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Research Article

To test the hypothesis that deficient interleukin 2 (IL-2) secretion may underlie the impaired capacity of T cells from patients with Acquired Immunodeficiency Syndrome (AIDS) and the AIDS-related complex (ARC) to generate the macrophage-activating lymphokine, gamma interferon (IFN-gamma), we used five specific microbial antigens to examine IL-2 production. Mononuclear cells from only one of 32 (3%) AIDS patients secreted normal levels of IL-2, and 21 (66%) failed to produce any detectable IL-2. For 36 ARC patients, IL-2 generation was normal in nine (25%) and absent in 11 (31%). Given these results, recombinant (r) IL-2 was tested for its capacity to stimulate or enhance IFN-gamma production. rIL-2 (10 U/ml) alone stimulated cells from controls, ARC, and AIDS patients to secrete 93 +/- 25, 99 +/- 33, and 7 +/- 3 U/ml of IFN-gamma, respectively. rIL 2 (10 U/ml) plus antigen induced no change in mean IFN-gamma levels for controls, a 4.4-fold increase for 17 AIDS patients (16 +/- 16 vs. 71 +/- 21 U/ml), and a 7.2-fold increase (18 +/- 5 vs. 130 +/- 27 U/ml) for 19 ARC patients with abnormal IFN-gamma generation to antigen alone. Individual responses indicated that six of the 17 (35%) AIDS patients with opportunistic infections and 12 of the 19 (63%) with ARC were apparent responders to 10-100 U/ml of rIL-2. These results (a) document […]

Production of and In Vitro Response to Interleukin 2 in the Acquired Immunodeficiency Syndrome

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Abstract

To test the hypothesis that deficient interleukin 2 (IL-2) secretion may underlie the impaired capacity of T cells from patients with Acquired Immunodeficiency Syndrome (AIDS) and the AIDS-related complex (ARC) to generate the macrophage-activating lymphokine, gamma interferon (IFN-γ), we used five specific microbial antigens to examine IL-2 production. Mononuclear cells from only one of 32 (3%) AIDS patients secreted normal levels of IL-2, and 21 (66%) failed to produce any detectable IL-2. For 36 ARC patients, IL-2 generation was normal in nine (25%) and absent in 11 (31%). Given these results, recombinant (r) IL-2 was tested for its capacity to stimulate or enhance IFN-γ production. rIL-2 (10 U/ml) alone stimulated cells from controls, ARC, and AIDS patients to secrete 93±25, 99±33, and 7±3 U/ml of IFN-γ, respectively. rIL 2 (10 U/ml) plus antigen induced no change in mean IFN-γ levels for controls, a 4.4-fold increase for 17 AIDS patients (16±16 vs. 71±21 U/ml), and a 7.2-fold increase (18±5 vs. 130±27 U/ml) for 19 ARC patients with abnormal IFN-γ generation to antigen alone. Individual responses indicated that six of the 17 (35%) AIDS patients with opportunistic infections and 12 of the 19 (63%) with ARC were apparent responders to 10–100 U/ml of rIL-2. These results (*a*) document profound impairment in antigen-induced IL-2 secretion by AIDS and ARC T cells, (*b*) indicate that, in vitro, mononuclear cells from certain patients can respond to rIL-2 with enhanced IFN-γ production, and thus (*c*) suggest that in selected patients rIL-2 might have a potentially beneficial therapeutic (AIDS) or prophylactic (ARC) effect against opportunistic infections.

