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Originals

Combination treatment with zidovudine, thymosin α_1 and interferon-α in human immunodeficiency virus infection

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Summary. We have investigated the effects of combination therapy with thymosin α_1 and natural human lymphoblastoid interferon-α in human immunodeficiency virus infection and have shown that in vitro this combination treatment: (1) synergistically stimulated the cytotoxic activity against natural killer-sensitive target cells of lymphocytes collected from human immunodeficiency virus-infected donors and (2) did not interfere with the antiviral activity of zidovudine. We thus studied the effects of combination therapy with thymosin α_1, interferon-α and zidovudine in patients with CD4+ lymphocytes ranging from 200 to 500/mm^3 in a randomized nonblinded study and found that the treatment was well tolerated after 12 months of therapy and was associated with a substantial increase in the number and function of CD4+ T cells. A similar effect was not observed in human immunodeficiency virus patients treated with zidovudine alone or associated with single agents. These data suggest the need for a controlled, double-blind clinical trial, recently initiated with the approval and the support of the Italian Ministry of Health.

Key words: Human immunodeficiency virus infection – Combination therapy – Zidovudine – Thymosin α_1 – Interferon-α

Introduction

Human immunodeficiency virus (HIV) infection causes chronic and progressive immunodeficiency syndrome (AIDS) characterized by immune dysfunction connected with the depletion of CD4+ cells and loss of T helper cell function. This immune dysfunction appears to be due to at least two major modifications: (1) progressive loss of CD4+ cell number and function and (2) a progressive loss of CD8+ specific cytotoxic T cell and natural killer (NK) cell function [2, 5, 22].

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The exact mechanisms by which HIV suppresses T cell responses are not yet fully understood. The severe and selective depletion of CD4+ T lymphocytes was, until recently, considered to be exclusively due to the cytopathic effect of HIV. There is now growing evidence to suggest that many other complex mechanisms could be responsible for the depletion and immune impairment of CD4+ cells. Among these, autoimmune responses by antibodies or by cytotoxic MHC-restricted and non-restricted cells [23]. More recently, programmed cell death or apoptosis has been indicated as a possible crucial event in CD4+ cell depletion [1]. The mechanism of loss of immune function is also at the moment unclear, and only partly explained by the reduction of cytokine production, particularly interleukin-2 (IL-2) [19]. In fact this cellular dysfunction doesn't appear to be totally dependent on the decline in the CD4+ cell count. Some authors have now shown that the decline in cellular immune response (in IL-2 production) precedes a significant reduction in CD4+ cell number by at least 12 months [18, 21]. To date treatment strategies have focused on the use of antiretroviral agents, based on the assumption that viral proliferation is a major factor involved in AIDS progression. The availability of zidovudine (AZT) reduced the risk of disease progression by slowing the rate of the loss of CD4+ cells. As a result, AZT therapy has been shown to increase the median survival of HIV-infected patients by 6–12 months [9, 13]. However, the beneficial effects of AZT are limited because it does not seem to affect the decline in immunological functions [24] and AZT-resistant strains have been described [16, 17]. As a consequence, the use of agents able to restore immune responses should be considered in association with antiretroviral therapy.

Since our previous studies demonstrated the high degree of immunopotentiation achieved by combination therapy with thymosin α_1 (Tα_1) plus interferon-α (IFN-α) in experimental animal models of immunodepression [6, 7, 11], we have examined the effects of this combination immunotherapy in association with AZT in preclinical experiments and in a randomized, non-blinded 1-year clinical study.

Materials and methods

Cytotoxic assay. Peripheral blood mononuclear cells (PBMC) from healthy seronegative donors or asymptomatic untreated HIV-positive volunteers were obtained under sterile conditions by density gradient centrifugation, as previously described. Cells were washed and resuspended in complete medium (CM) consisting of RPMI 1640 supplemented with 5% fetal calf serum (FCS), 200 mM L-glutamine, 25 mM Herpes and antibiotics (all reagents were obtained from Flow, Irvine, Ayrshire, UK). PBMC were cultured in 24-well plates (Flow) at 37°C in 5% CO_2/95% air for 24 h at 2×10^6/ml in 1 ml of CM in the presence or absence of 500 U/ml IFN-α with or without pretreatment with $T\alpha_1$. For $T\alpha_1$ pretreatment, PBMC were resuspended in serum-free RPMI and preincubated for 1 h at 37°C with 100 ng/ml $T\alpha_1$. FCS and IFN-α or diluent control were then added and the cultures were incubated as described above. At the end of culture, the cytotoxic activity against the NK-sensitive K562 cell line was tested according to the microcytotoxicity assay described by Herberman et al. [14]. The percentage cytotoxicity was expressed as specific ^{51}chromium release and was calculated as follows:

$$\frac{\text{test cpm} - \text{baseline cpm}}{\text{total cpm incorporated} - \text{baseline cpm}}$$

