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Effect of *In Vivo* Administration of Interferon Gamma on Expression of MHC Products and Tumour Associated Antigens in Patients with Metastatic Melanoma

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Abstract—*IFN-γ is an effective inducer of MHC class II antigen expression in cell lines of malignant melanoma. To investigate the possibility that IFN-γ may increase MHC class I and II and melanoma tumour associated antigens* in vivo, *immunohistochemical analyses of biopsies from six patients with metastatic disease undergoing IFN-γ treatment were performed. Before IFN-γ treatment, the melanomas were class I positive and class II negative. After treatment, class I expression was neither enhanced nor class II expression induced in any tissue sample regardless of biopsy time or dose of IFN-γ. TAA was similarly unchanged. However, in one of the six patients a primary cell line was established and IFN-γ induced expression of MHC products. The possible reasons for lack of MHC induction are discussed, although qualitative changes in antigen expression cannot be excluded on the basis of qualitative immunocytochemical techniques alone.*

INTRODUCTION

INTERFERON GAMMA has been evaluated in a number of clinical studies to determine its efficacy in the treatment of cancer [1–5]. Whilst anti-cancer activity has been reported, the mechanism of action is not well understood. A variety of biological responses in both host and tumour can be altered by interferon. Host natural killer and macrophage tumour cytotoxic activity have been shown to be enhanced [6], but there is no direct evidence that such enhancement results in a therapeutic effect. The biological activities of IFN on tumour cells may be important in their anticancer activity. Following binding to specific receptors on the cell surface, the receptor-bound IFNs are internalized and degraded [7]. This interaction results in the stimulation or inhibition in the synthesis of a variety of cellular proteins, for example, induction of 2′,5′-oligoadenylate activity, the induction of specific proteins, activation of a protein kinase and the modulation of expression of cell surface antigens such as major histocompatibility complex (MHC) antigens and tumour associated antigens [8–11]. MHC class I and II antigens are essential in the control of immune responses functioning in the recognition of antigens by T lymphocytes. Alterations of expression of MHC antigens may result in altered biological behaviour and enhanced expression may serve to increase host cellular immune response to the tumour [12].

Numerous *in vitro* studies with IFN-γ have shown increased expression of MHC on cultured melanoma cells [11, 13]. However, to date there have been no reports on the *in vivo* effect of IFN-γ on antigen expression in humans. In this study, recombinant interferon gamma was administered to patients with metastatic melanoma. Biopsy specimens were obtained before and at various times during treatment and these were examined for the effect of *in vivo* administration of IFN-γ on the expression of MHC and melanoma associated antigens.

MATERIALS AND METHODS

Patients

The patient characteristics are shown in Table 1. All patients had stage II or III malignant melanoma and four had had previous chemotherapy. Interferon gamma (IFN-γ) was supplied by the Schering Corporation via its Swiss subsidiary Werthenstein

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Table 1. Patient characteristics

Patient	Sex	Age	Stage	Immuno-chemotherapy
BG	f	54	III	No
CU	f	70	II	BCG, DTIC
JB	m	60	III	BCG, DTIC
ND	f	62	II	No
AP	m	47	II	VCR, CCNU, BLEO/Irrad.
CB	m	53	III	DTIC, Melphalan

Chemie AG, and was recombinant DNA produced human IFN-G (Schering 36850) with a specific activity of 2×10^6 IU/mg protein. The IFN-γ was supplied as a lyophilized powder, the vials contained either 0.5 or 1.0 mg of IFN-γ and were stored at 4°C. The IFN-γ was reconstituted by the addition of 1.0 ml of sterile water for injection and was administered immediately after reconstitution.

Subcutaneous injections of IFN-γ were given three times a week for up to 8 weeks and single doses were in the range 3–5 mg/m^2 (6–10 $\times$ 10^6 U IFN-γ). The maximum tolerated dose was 5 mg/m^2.

Biopsies of skin or soft tissue metastases were taken before and after a variable duration of still ongoing IFN-γ treatment from 50 h up to 8 weeks (in one patient, a pre-treatment biopsy was not available), as detailed in Table 2. The biopsies were snap-frozen in isopentane over liquid nitrogen and stored in liquid nitrogen for subsequent immunohistological analysis.