Introduction

Although T cells from patients with the acquired immunodeficiency syndrome (AIDS)[1] display a variety of defects in vitro (1), the failure to properly secrete gamma interferon (IFN-γ) in response to specific microbial antigen seems particularly relevant to the immunopathogenesis of AIDS-related opportunistic infections (2).[2] In addition to its well-recognized antiviral effects (3), for example, IFN-γ also appears to play a pivotal role in a number of antigen-initiated cellular immune reactions (reviewed in reference 4), including the capacity to induce macrophages and other potential host defense cells to kill or inhibit both intra- and extracellular pathogens (5–13). While interleukin 2 (IL-2), another soluble product (lymphokine) secreted by sensitized T cells (14–16), does not share IFN-γ's ability to activate macrophages directly (6), it nevertheless has been of considerable interest in AIDS research (17–23) because of its broad immunoregulatory effects, which include the stimulation and perhaps regulation of IFN-γ production (24–29). Since deficient IL-2 secretion may underlie the failure of AIDS T cells to generate IFN-γ, thereby contributing to susceptibility to various opportunistic infections (2), we examined the capacity of peripheral blood mononuclear cells from patients with both fully established AIDS and its prodrome (AIDS-related complex [ARC]) to produce IL-2 in response to a diverse group of microbial antigens. Because IL-2 is now available in recombinant (r) form as a potential immunotherapeutic agent, we also determined whether IL-2 can by itself either induce these patients' cells to secrete IFN-γ or enhance IFN-γ production in response to specific antigen.

Methods

Patients. The patients studied were homosexual men with abnormal $T4^+/T8^+$ cell ratios (<0.84) (2) who had either (*a*) full-blown AIDS (Kaposi's sarcoma or opportunistic infections) (2) or (*b*) ARC manifested by unexplained generalized lymphadenopathy with or without oral candidiasis and/or systemic constitutional symptoms (30).[2] 93% of the ARC patients studied were seropositive to the retrovirus, HTLV-III (31), in a spectrophotometric ELISA assay performed by Dr. Jacob C. Holper (Litton Bionetics, Charleston, SC). Healthy heterosexual laboratory personnel served as controls.

Production of IL-2 and IFN-γ. Ficoll-Hypaque–separated peripheral blood mononuclear cells were prepared as described (2), and suspended in RPMI 1640 medium containing 15% heat-inactivated heterologous normal human serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) (2). Cells were cultivated at 3×10^6/ml in 35-mm plastic tissue culture dishes at 37°C in 5% CO_2–95% air with the following optimal concentrations of specific antigens: (*a*) *Toxoplasma gondii* lysate, 50 μg/ml, for patients with a Sabin-Feldman dye test titer of ≥1:64 (2); (*b*) cytomegalovirus (CMV) (MA Bioproducts, Walkersville, MD), 1:50 dilution, for patients with CMV serum complement fixation titers ≥1:8; (*c*) *Herpes simplex* (hominis) virus (HSV) (MA Bioproducts), 1:50 dilution, for patients with serum titers ≥1:8; (*d*) *Candida albicans* sonicate, 150 μg/ml, for those with positive *Candida* skin tests or oral candidiasis (2); and (*e*) tetanus toxoid (Massachusetts Biologic Laboratory, Boston, MA), 4 μg/ml, for patients with a positive skin test or a history of previous immunization. After 48 h, the culture supernatants were removed, sterilized by filtration, and stored at 4°C. As described elsewhere (32), supernatant IL-2 activity was determined using IL-2–dependent CTLL-1 cells (33) and was measured in parallel with a laboratory standard provided by the National Institutes of Health (NIH) Biological Response Modifiers Program. IL-2 activity is expressed as NIH units. The IFN-γ activity of supernatants from cells stimulated with rIL-2 or *T. gondii* or CMV antigen was measured in a standard cytopathic effect–inhibition bioassay that

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1. *Abbreviations used in this paper:* AIDS, acquired immunodeficiency syndrome; ARC, AIDS-related complex; CMV, cytomegalovirus; HSV, herpes simplex virus; IFN-γ, gamma interferon; IL-2, interleukin 2; r, recombinant.

2. Murray, H. W., J. K. Hillman, B. Y. Rubin, C. D. Kelly, J. L. Jacobs, L. W. Tyler, D. M. Donelly, S. M. Carriero, J. H. Godbold, and R. B. Roberts, manuscript submitted for publication.