In vitro treatment of HIV-infected cells. PBMC from healthy donors were prepared under sterile conditions by density gradient centrifugation as previously described. The cells were washed in phosphate-buffered saline, resuspended in CM supplemented with 20% FCS and cultured in 24-well plates at a final concentration of 1×10^5/ml plus phytohemagglutinin at 10 μg/ml (Sigma, St. Louis, Mo., USA) for 48 h in 5% CO_2 at 37°C. Cultures were performed in triplicate. Twelve hours before infection, 1.5 ml of the supernatants were gently collected in order to minimize cell loss and replaced by 1.5 ml of CM with 20% FCS, 10 U/ml IL-2 and the various drugs. The final concentrations of the drugs were: AZT 0.2 μM, $T\alpha_1$ 100 μg/ml and IFN-α 20 U/ml. No cell toxicity was observed with these concentrations in control cultures of uninfected PBMC. The doses of the drugs were selected on the basis of preliminary experiments which showed that: (1) the dose of 0.2 μM AZT was able to efficiently inhibit HIV replication in vitro, (2) doses of $T\alpha_1$ ranging from 1 to 10 μg/ml (i.e., doses usually utilized to achieve immunomodulation in vitro) did not influence HIV replication in vitro and (3) 20 U/ml IFN-α was suboptimal in inhibiting HIV replication in vitro. Drugs were utilized singly or combined. Cells were then infected by adding 300 $TCID_{50}$/well ($TCID_{50}$ = time culture infectious dose 50%) of virus $HTLV_{IIIb}$ (HIV_{III}) obtained from cultures of H9 cells and titred on MT4 cells. Every 3 days 1.5 ml of the supernatants were replaced by fresh CM containing the various drugs as previously described. Twelve days after infection, virus production was assessed by measurement of HIV-p24 gag protein production (expressed in pg/ml) in supernatants of infected cultures, using a commercially available HIV kit (Abbott, Pomezia, Italy).

Patients, treatment and evaluation. Forty HIV-seropositive subjects (23 males and 17 females, aged 22–43 years) were enrolled in a randomized non-blinded clinical study between January and June 1991. Of the 40 patients, 27 were drug abusers, 9 were homosexuals and 4 were heterosexuals. Of the 4 heterosexuals, 2 had sexual partners who were drug abusers. Criteria for eligibility were: (1) a positive ELISA for HIV with confirmation by Western blot (Dupont de Nemours, Bruxelles, Belgium), (2) aged 18 years or older, (3) CD4+ lymphocytes ranging from 200 to 500/mm^3, (4) Centre for Disease Control classes II or III, Karnosky > 70, (5) no previous antiretroviral or immunomodulator treatment and (6) normal hematological, hepatic, renal and coagulation function. Written informed consent was obtained from each patient.

The subjects were randomly assigned to four groups which were homogeneous in terms of age, risk factor and sex. The first group received 500 mg/day AZT (Wellcome, London, UK) in two administrations; the second group, AZT plus 2 MU natural human lymphoblastoid IFN-α (Wellcome) intramuscularly twice weekly; the third group, AZT plus 1 mg $T\alpha_1$ (Sclavo Pharmaceuticals, Siena, Italy) subcutaneously twice weekly; the fourth group AZT plus $T\alpha_1$ and IFN-α. After 1 year, 28 patients (7 from each group) had completed the cycle of treatment and were ready for evaluation; after 18 months the number of volunteers continuing in the study was reduced to 23 (6, 6, 6 and 5 patients for the four groups, respectively). None of the patients interrupting treatment withdrew because of side effects or disease progression, but rather due to either admission to therapeutic communities or lack of compliance.

On enrolment and at 4-week intervals the patients were evaluated by interview, symptom questionnaire, physical examination and by laboratory analysis, which included hematological, virological and immunological studies. Laboratory evaluation included complete blood count with differential, platelet count, glucose, electrolytes, blood urea nitrogen, liver function tests, cholesterol, triglycerides, coagulation profile and β_2-microglobulin measured by turbidimetric immunoassay.

