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Research Article

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Downregulation of Interferon α but Not γ Receptor Expression In Vivo in the Acquired Immunodeficiency Syndrome

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Abstract

Interferons (IFN) elicit antiviral and antineoplastic activities by binding to specific receptors on the cell surface. In evaluating the role of IFN as therapeutic agents in AIDS, we investigated the expression of IFNα and γ receptors on peripheral blood mononuclear cells (PBM) from patients with AIDS, ARC, and heterosexual control subjects using radioiodinated IFNα_2 and IFNγ. The binding characteristics of the ^{125}I-IFNα and γ to PBM were analyzed to determine receptor numbers and dissociation constants. PBM from controls expressed 498±247 IFNα receptor sites/cell (n = 17). However, eight patients with ARC and seven patients with AIDS had a mean number of IFNα receptor/cell of 286±235 ($P < 0.05$) and 92±88 ($P < 0.001$), respectively. This was consistent with elevated levels of serum acid-labile IFNα and cellular 2-5A synthetase activity in patients. Treatment of PBM from the AIDS patients with exogenous IFNα in vitro resulted in minimal 2-5A synthetase induction in comparison to controls. In contrast, the expression of IFNγ receptors in ARC (n = 5) and AIDS (n = 4) patients remained normal. Thus the decrease in IFNα receptor expression and consequent hyporesponsiveness to IFNα raises the question of the usefulness of IFNα therapy in end-stage AIDS. The normal expression of IFNγ receptors in AIDS patients suggests that IFNγ may prove useful in attempts to provide immune reconstitution.

Introduction

The acquired immunodeficiency syndrome (AIDS) is associated with infection by a retrovirus, human immunodeficiency virus (HIV), and is characterized by progressive immune defects and consequent opportunistic disease (1–6). The patients with HIV infections have a constellation of immunoregulatory defects at multiple levels of the immune system.

In vitro studies of immune function on lymphocytes isolated from AIDS patients have demonstrated many abnormalities of B and T cell function (7–12). Other reports have indicated defects in synthesis of lymphokines including interleukin 1 (13), interleukin 2 (14), and interferon (IFN) γ (13), and decreased production of conventional α IFNs in response to experimental infections in vitro (15). In contrast, high levels of an unusual acid-labile IFN-α subtype in sera from ~ 65% of homosexual AIDS patients have been reported (16). The acid-labile IFN was also detected in two hemophiliac patients before the onset of AIDS (17). We have previously reported elevated levels of an IFN-induced intracellular enzyme, 2′-5′ oligoadenylate (2-5A) synthetase,[1] activity in AIDS and AIDS-related complex (ARC) patients (18). Although the physiological and immunological significance of these elevated levels of IFN and 2-5A synthetase activity in the pathogenesis of AIDS is not known, there appears to be an association between persistent elevation of 2-5A synthetase activity in ARC patients and increased risk of developing AIDS (19).

Recent advances in recombinant DNA technology have provided sufficient supplies of highly purified IFN preparations for therapeutic trials. Clinical efficacy of IFN on several viral infections and neoplastic diseases has been demonstrated (20, 21). Recent studies reported that some AIDS patients with Kaposi's sarcoma showed tumor regressions in response to recombinant leukocyte α IFN therapy (22–24). In addition, enhancement of monocyte/macrophage killing function has been observed when IFNγ is administered to AIDS patients (25). While many of the basic mechanisms involved in the antiviral, antineoplastic, and immunoregulatory activities of IFN in vivo remain to be elucidated, it is well documented that IFN elicits its activity by binding to specific high affinity receptors on the cell surface. As a consequence, knowledge of the expression of IFNα and γ receptors in AIDS patients in vivo is important, as it provides crucial information regarding the availability of IFN receptors at the cell surface for the mediation of IFN actions during IFN therapy.

In this study, we have investigated the expression of IFNα and γ receptors on freshly isolated peripheral blood mononuclear cells (PBM) from AIDS and ARC patients, and correlated this with the levels of the IFN-induced enzyme, 2-5A synthetase.