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employed WISH cells and vesicular stomatitis virus (34). For supernatants from cell cultures stimulated by rIL-2 alone or *T. gondii* antigen, antiviral activity was completely neutralized by a monoclonal anti-human IFN-γ antibody (2, 5, 7, 34), which does not neutralize either IFN-α or -β (34), indicating the presence of IFN-γ only. For CMV antigen–stimulated supernatants, this antibody did not consistently neutralize all antiviral activity; therefore, we employed a radioimmunoassay test kit (Centocor, Malvern, PA) that specifically measures IFN-γ (28, 35) for the experiments in which CMV antigen was used as the T cell stimulus. In both the bioassay and the radioimmunoassay, IFN-γ activity was measured in parallel with an NIH laboratory standard (Biological Response Modifiers Program), and activity is expressed as NIH IFN-γ units. For the *T. gondii* antigen–induced[2] and the rIL-2–stimulated supernatants, comparable results were obtained for IFN-γ activity in both the radioimmunoassay and the bioassay. In addition, since IFN-γ generation in response to CMV and *T. gondii* antigens is similar in our laboratory for healthy seropositive controls (CMV, 773±140 U/ml [12 subjects, 37 experiments]; *T. gondii*, 986±149 U/ml [7 subjects, 21 experiments]),[2] the results for CMV and *T. gondii* antigen–induced IFN-γ have been grouped together and are presented as "antigen."

In separate experiments (not shown) using the five antigens listed above with cells from healthy controls who either (*a*) were seronegative to *T. gondii* (7), CMV, or HSV, (*b*) had nonreactive *Candida* skin tests, or (*c*) had never received tetanus toxoid, the culture supernatants did not contain detectable IL-2 (<0.1 U/ml) or IFN-γ (<10 U/ml). These results indicated the specificity of the antigen-induced responses observed in the "positive" controls shown in the tables.

rIL-2. Escherichia coli–derived human rIL-2, obtained from Dr. Michael Palladino (Genentech, Inc., S. San Francisco, CA), was provided as material with a titer of 1×10^6 U/ml and a specific activity of 1×10^7 U/mg (36). Using our laboratory standard and IL-2 assay (32), this preparation also contained 1×10^6 U/ml. To stimulate IFN-γ production, we added 1–100 U/ml of rIL-2 for 48 h to mononuclear cell cultures either by itself or with CMV or *T. gondii* antigen.

T Lymphocyte subset enumeration. Peripheral blood helper and suppressor T cells were identified using monoclonal antibodies to OKT4 and OKT8 in a standard indirect immunofluorescent assay, as previously described (2).

Results

Production of IL-2. Peripheral blood mononuclear cells from healthy controls readily generated IL-2 in response to stimulation with each of the five specific microbial antigens employed (Table I). In contrast, IL-2 production by cells from AIDS patients was profoundly impaired irrespective of clinical manifestations (Kaposi's sarcoma or opportunistic infections) or the antigen tested. Overall, cells from only one of 32 (3%) AIDS patients showed a normal response to any antigen, and 21 (66%) failed to secrete any detectable IL-2 (<0.1 U/ml). In eight of the latter subjects, extending the incubation period with antigen to 72 h did not alter these results (not shown). As also indicated in Table I, defective antigen-induced IL-2 production was not limited to patients with fully established AIDS. Although the mean IL-2–generating responses of cells from ARC patients were five- to 68-fold higher than those of AIDS patients (depending upon the stimulus tested), cells from only nine of 36 (25%) ARC patients produced normal levels of IL-2 in response to any antigen, and 11 (31%) secreted undetectable amounts.

IFN-γ production in response to IL-2. Since IL-2 is believed to play a role in the production of IFN-γ by T cells (24–29), the preceding results suggested that deficient IL-2 secretion might explain the impaired generation of IFN-γ documented in previous studies of both AIDS and ARC patients (2).[2] Therefore, we next examined whether IL-2 could by itself or with antigen either induce or enhance IFN-γ secretion. As shown in Table II, rIL-2 alone stimulated cells from controls to produce IFN-γ in a dose-dependent fashion. The IFN-γ–generating responses of controls to rIL-2 were, however, highly variable, and ranged from <10 U/ml (in two of the 10 controls) to 300 U/ml and from 10 to 1,000 U/ml after stimulation with 10 and 100 U/ml of rIL-2, respectively. 10 U/ml of rIL-2 also induced IFN-γ secretion by cells from 19 of 35 ARC patients (range, 10 to 1,000 U/ml), and the mean IFN-γ level for this group was similar to controls. For AIDS patients, only three of the 12 tested responded to 10 U/ml of rIL-2 with detectable IFN-γ generation (range, 10 to 30 U/ml); and as a group, AIDS patients' cells secreted 13- to 14-fold less IFN-γ than either controls or ARC patients. In response to 100 U/ml of rIL-2, IFN-γ production increased slightly for AIDS patients but declined somewhat for ARC patients, in contrast to the augmented activity displayed by control cells (Table II).