Melanoma cell cultures

If sufficient tumour material was available, attempts were made to establish melanoma cell cultures. Briefly, necrotic and connective tissue were removed aseptically, the remainder cut into small pieces and half were enzymatically digested with a mixture of collagenase (2 mg/ml), hyaluronidase (0.1 mg/ml) and DNase (0.01 mg/ml) in RPMI. The mixture was agitated for 2 h at room temperature whence cells in suspension were aspirated. Disaggregation was continued overnight after which cells were again aspirated, pooled, washed and resuspended in CM consisting of RPMI 1640, supplemented with 10% heat inactivated foetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and glutamine. The yield of tumour cells was too low to freeze down and cell cultures were set up, either initially as explants from the tumour or from tumour cell suspensions. Melanoma cells were cultured in tissue culture flasks, trypsinized when confluent and transferred into new flasks in CM. Short-term melanoma cell cultures were only successfully established from one pre-interferon biopsy in this study (BG).

Table 2. IFN-γ application and time of second biopsy*

Patient	Dose s.c. 3 × weekly for 8 weeks	Second biopsy
BG	6×10^6U/m^2	4 weeks
CU	6×10^6 U/m^2	4 weeks
JB	6×10^6 U/m^2	8 weeks
DN	8×10^6 U/m^2	50 h
AP	8×10^6 U/m^2	3 weeks
CB	10×10^6 U/m^2†	7 weeks

*rIFN-γ: Shering 36850.
†Maximum tolerated dose: 10×10 U/m^2.

In vitro *treatment with IFN*

Tumour cell cultures were trypsinized and transfered to two small culture flasks in CM. After 24 h of culture, CM was discarded and the monolayer cultures were further incubated in 10 ml CM with or without the addition of 500 IU U/ml IFN-γ (Biogen). After 72 h tumour cells were harvested and cytospins made for subsequent immunohistochemical analysis.

Immunoenzyme staining method

Cryostat sections were stained by an unlabelled antibody bridge immunoalkaline phosphatase procedure (APAAP) as previously described [14]. The alkaline phosphatase reaction was visualized with Fast Red and Naphthol AsMx phosphate as substrate.

Details of the monoclonal antibodies used in the study are shown in Table 3.

Pharmacokinetics

Pharmacokinetics were performed on three patients. Blood (10 ml) was taken at 0, 0.5, 1, 2, 4, 8, 12 and 24 h after the first injection of IFN-γ. The serum was separated, stored frozen at −70°C and subsequently assayed for IFN-γ content using an IRMA assay (Boots-Celltech Ltd., Berkshire, U.K.). A polystyrene bead coated with anti-human IFN-γ polyclonal sheep antibody was added to appropriate dilutions of sera. After a 3 h incubation period, the fluid was removed and the beads washed once. Then a monoclonal anti-IFN antibody labelled with ^{125}I was added and incubation was continued at ambient temperature for 1 h followed by 2–8°C overnight. The beads were then washed, the amount of bound radioactivity determined and compared to that of a standard curve.

Immunoscintigraphy

Imaging was performed with ^{99m}Tc labelled F(ab')$_2$ fragments of the anti-melanoma monoclonal antibody (225.28S) before and after rIFN-γ treat-

Table 3. *The monoclonal antibodies used in this study*

Antibody	Specificity	Source
MAA		
376.96S	Glycoprotein, MW 94 kD (LMW-MAA)	Sorin Biomedica
225.28S and 763.24T	Glycoprotein complex (HMW-MAA)	Sorin Biomedica
MHC		
Class I		
W6/32	Class I alpha	Sera Lab
BBMI	Beta2-microglobulin	ATCC
Class II		
CR3/43	DR/DP/DQbeta	D.Y. Mason
DA6.231	DR/DP>DQ beta	K. Guy
Tu35	DR/DP	D.Y. Mason
DA6.147	DR alpha	K. Guy
DA6.164	DR beta	K. Guy
TDR31.3	DR beta	W.F. Bodmer
B7/2.1	DP	J.G. Bodmer
Tu22	DQ	A. Ziegler
Leu10	DQ	Becton Dickinson
Leucocyte		
UCHTI	PanT	Sera Lab
DAKO-T4	T4	Dako
DAKO-T8	T8	Dako
Tac	IL-2 receptor	T. Waldman
OKM1	Macrophages	Ortho

ment in four patients. Full details of the technique used is described elsewhere [15].

