 12 | 0 | 5·45 | 133 | 6·57 | 170 | 185 | 122 |
| | 1 | 12·68 | | 17·73 | | 410 | |
| | 21 | | | | | 430 | |
| Mean ± s.d. | 0 | 7·53 ± 1·3 | — | 11·44 ± 2·41 | — | — | — |
| | 1 | 11·85 ± 2·22 | | 18·43 ± 2·98 | | | |
| Mean increase ± s.d. | | 4·32 ± 2·59 | 62% ± 42% | 6·99 ± 3·24 | 68% ± 45% | 248 ± 102 | 95% ± 51% |

au = arbitrary units.
Patients 1–6 small cell carcinoma, patients 7–12 squamous cell carcinoma.

**Table 2.** Estimation of HLA-ABC antigens and $\beta_2$m on lymphocytes from healthy individuals by quantitative immunofluorescence on FACS IV and of serum $\beta_2$m by radioimmunoassay

| Healthy individual No. | Day | Sample No. | HLA (au) | $\beta_2$m (au) | Serum $\beta_2$m (nmol/l) |
|---|---|---|---|---|---|
| 1 | 0 | 1 | 6·16 | 10·10 | — |
| | | 2 | — | 10·15 | |
| | 1 | 1 | 6·06 | 10·26 | — |
| | | 2 | 5·85 | — | |
| 2 | 0 | 1 | 5·65 | 9·88 | 107 |
| | | 2 | — | 10·57 | |
| | 1 | 1 | 5·68 | 9·71 | 99 |
| | | 2 | 5·82 | — | |
| 3 | 0 | 1 | 4·79 | 8·75 | 159 |
| | | 2 | — | — | |
| | 1 | 1 | 4·72 | 6·94 | 167 |
| | | 2 | 4·65 | 8·20 | |
| 4 | 0 | 1 | 4·11 | 7·73 | 113 |
| | | 2 | 4·05 | — | |
| | 1 | 1 | 4·65 | 8·67 | 113 |
| | | 2 | — | 8·13 | |
| 5 | 0 | 1 | 4·62 | 8·89 | 93 |
| | | 2 | — | — | |
| | 1 | 1 | 4·78 | 8·62 | 95 |
| | | 2 | 4·70 | 8·70 | |
| 6 | 0 | 1 | 4·86 | 8·96 | 97 |
| | | 2 | 4·93 | 9·02 | |
| | 1 | 1 | 4·49 | 8·56 | 101 |
| | | 2 | — | — | |
| 7 | 0 | 1 | 4·96 | 9·03 | 181 |
| | | 2 | — | — | |
| | 1 | 1 | 5·86 | 10·24 | 167 |
| | | 2 | 5·41 | 9·93 | |
| 8 | 0 | 1 | 4·83 | 11·49 | 171 |
| | | 2 | — | — | |
| | 1 | 1 | 4·78 | 11·50 | 167 |
| | | 2 | 6·26 | 10·95 | |
| 9 | 0 | 1 | 4·62 | 10·03 | 136 |
| | | 2 | 4·01 | 9·43 | |
| | 1 | 1 | 5·06 | 9·52 | 146 |
| | | 2 | — | — | |
| 10 | 0 | 1 | 3·85 | 8·45 | 117 |
| | | 2 | — | — | |
| | 1 | 1 | 3·68 | 8·76 | 117 |
| | | 2 | 4·63 | 8·19 | |
| Mean ± s.d. | 0 | — | 4·85 ± 0·67 | 9·33 ± 1·06 | 130 ± 33 |
| | 1 | — | 4·98 ± 0·72 | 9·28 ± 1·25 | 130 ± 31 |
| Mean ± s.d. | — | 1 | 4·92 ± 0·75 | 9·37 ± 1·25 | — |
| | — | 2 | 5·03 ± 0·77 | 9·33 ± 1·04 | — |

au = arbitrary units.

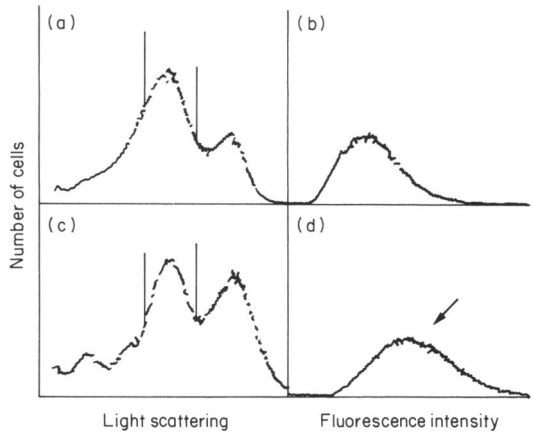


**Fig. 1.** Two parameter histograms of light scattering and fluorescence intensity of HLA-ABC antigens (HLA-ABC) from FACS IV of a patient (patient No. 8, Table 1) prior to (A & B) and 24 h after (C & D) initiation of IFN-α treatment. Gates for light scattering (region between markers in A & C) were set to include only small lymphoid cells ('lymphocytes', left peak) for fluorescence analysis. Twenty-four hours after treatment with IFN-α the mean fluorescence intensity of HLA-ABC has increased as reflected by the right ward transposition of the curve D (arrow) compared with B. The relative number of large lymphoid cells ('monocytes', right peak A & C) has also increased.