Table I. Antigen-induced IL-2 Production

		AIDS patients			
	Healthy controls	Total group	KS	OI	ARC patients
No. studied	19	32	11	21	36
IL-2 (U/ml)					
T. gondii	33.9±2.7 (15)	0.16±0.11 (9)	0.50±0.5 (2)	0.06±0.04 (7)	9.3±4.7 (12)
HSV	9.9±1.7 (9)	0.50±0.40 (9)	0.90±0.1 (5)	0 (4)	4.1±1.4 (12)
CMV	3.6±0.5 (7)	0.04±0.04 (11)	0.08±0.08 (5)	0 (6)	0.18±0.08 (12)
Candida	20.2±4.2 (9)	0.06±0.07 (13)	0 (3)	0.08±0.08 (10)	4.1±2.0 (11)
Tetanus	11.9±1.8 (13)	0.06±0.03 (14)	0.03±0.03 (7)	0.10±0.05 (7)	1.6±1.0 (17)
Peripheral blood T cells/mm^3					
T4$^+$	853±53	90±22	186±46	49±7	267±28
T8$^+$	579±45	358±51	528±78	276±40	715±99
T4/T8 ratio	1.6±0.1	0.29±0.05	0.32±0.07	0.26±0.06	0.37±0.03

Mononuclear cells (3×10^6/ml) were stimulated for 48 h with the indicated specific antigens (see Methods). Results for AIDS (KS, Kaposi's sarcoma; OI, opportunistic infections) and ARC patients are the means±SEM of (*n*) patients studied. For controls, the results indicate the means±SEM of (*n*) experiments using cells from 3–8 separate donors for each antigen. For purposes of calculating mean values, undetectable IL-2 levels (<0.1 U/ml) were assumed to equal 0 U/ml.

Table II. IFN-γ Generation Induced by rIL-2

Stimulus	IFN-γ production		
	Control	ARC	AIDS
U/ml	*U/ml*	*U/ml*	*U/ml*
rIL-2: 1	0 (11)	14±8 (7)	2±2 (7)
10	93±25 (29)	99±33 (35)	7±3 (12)
100	355±64 (29)	63±16 (35)	25±12 (12)

Mononuclear cells (3×10^6/ml) were stimulated for 48 h with 1–100 U/ml of rIL-2 alone. Results for ARC and AIDS indicate the mean±SEM of (*n*) patients studied, and for controls, indicate (*n*) experiments using cells from 10 healthy donors. For calculating mean values, undetectable IFN-γ levels (<10 U/ml) were assumed to equal 0 U/ml. $T4^+$ cells/mm^3, $T8^+$ cells/mm^3, and T4/T8 cell ratios were as follows (means±SEM): (*a*) controls, 853±53, 579±45, and 1.6±0.1; (*b*) ARC patients, 348±42, 901±88, and 0.38±0.03; and (*c*) AIDS patients (all of whom had opportunistic infections), 42±10, 312±40, and 0.18±0.05.

