Immunological Effects of Recombinant Interferon Alfa-2a in Patients With Disseminated Melanoma

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Twenty patients with disseminated melanoma were treated with interferon alfa-2a, given by intramuscular (IM) injection three times a week in escalating doses from 15 to 50 × 10^6 U/m^2. Of 18 patients considered evaluable, two had complete remission and in two others the disease was stabilized. Laboratory tests 6 hours after injection of interferon alfa-2a indicated a marked lymphopenia and a reduction in natural killer (NK) cell activity. Sequential changes (measured before injection of interferon alfa-2a on days 3, 10, and 31) consisted of neutropenia, thrombocytopenia, and a slight increase in OKT4 positive T cells compared with OKT8 positive T cells. NK activity against the K562 target cells was increased in most patients during the first week of treatment, returning to near or below pretreatment levels thereafter. This response contrasted with a delayed increase against melanoma target cells in 10 patients. The latter correlated with an increase in mitogen-stimulated interleukin-2 (IL2) production, and may indicate that the cytotoxic activity resulted from lymphokine-activated killer (LAK) cells. Changes in cortisol levels may explain some effects on the immune system, such as depression of IL2 and immunoglobulin production *in vitro*, and the differences noted in clinical responses during the present study compared with those observed with interferon alfa-2b given by intravenous (IV) injection in 5-day cycles. These results suggest that interferon alfa-2a has antitumor activity in certain melanoma patients, in particular those with metastases to pulmonary or subcutaneous sites. Assays of IL2 production and LAK activity may assist in the selection of patients who respond to interferon alfa-2a and help to optimize treatment regimens.

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SEVERAL STUDIES have demonstrated that alpha interferons from lymphoblastoid cells[1] or leukocytes[2] have minimal activity against melanoma when given systemically, although higher response rates were reported when leukocyte-derived interferon alpha was given in combination with oral cimetidine.[3] Results of treatment with higher quantities of recombinant interferon have been more promising. Ernstoff and colleagues[4] treated 16 patients with various dose levels of interferon alfa-2B, and reported complete remission in two patients and stabilization of disease in five. In studies by Creagan and coworkers,[5,6] there were three complete remissions (CR) and four partial remissions (PR) in a group of 31 patients given 50 × 10^6 U/m^2 of interferon alfa-2a by intramuscular (IM) injection three times a week, and one CR and five PR in 30 patients given 12 × 10^6 U/m^2 of interferon alfa-2a, three times a week. In the current study of 20 patients treated with the latter preparation at dosages of up to 50 × 10^6 U/m^2 tiw (IM), there were two patients with CR and two with stabilization of disease.[7]

Some studies suggest that the route of interferon administration may be important. Robinson and colleagues[8] reported only two responses in 31 patients given interferon alfa-2b by intravenous (IV) injection daily for 5 days, every 3 weeks. However, six responses (CR and PR) were reported by the same group when interferon alfa-2b (10 × 10^6 U) was given by subcutaneous (SC) injection three

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TABLE 1. Summary of Clinical Details of Melanoma Patients Treated with Interferon Alfa-2a

	No. of patients	Age (mean ± S.D.)	Treatment duration (wks)		Outcome PD	Outcome SD	Outcome CR	Median survival from treatment*
Total:	20	43.2 ± 14	7.5	(2–20)	14	2	2	26
Performance								
(ECOG 0, 1):	12	45.4 ± 16	9.6	(4–20)	8	2	2	30
(ECOG 2):	8	39.9 ± 11	4.4	(2–6)	6	0	0	6
Lung + SC:	7				5	1	2	30
Visceral:	13				11	1	0	24

* Median survival from diagnosis of metastases was 38 weeks.
ECOG: Eastern Cooperative Oncology Group (US), SC: subcutaneous sites; PD: progressive disease; SD: stable disease; CR: complete remission; S.D.: standard deviation.

times a week. This result was similar to that of Pouillart and colleagues[9] in patients given the same preparation by the SC route.

The mechanism of action of interferon against melanoma is largely unknown at present. Direct effects of interferon alfa-2a were observed on approximately half of 60 melanomas tested *in vitro* and, in some instances, growth inhibition was marked.[10] Recent studies also suggest that the interferons may inhibit the expression of certain oncogenes and cause reversion to a normal cell phenotype,[11–13] but whether this reaction occurs in melanoma is unknown. A third possible mechanism of action is the effect of interferon on the immune system. *i.e.*, increased natural killer (NK) cell activity, or cytotoxic T-cell activity.[13–15] The current studies were carried out to determine whether effects on the immune system could be demonstrated *in vivo* during treatment with interferon alfa-2a and whether these effects could be correlated with clinical responses in tumor growth.