Methods

Patients. 15 homosexual patients with HIV infections were studied: 7 of the patients had clinically defined AIDS (HIV group IVc) and 8 had ARC (HIV group IVa), according to the surveillance criteria established by the Centers for Disease Control (26). They were all homosexual males aged between 25 and 39 yr, and were followed at the Toronto General Hospital AIDS Clinic. Two of the AIDS patients (patients 1 and 2, Table I) were very ill with opportunistic infections including disseminated cytomegalovirus and severe *Pneumocystis carinii* pneumonia. The third sick individual (patient 7) was a 22-yr-old with severe Pneumocystis pneumonia. They were all hospitalized and treated with intravenous antibiotics, i.e., trimethoprim/sulfamethoxazole, at the time of the study. The other four AIDS patients were

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1. *Abbreviations used in this paper:* ARC, AIDS-related complex; 2-5A, 2′-5′-linked oligoadenylates.

stable without active infections. Two of them had recovered from opportunistic infections: one from perianal Herpes simplex and mild Pneumocystis pneumonia (patient 3), and the other one from a mild form of *P. carinii* pneumonia (patient 5). Both were successfully treated with trimethoprim/sulfamethoxazole per os. The remaining two AIDS patients had the cutaneous form of Kaposi's sarcoma without any dissemination or signs of opportunistic infections.

The ARC patients (Nos. 8-15, Table I) were stable with constitutional disease without any opportunistic infections or evidence of malignancy. They are therefore classified as group IVa infections. All patients had positive IgG antibodies to HIV by ELISA assay at the time of the study. The control subjects consisted of healthy heterosexual laboratory personnel, medical students, and residents.

Cells and serum samples. PBM were isolated from heparinized blood samples using Ficoll-Hypaque density gradient centrifugation (27). The cells were incubated in a tissue culture dish (Falcon Labware, Oxnard, CA) at 37°C for 30 min for monocyte separation. B and T lymphocytes were isolated by immunorosetting using neuraminidase treated sheep erythrocytes (28). The resulting T, B, and monocytic cells were resuspended in RPMI medium for AIDS sera treatment and subsequent IFNα receptor binding experiments. Serum samples obtained simultaneously were stored at −70°C until the time of assay for IFN titers or use in other experiments.

IFNα receptor binding assay. The binding of IFNα to PBM was studied using IFNα2 (kindly supplied by Drs. P. Trotta and T. Nagabushan, Schering Corp., Kenilworth, NJ, sp act 2×10^8 IU/mg protein), labeled to high specific radioactivity (90–120 Ci/g) with ^{125}I by the solid-phase lactoperoxidase procedure (29). The receptor binding assay was performed as described (30). In brief, 4×10^5 cells (PBM, T, B, or monocytes) were incubated with indicated concentrations of ^{125}I-IFNα2 in triplicate, in a final volume of 200 μl in RPMI 1640 medium at 4°C for 4 h. The binding reactions were centrifuged over a layer of phthalate oil and cell pellets obtained were analyzed for cell bound radioactivity. Binding specificity was determined in parallel incubations including a 100-fold excess of unlabeled IFNα2 at each ^{125}I-IFNα2 concentration. The nonspecific counts obtained were subtracted from the corresponding total cell bound radioactivity to calculate the specific binding activities reported. These specific binding data were plotted according to the method of Scatchard and analyzed by the LIGAND program of P. Munson and D. Rodbard (31, 32). The program computes binding parameters on the basis of the laws of mass-action binding and objectively determines the number of receptor sites and the dissociation constant, K_d, characterizing the binding reactions.

IFNγ receptor binding assay. Similarly, the binding of IFNγ to PBM was studied using IFNγ4A (kindly supplied by Dr. N. Stebbing, AmGen, Inc., Thousand Oaks, CA, sp act 2×10^7 IU/mg protein), labeled to high specific radioactivity (35 to 45 Ci/g) with ^{125}I by chloramine *T* oxidation method (33). In brief, 20 μg (4×10^5 U) of IFNγ4A was allowed to react with 1 mCi Na ^{125}I, and 40 μg chloramine *T* in a final volume of 100 μl of 0.1 M ammonium acetate buffer (pH 7.3) containing 400 mM urea, at 4°C for 5 min. The iodination reaction was terminated by the addition of 80 μg of sodium metabisulphite. The iodinated IFNγ4A was separated from unincorporated ^{125}I by chromatography and titrated for antiviral activity (29). Binding reactions and analysis of data were performed as described above.