Virological studies included determination of serum antibody and p24 by ELISA and HIV DNA analysis by the polymerase chain reaction (PCR) (after 12 months only). For this assay, blood samples from patients treated with AZT alone or AZT plus $T\alpha_1$ and IFN-α were collected in EDTA and mononuclear cells were obtained after medium separation in Ficoll gradient. DNA was obtained by enzymatic action with proteinase K (120 μl/ml, 1 h at 60°C and 10 min at 95°C) in a final volume of 600 μl. Aliquots of 12.5 and 25 μl (about 0.5 and 1 μg of genomic DNA) were subjected to the PCR with SK 38–39 primers. PCR was performed in a final volume of 100 μl with 2.5 U of Amplitaq polymerase (Perkin Elmer Cetus), 200 μM of each deoxynucleotide, in a buffer composed of 50 mM potassium chloride, 10 mM TRIS-hydrochloric acid (pH 8.3), 0.005% gelatin, 2.5 mM magnesium chloride, 0.25% Tween 20 and 0.25% NP-40. DNA amplification was accomplished with 20 pmol of each primer and 35 cycles in an automatic thermal cycling heat block (annealing temperature was set at 55°C). Amplified products were detected by electrophoresis on 2% agarose gels stained with ethidium bromide. They were also hybridized with internal oligonucleotide SK 19. The hybridization was revealed by the DEIA technique [4] (DNA Enzyme Immunoassay, Sorin Biomedica, Saluggia, Italy). PCR was quantified by comparison with a serial dilution of HIV genome (HIV control, Perkin Elmer Cetus). This positive control was run at the same time as the DNA samples.

Immunological studies included the phenotypic characterization of peripheral blood lymphocyte subpopulations by monoclonal antibodies and flow cytometry and the proliferative response of PBMC to glycoproteins from the cell wall of *Candida albicans* (GMP, kindly provided by Professor A. Cassone, Istituto Superiore di Sanità, Rome, Italy) and to tetanus toxoid (TT, Wyeth, Marietta, Pa., USA). Lymphoproliferative responses to GMP and TT were measured as described by Quinti et al. [20]. Briefly PBMC from healthy donors or from patients, prepared under sterile conditions by Ficoll-Hypaque density gradient centrifugation (Nycomed, Oslo, Norway) from heparinized venous peripheral blood, were incubated in 5% CO_2 at 37°C in the presence of GMP (final concentration 50 μg/ml) or TT (final concentration 2 Lf/ml) and harvested on day 7 of culture. Eighteen hours before harvesting radiolabelled thymidine was added at a final concentration of 1 μCi/well.

Results

In vitro effect of $T\alpha_1$ and IFN on cytotoxic activity of PBMC from HIV patients

Pretreatment with $T\alpha_1$ significantly potentiated the cytotoxic activity of IFN-α-stimulated PBMC collected from HIV-positive patients (Table 1). PBMC from healthy controls also responded to $T\alpha_1$ pretreatment. Treatment

with $T\alpha_1$ alone had no effect on cytotoxicity in either group. In contrast, as expected treatment with IFN alone stimulated the cytotoxic activity of cells collected from healthy donors as well as those from HIV patients. After 24 h in culture, we also observed an impairment of cytotoxicity against NK-sensitive target cells associated with HIV infection. A similar effect has been previously described by other authors with fresh PBMC [2]. Interestingly, in vitro pretreatment with $T\alpha_1$ of PBMC from HIV patients increased the IFN-induced cytotoxic activity to levels which were higher than those observed in PBMC from healthy donors when stimulated by IFN-α alone, indicating a complete restoration of the NK cell response to this cytokine. No significant difference in the total number of cells recovered after 24 h of culture was observed among the different groups (data not shown).

In vitro effect of combination treatment with $T\alpha_1$, IFN-α and AZT on HIV replication

Table 2 show the effect of combination treatment with $T\alpha_1$, IFN-α and AZT on HIV viral antigen production by human primary cultures of PBMC infected with HIV in vitro. AZT exerted its well-known antiviral effect when used singly, while no or very little antiviral effect was exerted by $T\alpha_1$ or IFN-α (at suboptimal doses) when used singly or in combination. The addition of $T\alpha_1$ or IFN-α, or both, to an AZT treatment regimen, slightly modified the antiviral action of this drug. Treatment with $T\alpha_1$ in vitro did not potentiate the direct antiviral activity of IFN-α at a suboptimal dose, whether or not it was associated with AZT. However, $T\alpha_1$ and IFN-α treatment, when used as single agents or in combination, did not interfere with the direct antiviral activity of AZT. Hence $T\alpha_1$ and IFN-α could be used in association with AZT in HIV patients.