Materials and Methods

Patients

The study population consisted of 15 patients with disseminated melanoma attending the Sydney Melanoma Unit and five patients attending the Newcastle Melanoma Unit. Full details of the patients are described elsewhere[7] and are summarized in Table 1. Twelve patients were in good physical condition, whereas eight were restricted in their activities due to the disease. In seven of the 20 patients, metastases were confined to lung and subcutaneous sites and in 13 patients there was visceral involvement.

Interferon Administration and Study Design

Interferon alfa-2a (Roferon®-A) was supplied by Roche Products Pty., Ltd., Dee Why, NSW, Australia, as a freeze-dried preparation and given by IM injection in the gluteal region in an escalating dosage (15–50 × 10^6 U/m^2) tiw, as described previously.[7]

Criteria of Response

Following the recommendations of Miller *et al.*[16] CR was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks. PR was defined as a 50% or greater decrease in the products of the perpendicular diameters of measurable lesions with no progression of existing lesions or development of new lesions. Stable disease (SD) was defined as no response, the absence of new lesions, and less than a 25% increase in the size of existing lesions. Progressive disease (PD) was defined as an increase of greater than 25% in the size of measurable lesions or the appearance of new lesions.

Immunologic Tests

Estimation of lymphocyte subpopulations in blood: Mononuclear cells were separated from defibrinated blood samples by centrifugation on Hypaque-Ficoll (B.D.H., Kilsyth, Vic.) using standard techniques. T-cell populations were defined by the use of monoclonal antibodies OK3, OKT4, and OKT8 (Ortho Diagnostics, North Ryde, NSW, Australia) and peroxidase-labeled rabbit anti-mouse immunoglobulin (Dako Patts, Code, P. 161 Bioscientific, Gymca, NSW).

Cells (3–5 × 10^5) in 30 μl were placed on a slide, air dried, and fixed in acetone for 5 minutes. They were washed in phosphate-buffered saline (PBS), incubated with 0.03% H_2O_2 for 5 minutes, and washed with PBS + 1% bovine serum albumin (BSA). Twenty μl of a 1:4 dilution of the monoclonal antibody was added for 30 minutes at room temperature and, after washing, the cells were incubated in a 1:20 dilution of the second antibody. They were washed and then incubated with substrate, 3-amino-9-ethyl-ethylcarbazole, prepared as described elsewhere,[17] for 15 minutes, then stained with hematoxylin and mounted in Glycergel (Dako) for examination by bright field microscopy.

Measurement of NK Activity

The methods used to measure NK activity against the MM200 and K562 myeloid cells in chromium 51(^{51}Cr)

release assays are described elsewhere.[18] Blood mononuclear cells (3×10^5, 10^5, and 3×10^4 in 0.5 ml) were incubated with 3×10^3 ^{51}Cr-labeled MM200 or 10^4 K562 cells (in 0.5 ml) in overnight 16-hour assays in duplicate round-bottom tubes. Supernatants (0.5 ml) were harvested after centrifugation at 500 g for 7 minutes and counted. Percentage of ^{51}Cr release was calculated by the formula $2a/a + b \times 100$, where a = counts in tube with supernatant alone minus machine background, and b = counts in tube with target cells and half the supernatant. Percent specific cytotoxicity was calculated as follows:

$$\frac{\%\ ^{51}\text{Cr release test} - \%\ \text{spontaneous}\ ^{51}\text{Cr release}}{\text{Maximum}\ \%\ ^{51}\text{Cr release} - \%\ \text{spontaneous}\ ^{51}\text{Cr release}} \times 100$$

Lytic units were defined as the number of effector cells required to lyse 20% of the target cells and were expressed per 10^6 of the lymphocyte population (LU[20%/10^6]).[19] These values were compared with the mean value of two frozen and thawed controls carried out in parallel with the test subjects. If the value of the controls was outside the mean + 2 standard error (SE) of the mean control value (estimated from ≥20 tests), the test value was multiplied by a multiple obtained by dividing the control value on that day by the mean control value.

Measurement of IL2 Production and Assay of IL2

Blood lymphocytes (4×10^6 in 2 ml of Roswell Park Memorial Institute 1640 medium fetal calf serum), were incubated with 1% phytohemagglutinin (PHA) (Wellcome Pharmaceuticals, Code HA15) for 36 hours in flat-bottom Bijou bottles. The supernatants were collected and assayed at 4 dilutions for mitogenic activity against the NK-7, interleukin-2 (IL2)-dependent, murine cell line (111-E3) provided by Professor Kumagai and described elsewhere.[20] In brief, supernatant (100 μl) was added in doubling dilutions to triplicate wells with 2×10^4 NK-7 cells for 18 hours, and pulsed with 1 microcurie (μCi) of iodine 125-labeled iododeoxyuridine (^{125}IUDR) for 6 hours. A unit of IL2 was defined as the reciprocal dilution that induced 50% of the maximum ^{125}IUDR uptake of a standard IL2 preparation included in each assay. To reduce variability, supernatants from IL2 production assays from each patient, as described above, were stored until the treatment with interferon was complete, and all supernatants were assayed at once against the NK-7 cells.