**Table 3.** Estimation of blood leucocytes on Hemalog 8, lymphocytes and monocytes by automated differential count on Hemalog D in lung cancer patients before and after treatment with IFN-α.

| Patient No. | Day | Leucocytes ($10^9$ cells/l) | Lymphocytes ($10^9$ cells/l) | Monocytes ($10^9$ cells/l) |
|---|---|---|---|---|
| 1 | 0 | 7·2 | 0·49 | 0·5 |
| | 1 | 4·3 | 0·39 | 0·75 |
| 2 | 0 | 6·7 | 0·76 | 0·57 |
| | 1 | 5·0 | 0·4 | 1·18 |
| 3 | 0 | 13·4 | 1·16 | 1·13 |
| | 1 | 7·7 | 0·41 | 1·21 |
| 4 | 0 | 8·7 | 1·53 | 0·68 |
| | 1 | 4·2 | 0·47 | 0·57 |
| 5 | 0 | 6·2 | 0·9 | 0·67 |
| | 1 | 4·2 | 0·64 | 0·82 |
| 6 | 0 | 7·2 | 0·97 | 0·46 |
| | 1 | 7·6 | 1·06 | 0·82 |
| 7 | 0 | 7·6 | 1·73 | 0·31 |
| | 1 | 4·2 | 0·46 | 0·29 |
| 8 | 0 | 4·1 | 1·03 | 0·31 |
| | 1 | 3·1 | 0·5 | 0·45 |
| 9 | 0 | 9·9 | 1·47 | 0·66 |
| | 1 | 6·8 | 0·81 | 0·71 |
| 10 | 0 | 5·8 | 0·9 | 0·7 |
| | 1 | 3·2 | 0·4 | 0·7 |
| Mean increase ± s.d. | | −2·65 ± 1·73 | −0·54 ± 0·42 | +0·15 ± 0·21 |

**Table 4.** Expression of HLA-ABC antigens and $\beta_2$m (arbitrary units) on lymphoid cells from healthy individuals and patients before start of IFN-α treatment

| No. | | HLA-ABC antigens (mean ± s.d.) | $\beta_2$m (mean ± s.d) |
|---|---|---|---|
| 10 | young healthy individuals | 4·85 ± 0·67 (range 3·68–6·16) | 9·33 ± 1·06 (range 6·94–11·50) |
| 10 | age matched healthy individuals | 4·91 ± 0·52 (range 4·33–6·11) | 8·40 ± 0·87 (range 7·08–9·87) |
| 6 | patients with small cell carcinoma of the lung | 8·41 ± 1·00 (range 7·09–9·51) | 13·22 ± 1·37 (range 11·46–14.61) |
| 6 | patients with squamous carcinoma of the lung | 6·66 ± 0·95 (range 5·45–7·48) | 9·67 ± 1·83 (range 6·57–11·49) |

This was observed both in patients and healthy individuals. The mean ratio in patients of $\beta_2$m to HLA-ABC was 1·54 (range 1·24–1·87) in young healthy individuals 1·92 (range 1·64–2·38).

Serum $\beta_2$m estimated by radioimmunoassay showed a median increase of 90% (range 12–95%) 24 h after initiation of IFN-α treatment (Table 1). This elevation persisted for 3–4 weeks after initiation of treatment in five patients examined during continuous IFN-α treatment (Table 1).

## DISCUSSION

We have demonstrated a significant increase in the expression of HLA-ABC and $\beta_2$m on human lymphoid cells induced within 24 h after treatment of lung cancer patients with IFN-α. These findings are consistent with the effects of IFN *in vitro* and *in vivo* on murine lymphoid cells (Lindahl, Leary & Gresser, 1974; Lindahl *et al.*, 1976) and on human lymphoid cells *in vitro* (Heron *et al.*, 1978; Plesner, Nørgaard-Pedersen & Boenish, 1975). Statistical analysis of the results from the patients and the control group showed that the increased expression of HLA-ABC and $\beta_2$m is highly significant ($P < 0·0001$) in the IFN-α treated group, whereas HLA-ABC ($P > 0·1$) and $\beta_2$m ($P > 0·5$) remained unchanged by day-to-day analysis of the control group. The magnitude and variability of the elevation of HLA-ABC (3–170%) and $\beta_2$m (−12–133%) is similar to the results obtained after IFN-α treatment *in vitro* (Hokland, Heron & Berg, 1981).