RESULTS

Analysis of cell surface antigens

In five patients biopsies were obtained before and after a variable duration of IFN-γ treatment (Table 2). In a sixth patient a snap-frozen biopsy was only available after interferon treatment. Immunohistochemical staining with three monoclonal antibodies to melanoma associated antigens (225.28S and 763.24T to a HMW-MAA and 376.96S to a LMW-MAA) showed no detectable changes in staining pattern or intensity of antigen expression in paired biopsy specimens from four patients. In one case the pretreatment biopsy was negative for the HMW.MAA and the second biopsy showed a small foci of positive staining (Table 4). Biopsies taken before IFN-γ treatment were MHC class I positive, the stain was uniform throughout the lesion, and MHC class II negative. Macrophages were, however, class II positive. After IFN-γ treatment, MHC class I expression was neither enhanced nor MCH class II expression induced on melanoma cells in any tissue sample regardless of biopsy time or dose of IFN-γ. However, in one case keratinocytes were positive for HLA-DR antigens in the second biopsy.

Analysis of the leucocytic infiltrate showed a reduction in the lymphocytic response in one case and an increase in another case after treatment. There were no significant changes in the number of activated T lymphocytes, detected by an antibody to the IL-2R after treatment (Table 5).

Effect of IFN on cultured melanoma cells in vitro

Melanoma cells were analysed for cell surface antigens after short-term culture *in vitro* from one patient (BG). Untreated cultured cells expressed melanoma associated antigens and MHC class I, which correlated with their expression on tissue sections.

The culture was incubated with 500 U/ml of IFN-γ for 72 h. There was no significant change in the number of cells expressing MAA or MHC class I antigens after IFN incubation. Short-term cultured melanoma cells expressed MHC class II antigens on a small percentage of cells. After incubation with IFN-γ there was a significant increase in the number of cells expressing class II molecules detected by a monoclonal antibody to a monomorphic determinent on DR, DP and DQ.

Pharmacokinetics

IFN was measured by an immunoradioactive assay in serum samples at various times after the

Table 4. In situ *MHC and MAA expression before* (1) *and after* (2) *IFN-γ*

Patient		MHC class I* Alpha	Beta2-m	MHC class II DR	DP	DQ	HMW-MAA 225.28S	763.24T	LMW-MAA 376.96S
BG	(1)	+	+	—	—	—	+wk	+wk	+
	(2)	+	+	—	—	—	+wk	+wk	+
CU	(1)	+	+	—	—	—	+	+	+
	(2)	+	+	—	—	—	+	+	+
JB	(1)	+	+	—	—	—	—	—	+
	(2)	+	+	—	—	—	—†	—†	+
DN	(1)	+	+	—	—	—	+	+	+
	(2)	+	+	—	—	—	+	+	+
AP	(1)	+	+	—	—	—	+	+	+
	(2)	+	+	—	—	—	+	+	+
CB	(1)	nd							
	(2)	+	+	—	—	—	+	+	+

*No significant quantitative difference in Class I expression.
†One tiny area positive.

first dose of IFN-γ. The measurement and area under the curve for three patients are shown in Table 6.

As this measurement shows that immunoreactive IFN is in the serum, but not if it is biologically active, in patient BG (with the highest IFN-γ level by RIA), the anti-viral activity was analysed. Low levels of anti-viral activity were found (data not shown).

Table 5. Mononuclear inflammatory infiltrate before (1) *and after* (2) *IFN-γ*

Patient		Pan T	T4/T8	IL-2R	Macrophages
BG	(1)	++	1:1	±	++
	(2)	++	1:1	±	++
CU	(1)	+++	2:1	±	++
	(2)	++	2:1	±	++
JB	(1)	+	1:1	±	++
	(2)	++	1:1	±	++
DN	(1)	++	1:1	±	++
	(2)	++	1:1	±	++
AP	(1)	+	1:1	±	++
	(2)	+	1:1	±	++
CB	(1)	nd			
	(2)	+	1:1	±	++

Staining reactions are scored semi-quantitatively (0 to +3).
nd—not determined.

Table 6. The AUC during the first 24 h after s.c. administration of rIFN-γ

Patient	Dose of IFN (mg/m²)	AUC	Peak h
BG	3.0	708.9	2
CU	3.0	388.75	24
CB	5.0	643.4	8

Immunoscintigraphy

In four patients, immunoscintigraphy with ^{99m}Tc labelled F(ab')$_2$ fragments of the anti-melanoma monoclonal antibody (225.28S) were performed before and repeated 3–4 weeks after treatment with IFN-γ. No significant change was observed in the pattern of uptake of antibody.