Statistical Analysis

The complete remission rate and 95% confidence limits were obtained from the binomial distribution tables. Sequential changes in leukocyte populations were tested for significance by analysis of one-way variance performed on data collected on days 1, 3, 10, and 31, using the "minitab" statistical package (Pennsylvania State University). Median survival was estimated from life tables constructed as described by Kaplan and Meier.[21] Analysis of the significance of short-term changes in NK activity during treatment with interferon alfa-2a was carried out by the paired *t* test.

Assay of Immunoglobulin Production in Pokeweed Mitogen (PWM)-Stimulated Cultures of T- and B-Cells

The methods for these assays have been described fully.[22] In brief, 3×10^4, 10^5, or 3×10^5 E-rosetting (T) cells were added to 10^5 non-E-rosetting ("B") cells in triplicate 30 × 10 mm round-bottom tubes and stimulated with PWM (batch A771101; GIBCO, Grand Island, NY) at a final dilution of 1:240 (final volume, 0.6 ml). A parallel set of cultures was established in which the E-rosetting cells had been irradiated at 100 rad/min from a ^{137}Cs source to a maximum dose of 2000 rad. At 7 days, the cultures were harvested and assayed for IgA, IgG, and IgM production by using a Hyland PDQ laser nephelometer (Travenol Laboratories, Toongabbie, NSW). Suppressor T-cell activity in these cultures was estimated by comparison of immunoglobulin (Ig) production in cultures where suppressor T-cells had been inactivated by irradiation with that in cultures where suppressor activity was still present. Ratios greater than 1 indicated significant suppressor T-cell activity.

Cortisol Levels

Cortisol levels in sera were assayed using the Amerlex Cortisol radioimmunoassay kit (Amersham Australia Pty. Ltd., Sydney, Australia). Assays performed by this method in this laboratory have an intra-assay coefficient of variation of 4.8%.

Results

Clinical Responses

The clinical outcome of treatment as described earlier[7] is summarized in Table 1. Two patients (Nos. 4 and 20) experienced CR lasting 12 and 10 months, respectively. Both patients had pulmonary metastases and one had extensive SC metastases. In patient No. 4, SC recurrence near the primary site was detected at 12 months and lymph node metastases in the iliac nodes at 20 months. Death occurred at 24 months from cerebral metastases. The second patient, No. 20, developed early signs of biliary obstruction at 10 months due to involved lymph nodes around the head of the pancreas and remains well after bypass surgery. One patient No. 8, with extensive liver metastases remained in a stable condition during therapy for a period of 8 months and deteriorated after cessation of therapy. Patient No. 9 experienced stabilization of SC and pulmonary metastases for 9 weeks before disease progression occurred, coincident with the development of antibodies to interferon alfa-2a. There was no response

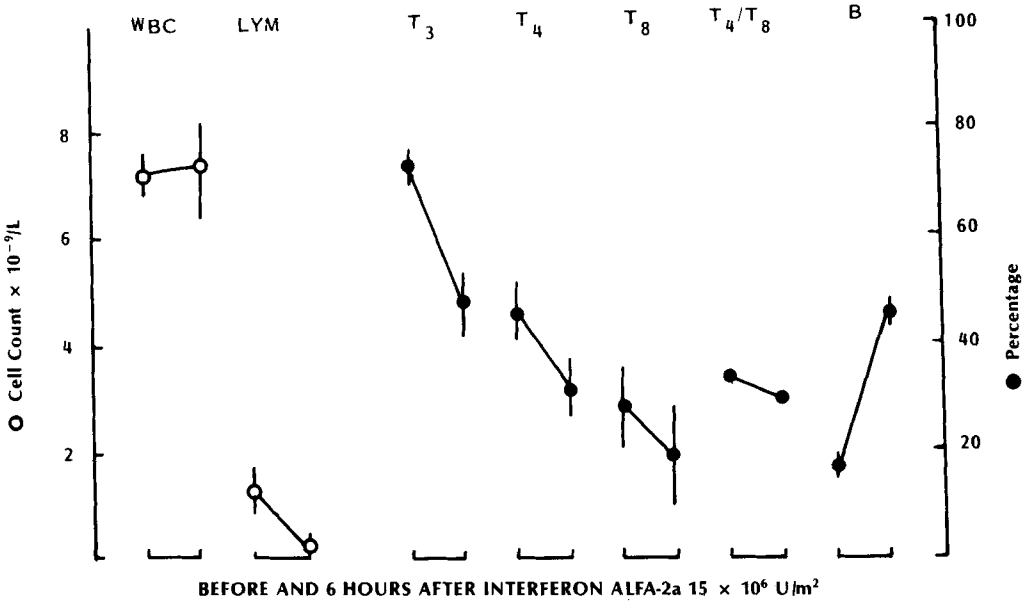

FIG. 1. Changes in total leukocyte (WBC) and lymphocyte (LYM) counts and percentages of lymphocyte subsets before and 6 hours after intramuscular injection of interferon alfa-2a. Values shown are means ± 1 standard deviation of data from 15 patients.

in patients with poor performance status or in those with visceral disease, except for patient No. 8 described above.