2-5A synthetase assay. To test the 2-5A synthetase response to IFN, freshly isolated PBM were resuspended into 1-ml aliquots of RPMI 1640 medium containing 1×10^6 cells, supplemented with 10% FCS to which 1,000 U of IFNα2 was added. Cells that were not treated with IFN were used as controls. The samples were incubated for 18 h at 37°C. Subsequent to washings, the cell pellets were frozen at −70°C until the time of assay. 2-5A synthetase activities of the cellular extracts were determined using poly r(I):r(C) coated agarose beads (18, 19). The results of the enzyme activities were expressed as picomoles per hour per 10^5 PBM.

Interferon assay. Human IFN titers in patients' sera were quantitated in 96-well microtiter plates by a cytopathic effect protection assay, using T98G cells (a glioblastoma cell line). In brief, 1×10^5 cells were seeded into each well and incubated with twofold serial dilutions of serum and then challenged with encephalomyocarditis virus (0.1 pfu per cell). Virus-induced cytopathic effects were assessed by microscopic examination and by staining cells with 0.1% crystal violet in 2% ethanol solutions. The IFN titer was defined as the reciprocal of the highest dilution of serum samples capable of protecting 50% of the cells from viral-induced cytopathic effects. In each assay, the reference IFNα standard (G023-901-527) from the National Institute of Allergy and Infectious Diseases (Bethesda, MD) was used for standardization. All titers are expressed as international units.

Monoclonal antibodies against IFNα (kindly provided by Dr. A. Hovanessian, Institut Pasteur) were used in neutralization experiments on sera from AIDS patients. 100 μl of serum samples was incubated with 100 μl of the monoclonal antibodies (final concentration 1 μg/ml) for 2 h at 37°C. Residual IFN activities after incubations were assayed as described above. Each assay was performed in triplicates.

Effects of AIDS sera on normal cells. 5 ml of AIDS serum were added to 10 ml of RPMI 1640 medium, supplemented with 10% FCS, containing 10×10^6 PBM, monocytes, B or T cells from normal heterosexual controls. The mixtures were incubated for 18 h at 37°C and subsequently washed with PBS. The cells were assayed for IFN receptor binding as described above. Aliquots of these cells were stored for 2-5A synthetase assay. Cells that were treated with normal serum were used as controls.

To account for the possibility that cellular IFNα receptors were occupied by the acid-labile IFNα in the patients' sera, and this was inhibiting the subsequent receptor assay with ^{125}I-IFNα2, the following experiments were performed. Subsequent to incubation at 37°C for 18 h with AIDS serum or RPMI medium containing 1,000 U/ml of IFNα2, the cells were washed with PBS at pH 5.5 for 5 min to release receptor-bound IFNα (30, 34). Cells treated with AIDS patients' sera or unlabeled IFNα2, but not acidic pH washings, were used as controls. All cells were subsequently assayed for IFNα receptor binding as described.

Statistics. The results were analyzed for statistical significance by using the two-tailed Student's *t* test.