The cause of the higher mean fluorescence intensity of both HLA-ABC and $\beta_2$m which could be demonstrated prior to IFN-α treatment in the patients when compared to the age matched control group is not clear. It cannot be due to previous chemotherapy because both patients with small cell carcinoma, which previously had received chemotherapy, and patients with squamous cell carcinoma, who had not received chemotherapy, revealed an enhanced expression of MHC class 1 antigens. It is not likely to be due to endogenous IFN, since elevated levels of (2′–5′) oligo (A) synthetase were not demonstrated in these cells.

A parallel increase in HLA-ABC and $\beta_2$m was found in individual patients after IFN-α treatment. The ratio of HLA-ABC to $\beta_2$m in samples from patients was estimated to approximately 1:1·5 and from healthy individuals 1:1·9. This could indicate an excess of $\beta_2$m over HLA-ABC on human peripheral blood lymphocytes as has been suggested previously (Plesner *et al.*, 1978). This explanation is, however, not supported by the findings of Brodsky, Bodmer & Parham (1979). They found a 1:1 molar ratio of $\beta_2$m to HLA-ABC using an indirect cell binding radioimmunoassay with monoclonal antibodies and $^{125}$I-F(ab)$_2$ rabbit anti-mouse immunoglobulin (Brodsky, Bodmer & Parham, 1979). One explanation for this discrepancy might be that different monoclonal antibodies (e.g. IgG1 and IgG2a) bind different stochiometric amounts of second antibody (radiolabelled or fluoresceinated).

Forward angle light scattering is generally used as an approximate measurement of the size of spheroid cells (Forni, 1979; Loken, Stout & Herzenberg, 1979). Peak intensities of the 'lymphocyte'

and 'monocyte' subpopulations were practically unaffected by IFN-α treatment. The enhanced expression of HLA-ABC and $\beta_2$m therefore for the main part must be due to an increased 'density' of these molecules on the cell surface (Burchiel *et al.* 1982). The biological implications of this is not known, but a number of suggestions have been offered (Hokland *et al.*, 1982; Sanderson & Beverley 1983). These include a higher natural killer cell activity proportional to the amount of $\beta_2$m on the cell surface (Hokland *et al.*, 1982). and augmentation of immunoselective pressure (Sanderson & Beverley, 1983).

The increased density of $\beta_2$m on the cell surface after IFN-α treatment was accompanied by a rise in serum $\beta_2$m, which persisted for 3–4 weeks during IFN-α treatment. This suggests that the synthesis and turnover rate of $\beta_2$m are also increased.

It is well known, that serum $\beta_2$m frequently is elevated in a variety of cancers, but a cause of this elevation has not been found. In the lung cancer patients studied by us elevated pre-treatment levels of serum $\beta_2$m was associated with enhanced expression of $\beta_2$m on lymphocytes. We therefore suggest, that the elevation of serum $\beta_2$m in malignant disease might be caused by an enhanced expression and production of lymphocyte $\beta_2$m but further studies are needed to substantiate this hypothesis.

One difficulty in comparing results obtained *in vivo* and *in vitro* is due to a significant redistribution *in vivo* of mononuclear cells 24 h after IFN-α treatment. The treatment resulted in lymphocytopenia and relative monocytosis. This caused a more pronounced increase in HLA-ABC and $\beta_2$m due to the contribution from the larger monocytes, when all mononuclear cells were included in the measurement. However, the scatter profile obtained on FACS IV allows discrimination between lymphocytes and monocytes, so that mononuclear cells of uniform size are selected for measurement. Redistribution within the heterogeneous population of lymphocytes has not been taken into account in the present study, where the lymphocytes were identified only by light scatter measurement. The homogeneity of the lymphocyte peak with respect to fluorescence intensity before and after IFN-α treatment, however, argues against the possibility that a major subpopulation of lymphocytes differs from the rest in not being induced by IFN-α to express more HLA-ABC and $\beta_2$m. This could be studied more directly by dual marker analysis applying one fluorochrome for HLA-ABC and $\beta_2$m measurements and another to define T- and B-lymphocytes and subsets of T lymphocytes. Such studies are now in progress.

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## REFERENCES

BASHAM, T.Y. & MERIGAN, T.C. (1983) Recombinant interferon increases HLA-DR synthesis and expression. *J. Immunol.* **130,** 1492.

BRODSKY, F.M., BODMER, W.F. & PARHAM, P. (1979) Characterization of monoclonal anti $\beta_2$microglobulin antibody and its use in the genetic and biochemical analysis of major histocompatibility antigens. *Eur. J. Immunol.* **9,** 536.