Changes in Blood Leukocytes 6 Hours After Administration of Interferon Alfa-2a

Figure 1 summarizes studies on total leukocyte, lymphocyte, and lymphocyte subset counts in 15 patients attending the Sydney Melanoma Unit after the first injection of interferon alfa-2a (15 × 10^6 U/m²). Total leukocyte count remained unchanged, but there was a marked decrease in total lymphocyte count. Lymphocyte subset analysis revealed that this change affected both T4 (helper) and T8 (suppressor/cytotoxic) T-lymphocytes, and that B-cells showed a reciprocal increase in percentage. Similar changes were observed after doses of 30 and 50 × 10^6 U/m², as described previously.

Sequential Changes in Leukocyte and Platelet Counts During Treatment With Interferon Alfa-2a

Analysis of changes in blood taken 2 days after the first injection and before interferon alfa-2a administration on days 3, 10, and 30 during treatment are shown in Figure 2. Mean total leukocyte counts declined significantly ($P < 0.01$) due to a decrease in the neutrophil count. The total number of lymphocytes was reduced from a mean of 1.4 × 10^{-9}/1 to 1.0 × 10^{-9}/1, but this and the changes

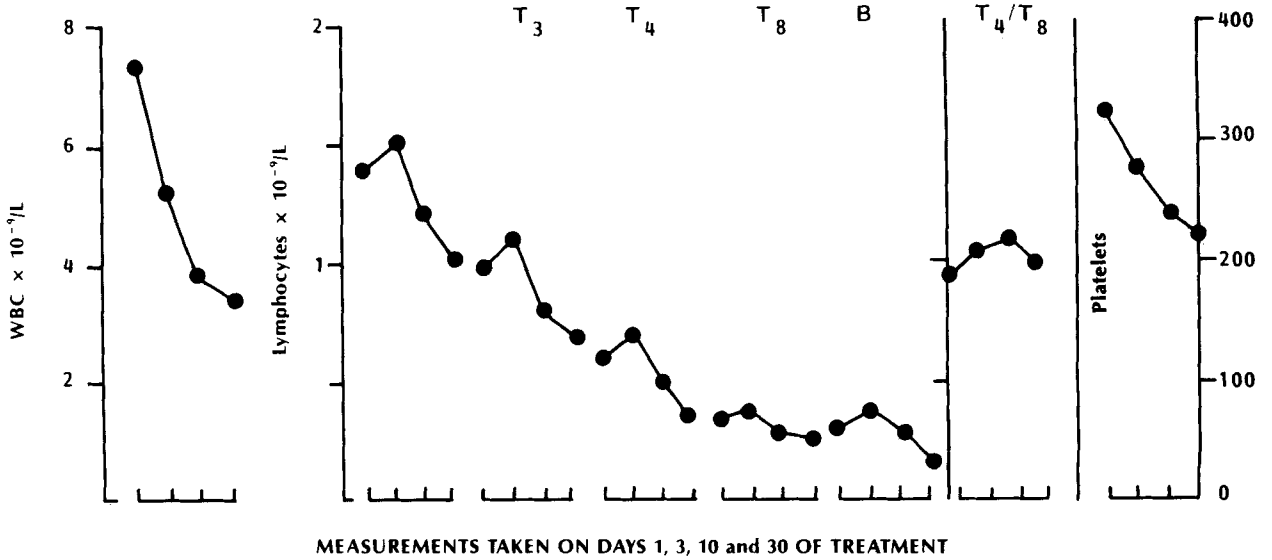

FIG. 2. Sequential changes in total leukocyte (WBC), total lymphocyte, lymphocyte subset and platelet counts during treatment with interferon alfa-2a given intramuscularly, three times a week, as measured before treatment and at days 3, 10, and 30 of treatment. Values shown are means of data from 15 patients.