Results

Patients

IFNα receptor expression. When PBM from heterosexual controls, ARC and AIDS patients, were incubated for 4 h at 4°C in the presence of increasing concentrations of ^{125}I-IFNα2, specific binding curves were generated (Fig. 1 *A*). These were analyzed by the LIGAND program to compute the number of receptor sites expressed on the cells. In representative experiments (Fig. 1 *A*), normal PBM showed IFNα receptor numbers of 546 sites/cell, whereas the ARC patient's PBM expressed 150 sites/cell (patient 13, Table I). Furthermore, the PBM of the AIDS patient (patient 2, Table I) did not show any detectable binding (Fig. 1 *A*). This patient was severely ill at the time of the study and subsequently died of *P. carinii* pneumonia. Data on IFNα receptor numbers from PBM freshly isolated from 17 controls, 8 ARC and 7 AIDS patients are summarized in Fig. 2. The PBM from heterosexual controls exhibited a mean of 498±247 sites per cell, whereas ARC patients exhibited a wide range of IFNα receptor sites/cell (36 to 800, with a mean of 286±235, Fig. 2 *A*). One of the ARC patients (patient 15, Table I), in fact, expressed only 36 receptor sites/cell. This finding correlates inversely with his persistently elevated levels of 2-5A synthetase and exceptionally high serum levels of acid labile IFNα. On the other hand, PBM of two other ARC patients (Table I, patients 8 and 9) exhibited IFNα receptor numbers well within normal limits, i.e., 320 and 800, respectively.

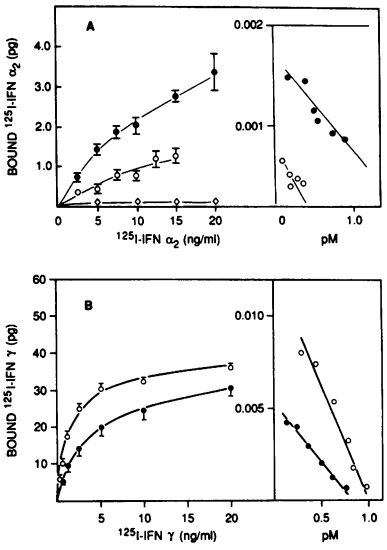

Figure 1. (*A*) In vivo reduction in IFNα receptor binding in PBM cells in AIDS patients. IFNα receptor binding assays were performed with 4×10^5 cells in a volume of 200 μl, 4 h at 4°C, with the indicated concentrations of ^{125}I-IFNα2. Linear and nonsaturable, nonspecific binding represented, typically, 50% of the total binding for PBM. Each point represents the mean of triplicate incubations for each ^{125}I-IFNα2 concentration, with error bars indicating one standard deviation about the mean. The Scatchard analysis (*inset* with ordinate as ratio of bound to free IFN ligand, and abscissa as pM bound) is illustrative of the binding curves resolved by LIGAND. These curves are representative of the data summarized in Fig. 2 *A*. ● control; ○ ARC; ◇ AIDS. (*B*) IFNγ receptor expression on PBM in ARC/AIDS patients. IFNγ receptor binding assays were performed as described in A, using ^{125}I-IFNγ4A. The binding curves are representative of the data summarized in Fig. 2 *B*. ● control; ○ ARC.

Table I. Summary of 2-5A Synthetase Levels in PBMC and Interferon α Titers in Sera of Patients with AIDS and ARC

Patients		2-5A Synthetase basal	+ IFN	Serum IFN	IFNα receptors per cell
			pM/h per 10^5 PBM	*U/ml*	
Heterosexual controls (n = 13)		8.0±4.5	46±17.8	0	
AIDS	1	18	9	256	30
	2	27	33	512	0
	3	46	92	256	147
	4	57	46	192	132
	5	31	41	64	240
	6	88	105	64	96
	7	—	—	32	0
Mean		45±25	54±37	196±167	92±88
ARC	8	4	23	0	320
	9	14	22	0	800
	10	35	89	16	405
	11	5	29	16	160
	12	2	8	8	200
	13	4	8	8	150
	14	31	40	8	220
	15	117	138	512	36
Mean		27±38	45±45	71±178	286±235

The patient population and their respective clinical status are described in Methods. 2-5A synthetase levels, IFN α titers, and IFN α receptors were assayed as described.

This is consistent with the lack of detectable circulating IFN and normal levels of 2-5A synthetase in their PBM.

In contrast, PBM from AIDS patients showed a uniform and remarkable reduction in IFNα receptor expression, i.e., with a mean of 92±88 sites/cell (n = 7). Among the three severely ill AIDS patients (patients 1, 2, and 7, Table I), two had no detectable IFN receptor binding sites and one had only 30 sites/cell. All three of these patients died of opportunistic infections within 1 wk after the receptor study.