BURCHIEL, S.W., MARTIN, J.C., IMAI, K., FERRONE, S. & WARNER, N.L. (1982) Heterogeneity of HLA-A, B, Ia-like, and melanoma-associated antigen expression by human melanoma cell lines analyzed with monoclonal antibodies and flow cytometer. *Cancer Res.* **42,** 4110.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Invest.* **Suppl. 97,** 77.

FORNI, L. (1979) Reagents for immunofluorescence and their use for studying lymphoid cell products. In *Immunological Methods* (ed. by J. Lefkowits & B. Pernis) p. 151. Academic Press, New York.

GIDLUNG, M., ORN, A., WIGZELL, H., SENIK, A. & GRESSER, I. (1978) Enhanced NK cell activity in mice infected with interferon and interferon inducers. *Nature*, **273,** 759.

HEBERMANN, R.B., ORTALDO, J. & BONNARD, G.D. (1979) Augmentation by interferon on human natural and antibody dependent cell-mediated cytotoxicity. *Nature*, **277,** 221.

HERON, I., HOKLAND, M. & BERG, K. (1978) Enhanced expression of $\beta_2$microglobulin and HLA-antigens on human lymphoid cells by interferon. *Proc. Nat. Acad. Sci. USA.* **75,** 6215.

HOKLAND, M., HERON, I. & BERG, K. (1981) Increased expression of $\beta_2$microglobulin and histocompatibility antigens on human lymphoid cells induced by interferon. *J. Interferon Res.* **1,** 483.

HOKLAND, M., HERON, I., BERG, K. & HOKLAND, P. (1982) Natural killer cell activity correlates with the amount of $\beta_2$microglobulin on human peripheral blood lymphocytes. *Cell. Immunol.* **72,** 40.

KNIGHT, M., CAYLEY, P.J., SILVERMAN, R.H.,

WRESCHNER, D.H., GILBERT, C.S., BROWN, R.E. & KERR, I.M. (1980) Radioimmune, radiobinding and HPLC analysis of 2–5 A and related oligomucleotides from intact cells. *Nature*, **288,** 189.

LANIER, L.L. & WARNER, N.L. (1981) Paraformaldehyde fixation of hematopoietic cells for quantitative flow cytometry (FACS) analysis. *J. Immunol. Meth.* **47,** 25.

LINDAHL, P., GRESSER, I., LEARY, P. & TOVEY, M. (1976) Interferon treatment of mice: Enhanced expression of histocompatibility antigens on lymphoid cells. *Proc. Nat. Acad. Sci. USA*, **73,** 1284.

LINDAHL, P., LEARY, P. & GRESSER, I. (1974) Enhancement of the expression of histocompatibility antigens of mouse lymphoid cells by interferon *in vitro*. *Eur. J. Immunol.* **4,** 779.

LINDAHL-MAGNUSSON, P., LEARY, P. & GRESSER, I. (1972) Interferon inhibits DNA synthesis induced in mouse lymphocyte suspensions by phytohaemagglutinin or by allogeic cells. *Nature*, **273,** 120.

LOKEN, M.R. & STALL, A.M. (1982) Flow cytometry as an analytical and preparative tool in immunology. *J. Immunol. Meth.* **50,** R85.

LOKEN, M.R., STOUT, R.D., HERZENBERG, L.A. (1979) Lymphoid cell analysis and sorting. In: *Flow Cytometry and Sorting* (ed. by M. R. Melamed, P. F. Mullaney & M. L. Mendelsohn) p. 505. John Wiley and Sons, New York.

PLESNER, T., KARLE, H., RUBIN, B. & THOMSEN, M. (1978) Evidence for a change in the expression of $\beta_2$microglobulin associated membrane structures on leukaemic human cells. *Clin. exp. Immunol.* **31,** 269.

PLESNER, T., NØRGAARD-PEDERSEN, B. & BOENISH, T. (1975) Radioimmunoassay of $\beta_2$microglobulin. *Scand. J. clin. lab. Invest.* **35,** 729.

SANDERSON, A.R. & BEVERLEY, P.C.L. (1983) Interferon, $\beta_2$microglobulin and immunoselection on the pathway to malignancy. *Immunol. Tod.* **4,** 211.

SOBIN, L.H. (1979) The WHO histological classification of lung tumors: Reviewed edition. In *Advances in Medical Oncology Research and Education* (ed. by P. M. Wilkinson) vol. 11. *Clinical Cancer Principle Sites* 2. p. 5. Pergamon Press, Oxford and New York.

WALLACH, D., FELLOUS, M. & REVEL, M. (1982) Preferential effect of interferon on the synthesis of HLA antigens and their RNAs in human cells. *Nature,* **299,** 833.