The clinical study

Side effects of therapy. No adverse effects or major toxicity were observed in any patient and none of the volunteers had to discontinue treatment or reduce the dosage of drugs they were receiving. As shown in Table 3, hematological parameters and, in particular, median hemoglobin values and platelet counts did not significantly change with therapy in any treatment group. Other biochemical parameters were also unchanged in all patients (data not shown). None of the patients progressed to AIDS during the treatment.

Immunological and virological monitoring. During the course of therapy, CD4+ cell counts were measured monthly in all patients using monoclonal antibodies and flow cytometry. The mean absolute CD4+ cell counts of patients who completed the 1-year treatment regimen are shown at time 0 and after 3, 6, 9 and 12 months of treatment in Fig. 1 and Table 4. CD4+ lymphocytes in the AZT group averaged 382±61 (mean±SD) before treatment and 331±63 after 12 months. In the group

Table 1. Effect of combination treatment in vitro with thymosin α_1 ($T\alpha_1$) and interferon (IFN-α) on cytotoxicity in peripheral blood mononuclear cells (PBMC) from human immunodeficiency virus (HIV)-seronegative or -seropositive volunteers[a]

Group	% Cytotoxicity (mean±SD)[b]			
	In vitro treatment			
	Control	$T\alpha_1$	IFN-α	$T\alpha_1$+IFN-α
HIV-negative volunteers	45.0±7.0	40.2± 6.9	56.2±9.3[1]	76.2± 2.1[2]
HIV-positive volunteers	35.0±9.1	34.7±11.2	51.7±9.5[3]	61.1±16.8[4]

[1] $P=0.0025$ vs. control; [2] $P=0.0018$ vs. control and $P=0.0169$ vs. IFN-α; [3] $P=0.0001$ vs. control; [4] $P=0.0001$ vs. control and $P=0.0156$ vs. IFN-α

[a] Separated PBMC from 10 HIV-seronegative or -seropositive volunteers were cultured (2×10.6/ml, 1 ml/well) in complete culture medium alone (control) or in complete medium with the addition of $T\alpha_1$ (100 ng/ml), IFN-α (500 units/ml) or both ($T\alpha_1$+IFN)

[b] Cytotoxicity against K-562 cells was tested after 24 h of culture with a 4-h ^{51}chromium release assay. Results are expressed as mean values per group calculated from individual values of cytotoxicity obtained for each patient from quadruplicate wells at effector/target ratio of 50:1±SD

Table 2. In vitro antiviral activity of zidovudine (AZT) alone or in combination with $T\alpha_1$ and/or IFN-α[a]

Treatment[b]	% Viral inhibition[c]
Control	0 ±13.5
AZT	82.4± 4.3
IFN-α	17.4± 8.6
$T\alpha_1$	3.1± 1.1
$T\alpha_1$+IFN-α	8.6±14.1
AZT+IFN-α	93.0± 0.2
AZT+$T\alpha_1$	78.1± 0.6
AZT+$T\alpha_1$+IFN-α	90.6± 1.8

[a] Antiviral activity was evaluated after 12 days' culture of PBMC from healthy donors infected with HIV in vitro

[b] Drugs were added to the cultures every 3 days

[c] Results are expressed as mean values from triplicate cultures ±SD, calculated as follows:

$$100-\left(\frac{\text{p24 sample}}{\text{p24 control}}\times 100\right)$$

The p24 concentrations were evaluated with a commercial kit

Table 3. Platelet and hemoglobin levels and the start of the study and after 360 days of treatment

Group	Day 0		Day 360	
	Platelets ($\times10^3$/μl)	Hemoglobin (g/dl)	Platelets ($\times10^3$/μl)	Hemoglobin (g/dl)
AZT	182[a]	14.6	191	13.9
AZT+IFN-α	153	14.0	175	13.9
AZT+$T\alpha_1$	182	13.5	200	14.1
AZT+$T\alpha_1$+IFN-α	175	14.9	191	15.1