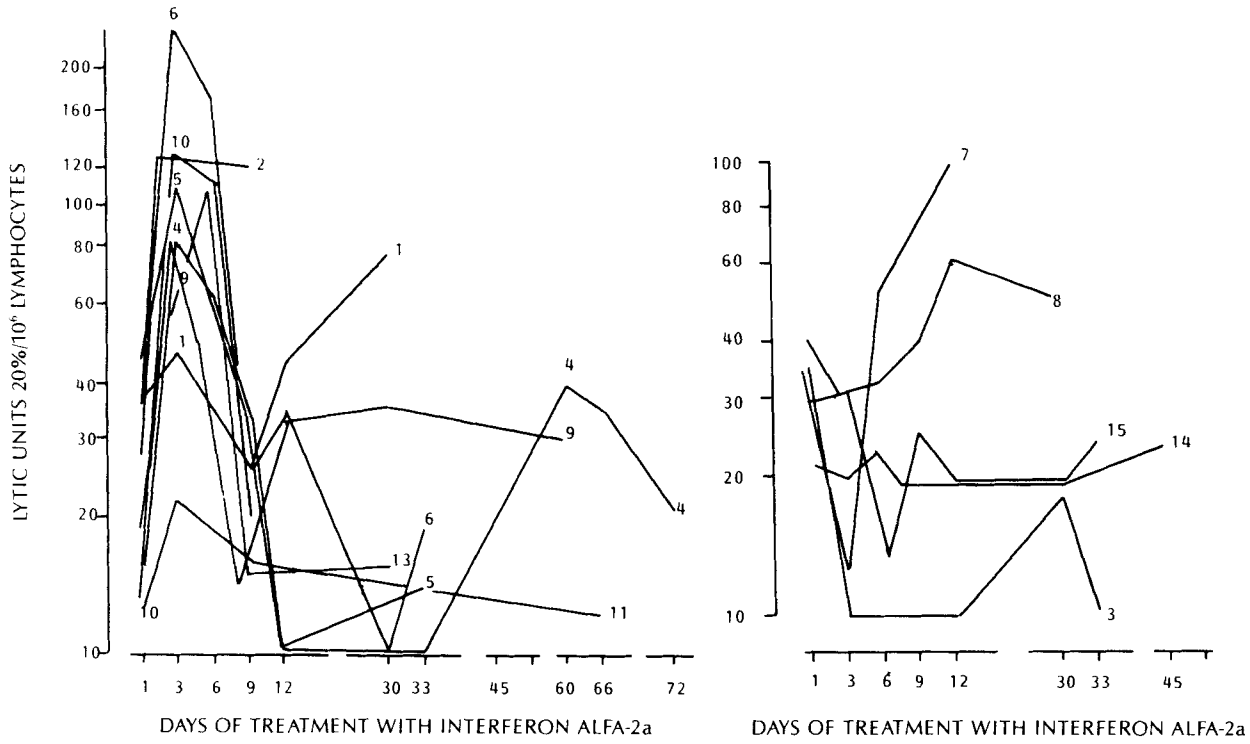

FIG. 3. Sequential changes in natural killer activity against the K562 target cell: (left panel) Patients showing an increase in the first week of treatment and (right panel) those showing a decrease or no change. Mean value ± 2 standard error of the frozen-thawed concurrent control peripheral blood lymphocytes against the K562 target cells was 20.3 + 4.2 LU/20%/10^6 (26 estimations). (Reproduced with permission from *Br J Cancer* 1985; 51:815–826.)

in the total number of T-cells or T-cell subsets were not significant as determined by one-way analysis of variance. There was, however, a significant decrease in the number of B-cells (from 0.30 to 0.14 × 10^{-9}/1), monocytes (from 0.38 to 0.21 × 10^{-9}/1) ($P < 0.01$), and platelets (from 326 to 222 × 10^{-9}/1) ($P < 0.01$). There was a small, though not statistically significant, increase in the T4/T8 ratio.

Changes in NK Activity During Treatment With Interferon Alfa-2a

As described previously,[7] NK activity measured in blood samples taken 6 hours after interferon alfa-2a administration was markedly reduced against the K562 myeloid target cell. For example, at the 15 × 10^6 U/m^2 dose, values (lytic units per 10^6 lymphocytes at the 20% level) were reduced from 30.7 ± 11 to 6 ± 4.6 and at the 30 × 10^6 U/m^2 dose, from 85 ± 72 to 19 ± 13. These effects were not evident against the MM200 target cell, *e.g.*, at the 15 × 10^6 U/m^2 dose there was a slight (not significant) increase from 16.4 ± 16.9 to 20.2 ± 34 lytic units.

Similarly, the sequential changes in NK activity during treatment differed against the K562 and MM200 target cells. As shown in Figure 3, there was a marked increase in NK activity against the K562 target cell at day 3 of treatment in 9 of 14 patients; however, this value fell to, or below, pretreatment levels on days 10 and 31. In contrast, NK activity against the MM200 target cell increased in 10 of 14 patients on days 3, 10, and 31, respectively, as compared with pretreatment levels (Fig. 4). This change was particularly evident in patients with low pretreatment levels. Mean values for the assays are shown in Table 2. Differences between the means were not statistically significant.