Interestingly, we have also observed an increase in the binding affinity of PBM from patients with ARC and AIDS, when compared to controls. The dissociation constants, K_d, as determined by the LIGAND program for controls, ARC, and AIDS patients were $6.1\pm5.0 \times 10^{-10}$ M, $7.5\pm7.0 \times 10^{-11}$ M ($P < 0.02$), and $1.91\pm1.9 \times 10^{-11}$ M ($P < 0.01$), respectively. This finding is in accord with our previous observation that IFNα pretreatment of PBM from normal individuals increases the subsequent binding affinity of IFNα receptors on the cell surface (30). On the basis of these findings, it is apparent that IFNα receptors could exist in different affinity states (30, 35), with a high affinity component exhibiting a K_d of $(1 - 10) \times 10^{-11}$ M, an intermediate affinity component exhibiting a K_d of $(1 - 10) \times 10^{-10}$ M, and a low affinity component exhibiting a K_d of $(1 - 10) \times 10^{-9}$ M (34). It appears that the existence of different affinity states of IFNα receptor depends on the cell line (35), presence of extracellular IFNα (30), and proliferative capacity of the cells (35). The K_d is practically defined as the concentration of ligand (IFN) that is required to saturate 50% of the receptor sites on the cell surface.

IFNα receptors in mononuclear subsets. The expression of IFNα receptors on peripheral blood mononuclear subsets from normal controls was determined (Table II). They exhibited a K_d of 1.6×10^{-10} M to 9.8×10^{-10} M, similar to that of the PBM from normal controls.

IFNγ receptor expression. Specific binding curves for IFNγ receptors were generated on PBM freshly isolated from heterosexual controls, ARC, and AIDS patients, in a manner similar to that of IFNα receptor assay. In representative experiments (Fig. 1 *B*), normal PBM revealed IFNγ receptor numbers of 2,800±250 sites/cell with a K_d of 1.80×10^{-10} M, while the ARC patient's PBM expressed 2940±180 sites/cell with a K_d of 7.1×10^{-11} M. Patient 15 (Table I) had high serum levels

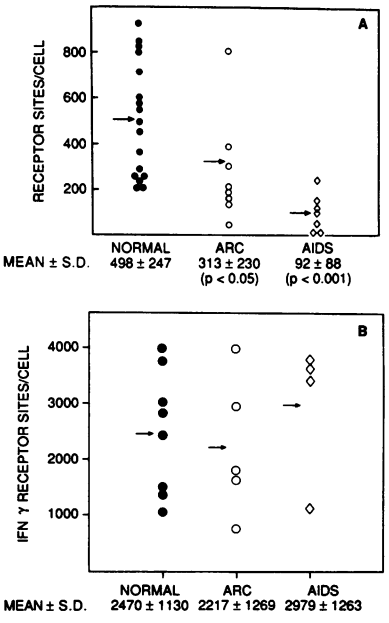

Figure 2. (*A*) In vivo downregulation of IFNα receptor expression in ARC and AIDS. ARC receptors significantly less than controls at $P < 0.05$ level and AIDS at $P < 0.001$. (*B*) In vivo expression of IFNγ receptors in AIDS/ARC. Arrow indicates the mean. *Refer to Fig. 1 for experimental details and Scatchard analysis. ● control; ○ ARC; ◇ AIDS.

Table II. Regulation of IFNα Receptor Expression on Normal Blood Mononuclear Subsets by AIDS Sera

Cell type	Total PBM	IFNα receptors per cell		
		Monocyte	B cell	T cell
Basal	607±66	1,540±460	390±120	410±100
+AIDS sera	190±40	354±95	144±29	160±53
	(n = 4)	(*n* = 3)	(*n* = 4)	(*n* = 3)

PBMC from normal heterosexuals were separated into its corresponding subsets, i.e., monocytes, B and T cells as described in Methods. After treatment with AIDS sera as described in text, the cells were assayed for IFNα receptor bindings as outlined in Fig. 1.