DISCUSSION

In this study the *in vivo* effect of IFN-γ on the expression of MAA, MHC CI and CII antigens was examined by an immunohistochemical technique by comparing staining reactions on biopsies taken before and during IFN-γ treatment. Whilst we were able to show that *in vitro* treatment of melanoma cells from the pre-interferon biopsy with IFN could induce class II expression, biopsies taken during therapy failed to show MHC class II expression and levels of MAA were similar to those in pre-treatment biopsies. One possible mechanism of how IFN-γ may act *in vivo* as an anti-tumour agent is the induction of class II antigens on tumour cells allowing host humoral and cellular reactions to the tumour by presentation of tumour associated antigens in association with MHC class II. Numerous *in vitro* studies have shown that IFN-γ can modulate expression of MHC class I and II antigens on a variety of cell lines, including human melanoma [9, 16–18]. Freshly isolated melanoma cells from biopsy material [13] and from serous effusions [19] have also been shown to have increased MHC antigen expression after *in vitro* incubation with interferon. Studies in experimental tumour models *in vivo* have provided further evidence that IFN might augment the expression of MHC antigens.

Balkwill *et al.* [20] reported that IFN-γ administered to mice with solid tumours resulted in strong induction of HLA-DR expression in two of three cell lines tested. The induction was dose-dependent and declined rapidly after cessation of therapy. In this report, biopsy samples were taken from patients at various times during IFN treatment. If the *in vivo* effect of IFN-γ on MHC class II expression is transient, serial biopsy specimens would be required to demonstrate this, and it is possible that the timing of biopsies in these patients was not optimal to detect any small change in MHC class II expression. A transient effect of IFN-γ would have important therapeutic implications because the drug may need to be used differently to get a continued sustained effect.

IFNs have also been shown to enhance expression of TAA on human breast, colon and melanoma cell lines. This effect has also been shown in experimental model systems. IFN-α increased imaging and anti-tumour effects of a Mab for nine human colon carcinoma xenografts [21]. A clinical study by Rosenblum *et al.* [22] showed increased uptake of ^{111}In-labelled Mab to melanoma in patients treated with IFN-α compared to an untreated control group. One explanation for this is that interferon induced increased TAA on melanoma cells may be the predominant effect for explaining increased tumour uptake in radioimmunoscintigraphy. However, other interpretations of their results must be considered [23]. Our own results with metastatic melanoma treated with IFN-γ do not support this hypothesis. Radioimmunoscintigraphy with ^{99m}Tc-labelled F(ab′)$_2$ fragments of a Mab 225-29S to HMW-MAA did not change in individual patients during treatment with IFN-γ compared to pre-treatment imaging. However, the two studies are not directly comparable as different interferons and different monoclonal antibodies were used.

Immunohistochemical analysis of biopsies before and under treatment did not show any detectable differences in MAA expression. The question of whether TAA is enhanced in patients treated with IFN needs to be further evaluated as this could have important clinical implications, diagnostically and therapeutically.

There are several possibilities why no effect of IFN-γ on MHC or TAA expression was observed. Previous studies have shown heterogeneity of MHC class II expression and TAA on histological sections of melanoma cells. This focal expression of class II may be due to the microenvironment and inaccessibility of some cells to putative external factors or due to clonal variation in the ability of cells to be induced. Results from *in vitro* studies support the hypothesis that class II can be induced by an external stimulus, IFN, although there is variation in induction. In this study, it is possible that melanoma cells are incapable of induction of class II with IFN or insufficient IFN-γ reached the tissues to induce class II.