Effect of Interferon Alfa-2a (and Interferon Alfa-2b) on IL2 Production

In 12 of 13 patients there was a marked inhibition of IL2 production at the beginning of interferon alfa-2a treatment on day 3, and then a gradual return to pretreatment levels or above (Table 3). The table also shows the effect of interferon alfa-2b (20 × 10^6 U/m^2) given on 5 consecutive days, every 14 days, by the IV route, as reported previously.[23] Before each cycle, IL2 production was within normal levels but fell to low levels by day 3 of each cycle, as described at the onset of treatment with interferon alfa-2a. During treatment with interferon alfa-2a some correlation between IL2 production and NK activity against the MM200 target cells was evident in that six patients who showed an increase in IL2 production also had an increase in NK activity against the MM200, but not the K562, target cells.

Cortisol Levels

Serum samples were assayed for cortisol levels from four patients in the current study and four melanoma

patients studied concurrently in the Phase II study of interferon alfa-2b (20×10^6 U/m^2 given IV on 5 consecutive days every 14 days), referred to above. The performance status of the patients in both studies was comparable (Table 4). In both studies, cortisol levels in serum samples taken 6 hours after interferon alpha administration tended to be higher than in samples taken before administration (at approximately 9 AM). This increase was more pronounced (twofold or greater) and statistically significant in the patients given interferon alfa-2b by the IV route compared with the increase seen in patients given interferon alfa-2a by IM injection. The mean increase in the latter group was one and a half times or less and not statistically significant as determined by paired *t* test or one-way analysis of variance.

Changes in Immunoglobulin Production

Immunoglobulin studies were carried out on two patients to determine whether interferon alfa-2a treatment was associated with an induction of suppressor T-cell activity. In both patients, there was a marked inhibition of Ig production during treatment with interferon alfa-2a (Table 5). This change did not appear to result from an induction of suppressor cells; when the latter were inactivated by gamma irradiation, the increase in Ig synthesis was no greater during interferon alfa-2a treatment than in pre- or posttreatment samples.

Immunoglobulin levels were also assayed by radial immunodiffusion on serum samples taken before and after 30 days of treatment with interferon alfa-2a. IgG, IgM, and IgA levels showed a decrease of 5% or greater in 5, 6, and 8, respectively, of 13 patients tested. Increases of 5% or more for IgG, IgM, and IgA levels were seen in 2, 2, and 1 of the patients, respectively. (All sera were stored at −20°C and assayed at one time on the same batch of immunoplates.)

Discussion

Analyses of the effects of interferon alfa-2a in several immunological tests were carried out to determine whether these effects may correlate with the clinical responses of tumor growth to interferon alfa-2a. Changes in T-cell subsets, for example, could indicate an increase in helper cells for Ig production or cytotoxic T-cell formation, and an increase in the cytotoxic/suppressor subset may reflect concomitant changes in functional activity against melanoma. Very little change was observed, however, in the total lymphocyte count or in particular T-cell subsets during treatment with interferon alfa-2a, even though platelet and neutrophil counts were significantly decreased. These results are in general agreement with some reports but not with others. Silver and colleagues[24] reported increased ratios of T4/T8 during IV treatment with lymphoblastoid interferon, whereas patients given

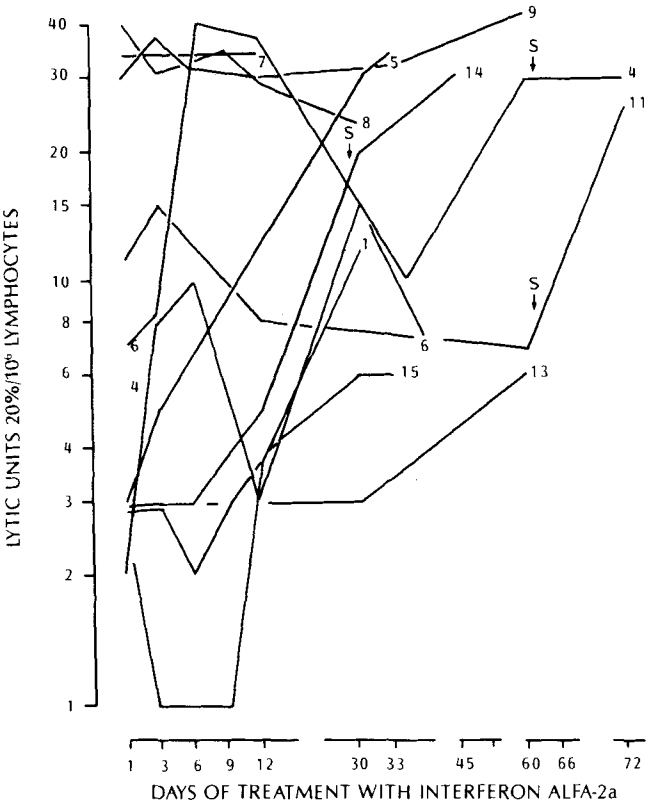

FIG. 4. Sequential changes in natural killer activity against the MM200 target cell showing the different pattern of activity from that against the K562 target cell. Increased NK activity was seen in most of the patients and this tended to be gradual and prolonged as compared with that against the K562 target cells. Mean value was ± 2 SE of the frozen-thawed concurrent control. Peripheral blood lymphocyte count against the MM200 target cells was 9.8 + 3.1 LU/20%/10^6 (26 estimations). S refers to date of cessation of inteferon alfa-2a treatment. (Reproduced with permission from *Br J Cancer* 1985; 51:815–826.)