of the acid-labile IFNα subtype and persistent elevation of 2-5A synthetase activity, and his PBM expressed only 36 IFNα receptor sites/cell. As summarized in Fig. 2 *B*, the PBM from controls, ARC, and AIDS patients exhibited a mean of 2,470±1,130 (*n* = 8), 2,220±1,270 (*n* = 5), and 2,980±1,260 (*n* = 4) IFNγ receptor sites/cell, respectively. Despite the apparent differences in binding affinity between the ARC patient and the control (Fig. 1 *B*), there was no significant changes in K_d of the IFNγ receptors on PBM isolated from ARC/AIDS patients relative to heterosexual controls (K_d = 1.75±0.80 × 10^{-10} M), when all the patients were taken into account (*n* = 9). Therefore, in contrast to the finding of reduction in IFNα receptor expression on PBM in AIDS, IFNγ receptor expression in both ARC and AIDS patients are normal when compared to that of the heterosexual controls.

2-5A synthetase activities

The levels of 2-5A synthetase activity were measured in the cellular extracts of PBM from these patients with ARC or AIDS as well as healthy heterosexual controls. In freshly isolated PBM, the basal 2-5A synthetase levels for controls, ARC, and AIDS patients were 12±6.2 (*n* = 13), 36±49 (*n* = 8), and 80±67 (*n* = 6) pmol/h per 10^5 PBM, respectively (data not shown). After overnight incubation without IFNα, the respective 2-5A synthetase levels decreased to 8.0±4.5, 27±38 and 45±25 pmol/h per 10^5 PBM (Table I). All the AIDS patients had persistent, significantly elevated levels of 2-5A synthetase ($P < 0.001$), consistent with earlier reports (18, 36). However, in ARC patients, the enzymatic activities were more variable; they ranged from normal to a > 10-fold increase in basal activity in one case (patient 15, Table I). This patient (No. 15) is particularly interesting. He is a 34-yr-old homosexual male with persistent lymphadenopathy in the absence of other symptoms. He had essentially a normal immunologic workup with the exception of a slightly depressed helper/suppressor (T4/T8) ratio of 1.3. Over a period of 9 mo, he has had persistently elevated levels of 2-5A synthetase (results not shown) without evidence of apparent clinical opportunistic disease.

2-5A synthetase responses of PBM to IFNα treatment in vitro were also measured. PBM from normal heterosexual controls had a sixfold increase in 2-5A synthetase activity after treatment with IFNα (Table I). In contrast, the PBM of AIDS patients had minimal in vitro responses to IFNα treatment, with an average increase of 20% from that of the AIDS basal levels. This finding is consistent with that of Preble et al. (36) and ours (19). Once again, the in vitro PBM responses of the ARC patients were more variable with an average increase of 67%.

Serum interferon titers

Normal individuals, in the absence of viral infections or autoimmune diseases, do not have detectable levels of IFN in the serum (16). However, consistent with previous reports (16, 17), the ARC and AIDS patients studies here had elevated serum levels of IFNα (Table I).

The IFN activity in the AIDS sera could be neutralized by monoclonal antibodies to IFNα. Preincubation of serum samples with 1 μg/ml of affinity-purified anti-IFNα monoclonal antibody, before the antiviral assay, resulted in complete neutralization or at least eightfold reductions in IFN activity (data not shown).

Downregulation of IFNα receptors by AIDS sera

In a representative experiment, normal PBM, when preincubated in vitro with AIDS serum for 18 h at 37°C, showed a marked and reproducible reduction in subsequent receptor expression from a level of 594 sites/cell to 178 sites/cell (Fig. 3 *A*). The final concentration of the acid-labile IFNα in the incubation medium was 170 U/ml. Data from four AIDS patients' sera on PBM and blood mononuclear subsets (monocytes, B and T cells) isolated from heterosexual controls showed the same phenomenon of downregulation of IFNα receptors (Table II). This phenomenon of downregulation of IFNα receptor expression on normal PBM can also be induced by pretreating the cells with unlabeled recombinant $IFN\alpha_2$ (30).