Responses to rIL-2 plus antigen. In a second set of experiments, we examined the capacity of rIL-2 to enhance IFN-γ secretion by acting in concert with specific antigen. Enhanced responses were arbitrarily defined as either (*a*) an increase to ≥100 U/ml, the lower limit of normal IFN γ-generating responses to either CMV or *T. gondii* antigens by seropositive healthy controls (19 subjects, 58 experiments [2])[2] or (*b*) a ≥3-fold increase in IFN-γ production to at least 50 U/ml (e.g., 50% of the lower limit of normal). As judged by mean values, IFN-γ production by controls was maximally stimulated by antigen alone (Table III); however, in four of 21 experiments, 10 U/ml of rIL-2 did induce ≥3-fold increases (range, three- to sevenfold) in IFN-γ levels. For AIDS patients, mean antigen-induced IFN-γ secretion increased by 4.4-fold (16±6 to 71±21 U/ml) in the presence of rIL-2, and four of 17 patients were designated as apparent responders to 10 U/ml of rIL-2 (Table IV). In contrast, as a group, the 41 ARC patients showed no overall enhancement with rIL-2 (Table III); however, cells from 22 of the 41 already generated normal levels of IFN-γ (≥100 U/ml) in response to antigen alone. Upon separating the ARC patients according to their responses to antigen (Table III), it was apparent that the 19 patients with abnormal (<100 U/ml) antigen-induced IFN-γ production were, however, responsive to rIL-2 and showed a 7.2-fold increase (18±5 to 130±27 U/ml) in mean IFN-γ generation. Eight of the 19 ARC patients were classified as apparent responders to rIL-2 (Table IV).

Increasing the concentration of rIL-2 to 100 U/ml did not appreciably augment mean antigen-induced IFN-γ levels for either controls ($n = 7$) or AIDS ($n = 8$) or ARC patients ($n = 34$) when compared with responses to antigen alone (not shown). However, as also shown in Table IV, six additional patients (AIDS, 2; ARC, 4), none of whom displayed enhanced responses to 10 U/ml of rIL-2, were designated by our criteria as responders to 100 U/ml of rIL-2. Thus, for patients whose cells failed to produce normal levels of IFN-γ in response to CMV or *T. gondii* antigen alone, six of 17 (35%) with AIDS and 12 of 19 (63%) with ARC demonstrated augmented IFN-γ generation in the presence of antigen plus 10–100 U/ml of rIL-2.

Correlation with numbers of $T4^+$ and $T8^+$ cells. Since $T4^+$ and $T8^+$ cells can both produce IL-2 and secrete IFN-γ in response to IL-2 (37–42), these peripheral blood T lymphocyte subsets were enumerated for the AIDS and ARC patients studied. As shown in each of the tables, AIDS patients (especially those with opportunistic infections) had strikingly low numbers of $T4^+$ cells and reduced numbers of $T8^+$ cells as well; ARC patients had considerably more cells of each subset. These data suggested that the differences between AIDS and ARC patients observed for both IL-2 production (Table I) and IFN-γ secretion in response to rIL-2 alone (Table II) may be explained by deficient numbers of responding cells. The results in Table IV, however, suggested that in individual patients, enhanced IFN-γ production induced by antigen plus rIL-2 may not be dependent upon the absolute numbers of $T4^+$ and/or $T8^+$ cells.

Discussion

These results extend our previous analysis of impaired lymphokine production in AIDS by demonstrating that mononuclear cells from patients with both the fully established syndrome and

Table III. IFN-γ Responses Induced by Antigen Plus rIL-2

Group	No. studied	IFN-γ production			Cells/mm^3		Ratio
		Antigen*	rIL-2	rIL-2 + antigen	$T4^+$	$T8^+$	T4/T8
		U/ml	*U/ml*	*U/ml*			
Healthy controls	10	671±121	85±23	825±154	853±53	579±45	1.6±0.1
AIDS							
Total group‡	17	16±6	13±7	71±21	53±9	289±42	0.23±0.06
ARC							
Total group	41	275±58	94±27	311±81	329±38	901±80	0.41±0.04
≥100 U/ml§	22	496±81	102±46	467±96	364±53	864±106	0.44±0.04
<100 U/ml§	19	18±5	80±20	130±27	288±61	928±169	0.34±0.08

Mononuclear cells (3×10^6/ml) were stimulated with either CMV or *T. gondii* antigen, rIL-2 (10 U/ml), or antigen plus rIL-2 for 48 h. Results for AIDS and ARC indicate the means±SEM of the number of patients studied, and for controls indicate the means±SEM of 21 experiments using cells from 10 healthy donors. The 17 AIDS patients had opportunistic infections. * Antigens used for the three groups were: healthy controls (*T. gondii*, 7; CMV, 14), AIDS (*T. gondii*, 4; CMV, 13), and ARC (*T. gondii*, 7; CMV, 34). ‡ Cells from all 17 AIDS patients produced <100 U/ml of IFN-γ in response to antigen alone. § ARC patients divided into two subgroups according to normal (≥100 U/ml) or abnormal (<100 U/ml) IFN-γ production in response to antigen alone. Cells from all healthy seropositive controls generated ≥100 U/ml in repsonse to CMV or *T. gondii* antigens (2).[2]