interferon alfa-2a by daily IM injection were found (after an initial decrease in the T4/T8 ratio) to have no significant change over 3 weeks of treatment.[21] Ernstoff and co-workers[25] reported a twofold increase in the T4/T8 ratio when interferon alfa-2b was given daily by the IM route and a 50% decrease in this ratio when it was given daily by the IV route.

The effects of interferon on NK activity have received attention in a number of studies because of the potential

TABLE 2. Changes in Natural Killer Activity During Treatment With Interferon Alfa-2a*

		Day of Treatment			
Target cell		1	3	10	31
MM200		12 ± 13	17 ± 15	14.5 ± 14.6	16.6 ± 11.7
	N	14	14	13	10
K562		28.6 ± 10.8	69 ± 58	35 ± 30	26.9 ± 22
	N	14	14	13	10

* Values indicated are lytic units/20%/10^6 lymphocytes (mean ± 1 SD).

TABLE 3. Interleukin-2 Production from PHA-Stimulated PBL of Interferon-Alpha-Treated Patients

	Alternate day IM route (interferon alfa-2a)					IV route 5-day cycles (interferon alfa-2b)							
	Days from start of treatment					Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	1	3	10	31	59	Day 1	Day 3	Day 15	Day 17	Day 29	Day 31	Day 43	Day 45
Units/ml (mean ± 1 SD)	16.68 ± 12.9	4.4 ± 3.18	8.7 ± 6.8	14.7 ± 6.8	12.1 ± 9	14.7 ± 17.5	9.4 ± 13.1	15.1 ± 18.4	8.8 ± 10.5	10.9 ± 13.6	2.0 ± 2.7	12.4 ± 9.8	5.5 ± 3.6
N	13	13	9	9	4	9		9		5		2	
Paired *t* values	4.26		2.5	1.8		.87		1.14		1.64			
P values	<0.001		<0.05	*ns*									

PHA: phytohemagglutinin; PBL: peripheral blood lymphocytes; IM: intramuscular; IV: intravenous; SD: standard deviation.

TABLE 4. Cortisol Values in Patients Given Interferon Alfa-2a IM (tiw) and Interferon Alfa-2b IV Daily for 5 Days Every 2nd Week

	Alternate day IM route (interferon alfa-2a)								IV route 5-day cycles (interferon alfa-2b)							
									Cycle 1				Cycle 2			
	Day 1		Day 3		Day 15		Day 17		Day 1		Day 3		Day 15		Day 17	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
nmol/l* (mean ± 1 SD)	343.0 ± 26.7	445.5 ± 184.9	398.2 ± 153	403.5 ± 197	289.0 ± 59	384.0 ± 129	279.5 ± 73	415.7 ± 125	151 ± 26	368.5 ± 77	164.8 ± 77	327.5 ± 106	168.0 ± 53	387.7 ± 112	143.0 ± 12	329.5 ± 111
Increase		(1.3×)		(1.01×)		(1.3×)		(1.5×)		(2.4×)		(2.0×)		(2.3×)		(2.3×)
P value (by paired *t* test)										0.013		0.05		0.024		0.045
F value *P* < 0.01	0.63								7.04							

* Normal range: 180–720 nmol/l (am): 60–500 nmol/l (pm)

IM: intramuscular; tiw: three times a week; IV: intravenous; SD: standard deviation.

TABLE 5. Sequential Study of Immunoglobulin Production in PWM-Stimulated Cultures of B- and T-Cells During Interferon Alfa-2a Administration

Patient		Day 0		Day 3		Day 31		Day 45	
		ng/ml	SR	ng/ml	SR	ng/ml	SR	ng/ml	SR
1	IgG 1:1	1437	0.8	729	0.87	536	1.6	—	—
	1:3	2264	1.2	628	1.07	382	1.6	3792	1.3
	IgM 1:1	3051	1.0	563	1.0	877	1.5	—	—
	1:3	3681	1.4	582	2.2	519	2.5	3131	2.1
	IgA 1:1	1302	1.6	224	2.2	1885	0.9	—	—
	1:3	1966	1.1	459	1.1	1576	1.08	4692	2.0
								(Day 59)	
2	IgG 1:1	3720	0.9	667	1.0	866	—	727	1.9
	1:3	5359	0.9	782	1.0	831	1.0	1140	1.4
	IgM 1:1	895	1.1	1037	0.9	1553	0.8	—	—
	1:3	1115	4.9	1123	1.1	1809	1.1	2965	1.2
	IgA 1:1	4244	0.8	623	1.2	948	1.2	1355	1.2
	1:3	5075	0.9	942	1.1	994	0.9	1943	1.5