2-5A synthetase activities were determined simultaneously on normal PBM that were pretreated with AIDS sera; the enzyme level increased to 46.4±17 pmol/h per 10^5 cells (*n* = 4) from that of 8.8±3.9 pmol/h per 10^5 cells (*n* = 4, $P < 0.01$).

The possibility that receptors at the cell surface might already be occupied by acid-labile IFNα, thereby inhibiting the assay for IFNα receptor expression, had to be considered. Therefore PBM, after pretreatment with AIDS sera or unlabeled IFNα2, were washed with acidic (pH 5.5) phosphate

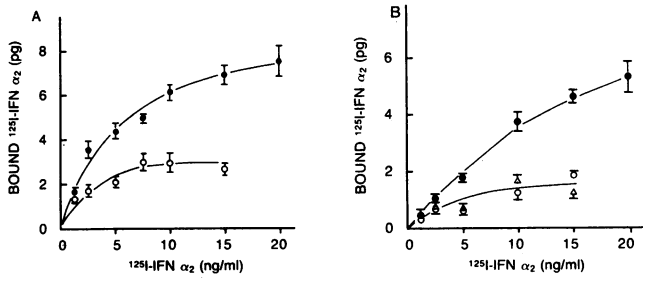

Figure 3. Reduction in IFNα receptor expression on normal cells by AIDS sera. (*A*) PBM from healthy heterosexual individuals were incubated with serum from AIDS patients and were subsequently assayed for IFN receptor binding and 2-5A synthetase activities. Controls were normal PBM treated with normal sera. ● control PBM (*n* = 4); receptor, 607±66 sites/cell; synthetase 8.8±3.9 pmol/h per 10^5 PBM. ○ treated PBM (*n* = 4); receptor, 190±40 sites/cell ($P < 0.001$); synthetase, 46±17 pmol/h per 10^5 PBM ($P < 0.01$). (*B*) Normal PBM were treated with AIDS serum. Cells were subsequently washed with PBS (pH 7.4) or PBS titrated to acidic pH (5.5). IFN receptor bindings were performed. ● control; ○ AIDS sera; △ AIDS serum plus acidic pH wash.

buffered saline before ^{125}I-IFNα2 receptor binding assays. This acid wash procedure ensured that prebound IFN was released from cellular receptors (30, 34). The results (Fig. 3 *B*) essentially showed identical binding isotherms and receptor sites, as resolved by the LIGAND program, obviating the involvement of prior receptor occupancy in the observed downregulation.

Discussion

On the basis of the viral etiology of AIDS and its associated immune dysregulation, investigators have attempted to explore the potential use of immunomodulators as therapeutic agents. In vitro studies have shown that human recombinant IL-2 partially reconstitutes the deficient immune functions of natural killer cells and augments proliferative responses of PBM from AIDS patients to mitogens and alloantigens (37). A recent report showed that overnight pretreatment of blood leukocytes in vitro with IFNα_A before HIV virus challenge significantly reduced reverse transcriptase activity, expression of viral antigens, and virus yield (38). Therefore, IFNs are particularly interesting because of their combined immunoregulatory, antiviral and antineoplastic effects. As IFNs elicit these cellular functions in target cells by binding to specific high affinity receptors, knowledge of IFN receptor expression in vivo in AIDS patients could be crucial for directing the therapeutic use of IFN.