Table IV. Individual Patient Responses to Antigen Plus rIL-2

Patients	Antigen used	IFN-γ production			Cells/mm³		Ratio
		Antigen	IL-2	Antigen + IL-2	T4⁺	T8⁺	T4/T8
AIDS No. 1	TOXO	<10	20	60	78	149	0.52
2	TOXO	<10	100	100	86	417	0.21
3	CMV	45	<10	190	11	304	0.04
4	CMV	52	44	256	23	561	0.04
5	CMV	75	<10	450	—‡	—	—
6	CMV	<10	78	135	42	286	0.14
ARC No. 1	CMV	<10	100	100	95	532	0.18
2	TOXO	<10	30	100	131	255	0.51
3	CMV	<10	235	430	374	1,016	0.37
4	TOXO	10	200	300	526	637	0.82
5	TOXO	30	30	600	124	239	0.52
6	TOXO	30	100	100	268	807	0.33
7	CMV	45	82	225	683	1,682	0.37
8	CMV	76	60	150	372	882	0.42
9	TOXO	<10	18	72	153	2,616	0.05
10	CMV	<10	<10	74	592	1,065	0.56
11	CMV	12	45	230	147	661	0.22
12	CMV	23	<10	75	301	772	0.42

Antigens used were either CMV or *T. gondii* (TOXO), to which cells from healthy seropositive controls (n = 19, 58 experiments) respond with ≥100 U/ml of IFN-γ (2).[2] rIL-2 was used at 10 U/ml, except where indicated. The AIDS patients had opportunistic infections. * Responses to 100 U/ml or rIL-2. ‡ This symbol, —, indicates not done.

its prodrome (ARC) not only fail to properly secrete IFN-γ in response to specific microbial antigen (2),[2] but also display a similar and striking defect in IL-2 generation as well. Since IL-2 may be a primary, naturally occurring stimulus for IFN-γ production triggered during antigen-induced T cell activation (24–29), these findings logically raise the possibility that defective IL-2 secretion may largely contribute to or be responsible for the impaired IFN-γ generation previously documented in both AIDS and ARC patients (2).[2]

IL-2 secretion by mononuclear cells from these two patient populations has also been examined in two previous studies (18, 20). In one (18), IL-2 production was impaired, while in the other (20) it was intact. In both studies, however, various mitogens were employed as T cell triggering agents. Since proliferative activity and IFN-γ generation in response to nonspecific mitogen stimulation are not infrequently preserved in AIDS and ARC patients (2)[2] (and thus may not be helpful in identifying T cell dysfunction), we elected to use specific antigens, most of which were derived from pathogens that infect these patients, as presumably more relevant stimuli for in vitro T cell testing. As demonstrated here, IL-2 production in response to a diverse group of antigens was uniformly impaired (97%) in patients with AIDS and, in fact, absent in two-thirds. In addition, absent IL-2 secretion was found in one-third of the ARC patients studied, indicating that a potentially key T cell defect, which is likely to be related to host susceptibility to opportunistic infections (2, 4), can also be identified in individuals who have not yet developed but are at high risk for AIDS (30).[2]