PWM: pokeweed mitogen; Ig: immunoglobulin; SR: suppressor ratio.

importance of this mechanism against tumors and, in particular, against metastatic static spread of tumors. Although it is well documented that interferon alpha enhances NK and antibody-dependent cell-mediated (ADCC) killing *in vitro,*[14,15,26] effects *in vivo* appear variable. In the current study, NK activity against the K562 target cells was markedly decreased in blood samples taken 6 hours after injection of interferon alfa-2a. Similar observations were made by Lotzova and colleagues.[27] This decrease in NK activity and the decrease in lymphocyte count may be the effects of corticosteroid release, as previous studies have shown that corticosteroids may cause sequestration of T-cells from the circulation and inhibit NK activity.[28,29]

NK activity against the K562 target cells in blood samples taken after the acute effects of interferon alfa-2a administration had subsided showed a marked increase in the first week of treatment and then decreased to or below pretreatment levels. Comparisons with other studies in the literature are difficult because of the different doses and dosage schedules employed. The route of administration may also be important. Ernstoff and colleagues[25] reported that interferon alfa-2b given IM resulted in an increase in NK activity, but had no effect by the IV route. When given daily by SC injection, there was no change or depression of NK activity. Maluish and co-workers[30] also found that interferon alfa-2a given by IM injection, either three times a week, or twice daily, resulted in little effect or in depression of NK activity. The latter appeared particularly prominent at high doses of interferon alfa-2a. Studies by Lotzova *et al.*[27] and Edwards *et al.*[31] suggested that the level of NK activity preceding treatment is another variable and that responses induced by interferon alfa-2a were inversely related to pretreatment levels.

A different pattern of cytotoxic activity by blood lymphocytes was seen against the MM200 melanoma cell during treatment with interferon alfa-2a, suggesting that a different population of effector cells was involved against this target cell. As reported elsewhere,[7] there was a broad correlation between this activity and IL2 production from mitogen-stimulated lymphocytes. This finding suggests that the lymphocytes mediating killing against the melanoma target cell were specific T-cells or lymphokine-activated killer (LAK) cells,[18,32] both of which are dependent on IL2 for their activity. Such a hypothesis would be consistent with previous studies showing that the MM200 target cells were relatively insensitive to NK cells, but were readily lysed by IL2-activated blood lymphocytes.[18]

Inhibition of IL2 production from mitogen-stimulated lymphocytes was noticed in practically all patients at the start of treatment with interferon alfa-2a; and IL2 then returned to or above pretreatment levels despite continued treatment. These results contrasted with those obtained during IV administration of interferon alfa-2b in 5-day cycles every 14 days; in that regimen, IL2 production was inhibited at the start of each treatment cycle. Such effects on IL2 production may reflect changes in endogenous cortisol production, as previous studies have shown that IL2 production is inhibited by cortisol.[33] This interpretation was supported by the changes observed in the cortisol levels in the two studies: interferon alfa-2b by the IV route appeared to stimulate a more marked increase in cortisol levels during each cycle than interferon alfa-2a administered IM on a more continuous basis. Cortisol production may also explain the suppression of Ig production seen in PWM-stimulated cultures *in vitro,* as previous studies have shown that cortisol may inhibit Ig production by several mechanisms.[34] Our conjecture is that administration of interferon alfa-2a by the IV as compared with the IM route may have a more marked effect on hypothalamic centers controlling adrenocorticotropic

hormone (ACTH) release and so account for the more marked increase in endogenous cortisol levels in patients receiving IV interferon alfa-2a.

Exact correlations between the *in vitro* immunological tests during interferon alfa-2a treatment and the clinical effects on tumor growth were not found in this study. Patients showing complete remission had good recovery of mitogen-stimulated IL2 production and increased lymphocyte cytotoxic activity against the melanoma target cell. Similar changes were, however, seen in three patients who did not show any clinical response. Other aspects, such as immunosuppressive factors from the tumor[35] or site of the metastases, may have been important to the clinical outcome in the latter cases. Our results suggest that measurement of NK activity against the K562 target cell was of little relevance, and that T-cell subset analysis was not helpful. Further studies are required to improve the selection of melanoma patients who may respond to interferon alfa-2a and to develop optimal treatment regimens. The effects of interferon alfa-2a on cortisol levels, IL2 production, and LAK- or T-cell-mediated cytotoxicity may be of value in these aims.

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