Another possibility is that the IFN did not reach the tissue in an active form. Antiviral assay of the sera from these patients failed to demonstrate levels of biologically active interferon gamma even though these were present using the monoclonal antibody assay (Boots-Celltech). IFN-γ can directly induce the intracellular activity of the enzyme 2′,5′-oligoadenylate synthetase (2,5A) and Schattner *et al.* [24] have suggested that 2,5A activity may reflect the biological activity of interferon *in vivo*. In this study, 2,5A could not be detected in tissue samples from two patients examined after IFN treatment (data not shown). Rosenblum *et al.* [25] found that induction of 2,5A activity in peripheral blood cells in patients treated with partially pure interferon gamma depended on the administration route. Intramuscular or bolus i.v. administration of IFN-γ resulted in little induction of 2,5A compared to continuous 6 h infusion. Poor absorption of IFN-γ after i.m. injection and the rapid clearance from the circulation after bolus i.v. injection may account for their observations. Another possible explanation for these and our observations is that after s.c. or i.m. administration of IFN the structure of the molecule undergoes conformational changes resulting in the production of varying degrees of monomers, dimers and oligomers. Monomers are not biologically active and this could explain the failure to detect antiviral activity or enhancement of cell surface antigen expression *in vivo*.

Subcutaneous administration of IFN-γ results in a local inflammation reaction with a consecutive drop in pH. Whilst it is practically difficult to measure pH values in tissues, IFN activity was measured at different pH values *in vitro* (T. Meager, personal communication). There was a dramatic loss of activity below pH 5.0. Hsu and Arakawa [26] demonstrated that native IFN-γ unfolds under acidic conditions to give a form, P1, which readily precipitates in phosphate buffers with a resultant irreversible loss of activity. If acute type inflammation lowers the extracellular pH down to pH 3–4, then most of the IFN-γ injected at such a site should be inactivated.

Structural differences between natural IFN-γ and recombinant IFN due to factors such as glycosylation, isoelectric points and C-terminal processing may be important in biological behaviour. Antigenic dissimilarities exist between nIFN and rIFN which may be accounted for by quantitative differences in expression of protein antigenic determinants, probably maked by oligosaccharide side-chains present in nIFN [27]. However, there is little evidence to suggest that rIFN would be more immunogenic than nIFN in man and preliminary published

results show very low incidence of human antibodies to rIFN in patients receiving rIFN by i.m. or i.v. routes [28].

Whilst this study failed to detect any changes in cell surface antigen expression during IFN treatment *in vivo*, this possibility cannot be excluded on the basis of the qualitative immunocytochemical technique. The question whether there is clinically relevant MHC and TAA enhancement in patients treated with interferons remains to be elucidated by further studies with type I and II interferons.