Given the availability of IL-2 as a potential immunotherapeutic agent (43–46), other investigators have examined the effects of various IL-2 preparations on AIDS and ARC cellular activities in vitro, and in certain patients, have demonstrated enhancement in natural killer cell activity, T cell cytotoxicity, proliferation to mitogen, and T cell colony formation (17–19, 21). In addition, Rook et al. (23) have recently reported that although cells from only two of nine AIDS patients responded to partially purified native IL-2 (100 U/ml) with detectable IFN-γ release, the presence of IL-2 nevertheless appreciably increased mitogen-induced IFN-γ generation in eight of the nine with an eightfold increase in mean IFN-γ levels. Not surprisingly in these studies (17–19, 21, 22), cells from less lymphopenic and less immune-deficient ARC patients have typically been most responsive to IL-2, while AIDS cells have shown highly variable and, for the most part, only partial responses. Our results, as judged by antigen-induced IFN-γ production in the presence of rIL-2, also showed a similar heterogeneous response. It remains to be seen whether this latter finding reflects deficient numbers or function of IFN-γ–secreting T cells or natural killer cells (47–50), suboptimal IL-2 receptor activity (20), either impaired expression of class II histocompatibility antigens or accessory cell function (51), defective interleukin 1 release (52), or that factors in addition to IL-2 are required for optimal IFN-γ production. Nevertheless, we were able to identify individual patients with impaired responses to antigen alone in whom rIL-2 appeared to have reasonable augmenting effects. In four of these 18 apparent responders (Table IV), these effects could largely be attributed to the presence of rIL-2 alone; in the remainder, rIL-2 appeared to act with antigen in either an additive or synergistic fashion. These distinctions seem relatively unimportant, however, since the desired goal of IL-2 treatment in this study was to attempt to increase IFN-γ production to normal or near-normal levels.

In addition, and to varying degrees, rIL-2 by itself at 10 U/ml also stimulated IFN-γ secretion by cells from 19 of 35 ARC

and three of 12 AIDS patients. Although more patients responded with detectable IFN-γ production to 100 U/ml of rIL-2 alone (ARC, 21 of 35; AIDS, 5 of 12) than to 10 U/ml, the calculated mean IFN-γ levels induced by 100 U/ml of rIL-2 shown in Table II indicated only a minimal increase for AIDS patients and a moderate decrease for ARC patients. For the latter group, these mean results are potentially misleading, since only eight of the 35 ARC patients actually showed decreased IFN-γ responses to 100 U/ml of rIL-2, and similar decreases were also observed in three of the 29 experiments with control cells. More pertinent, however, is the question of why AIDS and ARC cells failed to respond more effectively to the higher dose of rIL-2. For AIDS patients, and probably some of the lymphopenic ARC patients as well, a likely explanation is simply an absolute deficiency in the number of cells capable of responding to IL-2 and/or producing IFN-γ. However, since the numbers of $T4^+$ cells and $T8^+$ cells were normal or elevated in 50% and 88% of the ARC patients, respectively (not shown), and since IL-2 can stimulate both T cell subsets to secrete IFN-γ (42), additional, and as yet unidentified, cellular defects appear to be present in these patients to account for the ability to respond to low- (10 U/ml) but not high-dose (100 U/ml) rIL-2 (Table II). Since cells from healthy controls showed clearly augmented IFN-γ production in response to 100 U/ml of rIL-2, which presumably was accompanied by T cell expansion (14–16), a defect in the capacity of ARC patients' IFN-γ secreting cells to expand properly beyond a certain limit might be one explanation for the failure to respond to the higher concentration of rIL-2. Alternatively, since both high- and low-affinity IL-2 receptors may exist (44), it is also tempting to speculate that cells from certain ARC patients have normal high-affinity but impaired low-affinity IL-2 receptor activity that renders their cells unable to respond to high-dose rIL-2. Additional work, however, will be required to clarify these, as well as other, potential explanations for this particular observation in ARC patients.

When considered within the context that mononuclear cells from virtually all of our patients with AIDS and at least 50% of those with ARC fail to produce normal levels of IFN-γ (2)[2] or IL-2, the results achieved by using rIL-2 plus antigen as costimuli for IFN-γ secretion can be cautiously interpreted in a positive fashion. Thus, our in vitro observations suggest that in selected patients rIL-2 may hold some promise as either a potential immunotherapeutic agent in AIDS or as an immunoprophylactic agent in ARC. The capacity to respond to IL-2 with augmented IFN-γ production might, for example, through IFN-γ's direct macrophage-activating effects (5–10, 12, 53), as well as other diverse actions (4), possibly enhance host resistance to established opportunistic infections (2) or perhaps serve to reduce susceptibility and prevent the development of future infections.

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