Recent clinical trials with IFNα in AIDS patients with Kaposi's sarcoma have yielded variable results with 20–40% of patients showing significant tumor regression, including complete remission of the tumor in some patients (22–24). Reasons underlying this discrepancy in therapeutic responses are not known. On the basis of our previous findings of downregulation of IFNα receptors by prolonged IFNα treatment and thus rendering cells hyporesponsive to further IFN action (30), we postulated that this discrepancy in therapeutic efficacy of IFNα in Kaposi's sarcoma may be due to a similar mechanism in vivo by the endogenously produced acid-labile IFNα in AIDS patients. Accordingly, AIDS patients who had persistently elevated levels of endogenous IFNα before therapy were more likely to have tumor progression and not respond to treatment with exogenous IFNα (23). We therefore studied the IFNα receptor expression on PBM freshly isolated from AIDS patients. In this study, we have demonstrated a progressive reduction in IFNα receptor expression on PBM during the disease progression of AIDS, i.e., a significant decrease in IFNα receptor binding in ARC, and more dramatically in AIDS patients (Figs. 1 *A* and 2 *A*). The reduced binding represents downregulation of cell surface IFNα receptors in vivo. This phenomenon of downregulation is most likely due to the elevated serum levels of IFNα in these patients. Moreover, we have shown that normal PBM and blood mononuclear subsets, when treated in vitro with AIDS sera containing the endogenous IFNα, showed both a reduction in IFNα receptor binding, and a marked induction of 2-5A synthetase activities (Fig. 3 *A*, Tables I and II). This is in accord with our recent findings that IFNα induces reduction of its own receptor's expression in normal PBM both in vitro and during IFN therapy in vivo (30). Previous in vitro studies on tumor cells such as T98G and Daudi (a lymphoblastoid line) also indicated that IFN regulates its own receptor expression (34, 39, 40). It, therefore, appears that the downregulation of IFN receptor expression by IFN itself is a general phenomenon in cellular metabolism.

Concurrent with the elevated serum levels of endogenous IFNα and reduction of IFNα receptor expression in vivo are the elevated basal levels of the IFN-induced enzyme, 2-5A synthetase (Table I). PBM from AIDS and ARC patients showed minimal responses to IFNα in vitro as measured by induction of 2-5A synthetase activity, in contrast to the typical responses seen in PBM from normal heterosexual controls. Our results are consistent with earlier reports of hyporesponsiveness of some AIDS patients to IFNα therapy (23, 36). Our observation may assist in understanding the clinical failure of IFNα therapy in some cases of AIDS. In these cases, the failure of IFN therapy may be due to decreased numbers of IFNα receptors expressed on the cell surface, as a consequence of continuous in vivo exposure to endogenous IFNα (Figs. 1 *A*, 2 *A*). In addition, it is possible that the abnormal presence of endogenous IFNα may be involved in the pathogenesis of some immune disturbances in AIDS patients, such as suppressed natural killer cell activities, deficient proliferative responses to mitogens, or possible feedback inhibition of lymphokine production including IL-2 and IFNγ (3, 8, 9, 13, 14).

The documented deficiency of IFNγ production in AIDS may account for many of the immune disturbances. IFNγ is a T cell derived glycoprotein with a pivotal role in the regulation of the immune system (41–43). Recent in vitro studies on monocytes from AIDS patients showed that IFNγ improves antimicrobial function (13), and induces expression of HLA-DR antigen to a near normal level (41). Therefore, IFNγ has been suggested as a possible candidate to be used in attempts to provide immune reconstitution of AIDS patients.

To formulate a rationale for selecting patients for IFNγ therapy, we studied the IFNγ receptor expression in AIDS/ARC patients. Our results showed that the specific binding of ^{125}I-IFNγ to PBM from ARC and AIDS patients remained normal in comparison to heterosexual controls. This indicates that IFNγ receptors are expressed on the cell surface, and are available for IFNγ binding and mediation of IFNγ effects. Therefore, it appears that there is a downregulation of IFNα but not IFNγ receptor expression in vivo in AIDS/ARC patients. These observations are striking and provide in vivo evidence that IFNα/β and IFNγ do not share the same receptor sites, and the expression of IFNα and γ receptors is being regulated independently.

The phenomenon of in vivo downregulation of IFNα receptor expression and consequent hyporesponsiveness to IFNα treatment in PBM from AIDS patients is a unique observation with implications for the use of IFNα in therapeutic regimens. It raises the question of the usefulness of IFNα therapy in end-stage AIDS, but also suggests that the IFNα receptor assay may be particularly useful in directing IFNα therapy in ARC. Moreover, the normal levels of IFNγ receptor expression on PBM from these patients suggest that IFNγ may be useful in attempts to provide immune reconstitution in AIDS, perhaps in combination with IFNα.

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