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REFERENCES

1. Wagstaff J, Smith D, Nelmes P, Loynds P, Crowther D. A phase I study of recombinant interferon gamma administered by s.c. injection three times per week in patients with solid tumours. *Cancer Immunol Immunother* 1987, **25**, 54–58.
2. Foon KA, Sherwin SA, Abrams PG *et al.* A phase I trial of recombinant gamma interferon in patients with cancer. *Cancer Immunol Immunother* 1985, **20**, 193–196.
3. Kurzrock R, Rosenblum MG, Sherwin SA, Rios A *et al.* Pharmacokinetics, single-dose tolerance and biological activity of recombinant-interferon in cancer patients. *Cancer Res* 1985, **45**, 2866–2872.
4. Van den Burg M, Edelstein M, Gerlis G *et al.* Recombinant interferon-G: results of a phase I trial in patients with cancer. *J Biol Response Mod* 1985, **4**, 264–272.
5. Thompson JA, Cox WW, Lindgren CG *et al.* Subcutaneous recombinant gamma interferon in cancer patients: toxicity, pharmacokinetics, and immunomodulatory effects. *Cancer Immunol Immunother* 1987, **25**, 47–53.
6. Nathan CF, Murray HW, Wiebe HW, Rubin BY. Identification of interferon-G as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J Exp Med* 1983, **158**, 670–689.
7. Anderson P, Yip YK, Vilcek J. Human interferon-γ is internalised and degraded by cultured fibroblast. *J Biol Chem* 1983, **258**, 6497–6502.
8. Chapekar MS, Glazer RI. Effects of human immune interferon on cell viability, (2′,5′) oligoadenylate synthesis and polyamine-dependent protein phosphorylation in human colon carcinoma cells *in vitro*. *Cancer Res* 1984, **44**, 2144–2149.
9. Herlyn M, Guerry D, Koprowski H. Recombinant γ-interferon induces changes in expression and shedding of antigens associated with normal human melanocytes, nevus cells and primary and metastatic melanoma cells. *J Immunol* 1985, **134**, 4226–4230.
10. Houghton AN, Thomson TM, Gross D, Oettgen HF, Old LJ. Surface antigens of melanoma and melanocytes. Spcificity of induction of Ia antigens by human γ-interferon. *J Exp Med* 1984, **160**, 255–269.
11. Carrel S, Schmidt-Kessen A, Giuffre L. Recombinant IFN- can induce the expression of HLA-DR and DC on DR-negative melanoma cells and enhance the expression of HLA-ABC and tumour-associated antigens. *Eur J Immunol* 1985, **15**, 118–123.
12. Guerry D IV, Alexander MA, Elder DE, Herlyn MF. Interferon-γ regulates the T cell response to precursor nevi and biologically early melanoma. *J Immunol* 1987, **139**, 305–312.
13. Taramelli D, Fossati G, Mazzocchi A, Delia D, Ferrone S, Parmiani G. Classes I and II HLA and melanoma cells isolated from primary and metastatic lesions. *Cancer Res* 1986, **46**, 433–439.
14. Cordell JL, Falini B, Erber WW *et al.* Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984, **32**, 219–229.
15. Cerny T, Owens SE, Mackenzie SA *et al.* Immunoscintigraphy with ^{99m}Tc labelled F(ab′)$_2$ fragments of an anti-melanoma monoclonal antibody (225.28S) in patients with metastatic malignant melanoma. *Eur J Nucl Med* 1987, **13**, 130–133.
16. Ziai MR, Imberti L, Tongson A, Ferrone S. Differential modulation by recombinant immune interferon of the expression and shedding of HLA antigens and melanoma associated antigens by a melanoma cell line resistant to the antiproliferative activity of immune interferon. *Cancer Res* 1985, **45**, 5877–5882.
17. Basham TY, Merigan TC. Recombinant interferon-γ increases HLA-DR synthesis and expression. *J Immunol* 1983, **130**, 1492–1494.
18. Shaw ARE, Chan JKW, Reid S, Seehafer J. HLA-DR synthesis induction and expression in HLA-DR-negative carcinoma cell lines of diverse origins by interferon-γ but not by interferon-β. *JNCI* 1985, **74**, 1261–1268.
19. Guadagni F, Scholm J, Johnston WW *et al.* Selective interferon-induced enhancement of tumor-associated antigens on a spectrum of freshly isolated human adenocarcinoma cells. *JNCI* 1989, **81**, 502–512.
20. Balkwill FR, Stevens MH, Griffin DB, Thomas JA, Bodmer JG. Interferon gamma regulates HLA-D expression on solid tumours *in vivo*. *Eur J Cancer Clin Oncol* 1987, **23**, 101–106.
21. Greiner JW, Guadagni F, Noguchi P *et al.* Recombinant interferon enhances monoclonal antibody-targeting of carcinoma lesions *in vivo*. *Science* 1987, **235**, 895–898.

22. Rosenblum MG, Lamki LM, Murray JL, Carlo DJ, Gutterman JU. Interferon-induced changes in pharmacokinetics and tumor uptake of ^{111}In-labelled antimelanoma antibody 96.5 in melanoma patients. *JNCI* 1988, **80**, 160–165.
23. Borden EC. Augmented tumor-associated antigen expression by interferons. Editorial. *JNCI* 1988, **80**, 148–149.
24. Schattner A, Wallach D, Rosenberg H *et al.* Monitoring of interferon therapy by assay of (2′,5′) oligoisoadenylate synthetase in human peripheral white blood cells. *J Interferon Res* 1981, **1**, 587.
25. Rosenblum MG, Riso A, Gutterman JU. Activation of 2′,5′-oligoadenylate snythetase and B-2 microglobulin in cancer patients treated with partially pure gamma interferon: dependence of biological effect on administration route. *Cancer Chemother Pharmacol* 1986, **16**, 273–276.
26. Hsu YR, Arakawa T. Structural studies on acid unfolding and refolding of recombinant human interferon γ. *Biochemistry* 1985, **24**, 7959–7963.
27. Meager A, Leist T. Antigenic characteristics of glycosylated natural and unglycosylated recombinant human gamma-interferon. *J Interferon Res* 1986, **6**, 225–232.
28. Liang CM, Herren S, Fountoulakis M *et al.* Analysis of antibody response in patients receiving recombinant *Escherichia coli*-derived human interferon-gamma. *Clin Immunol Immunopathol* 1985, **36**, 95–100.