[a] Results are expressed as mean values obtained from all evaluable patients in the study. No significant difference was observed on day 360 compared with day 0 by paired Student's *t*-test

Table 4. Individual absolute number of CD4+ cells/mm^3 at enrolment (time 0) and after 6 and 12 months of therapy

Subject no.	CD4+ cells/mm^3											
	Group											
	AZT			AZT+IFN-α			AZT+Tα$_1$			AZT+Tα$_1$ +IFN-α		
	0	6	12	0	6	12	0	6	12	0	6	12
1	323	295	299	452	666	461	279	452	260	354	504	888
2	306	238	315	436	425	489	440	264	589	268	462	450
3	481	213	445	248	234	189	498	453	513	453	698	731
4	409	344	356	281	262	375	495	319	532	329	420	459
5	361	233	315	378	407	233	404	316	437	280	221	386
6	370	335	240	489	858	584	442	651	456	229	307	278
7	424	432	348	488	418	410	319	438	334	247	180	280

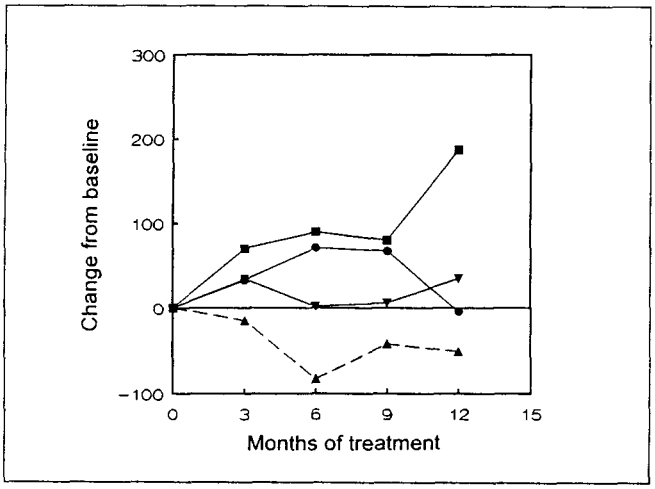

Fig. 1. Combination therapy with zidovudine, thymosin α, and interferon-α (AZT + Tα$_1$ + IFN-α): increase in CD4+ counts over 12 months. *Solid lines*, mean values obtained in patients treated with AZT + IFN-α (-●-), AZT + Tα$_1$ (-▼-) or AZT + Tα$_1$ + IFN-α (-■-); *dashed line*, mean values obtained in patients treated with AZT alone

receiving AZT and IFN-α, the CD4+ cell count changed from 396±98 to 392±140, and in the group receiving AZT and Tα$_1$ the cell count changed from 411±84 to 446±115. However, there were no significant differences in CD4+ values at time 0 and after 12 months in any group (paired Student's *t*-test). In contrast, in the group treated with AZT, Tα$_1$ and IFN-α, the CD4+ cell number averaged 309±77 before treatment and 496±230 after 12 months ($P=0.029$).

The proliferative responses of PBMC to both GMP (Fig. 2a) and TT (Fig. 2b) were significantly decreased in all four groups compared with PBMC from healthy donors, either at enrolment or after 1 year of treatment. However, the group receiving Tα$_1$ plus IFN-α and AZT showed an increased response to GMP and TT after 1 year of treatment compared with the other groups of patients after 1 year of treatment and the same group on entry ($P<0.05$ by Wilcoxon signed ranks non-parametric two-sided test).

Serum antibody and p24 assays gave no additional information on the status of patients. However, HIV DNA analysis by PCR (which was performed only in patients receiving AZT alone or AZT plus Tα$_1$ and IFN-α) demonstrated that the calculated number of viral copies per CD4+ cells (expressed as a percentage of the total CD4+ cell population) was significantly lower in patients receiving the combined treatment (0.61±0.16% vs. 1.24±0.55%, $P=0.013$ by Student's two-sided *t*-test).

Immunological results after 18 months of therapy. A smaller cohort of patients received treatment and was followed beyond 1 year. The CD4+ cell counts of this cohort of patients at 18 months are reported in Fig. 3. The results confirmed the trend previously observed, with the difference between patients receiving AZT plus Tα$_1$ and IFN-α and other groups being more evident than observed after 12 months of therapy. In fact, while the mean number of CD4+ cells was essentially stable or decreased in the patients treated with AZT alone or AZT plus single agents, it increased progressively from 583±

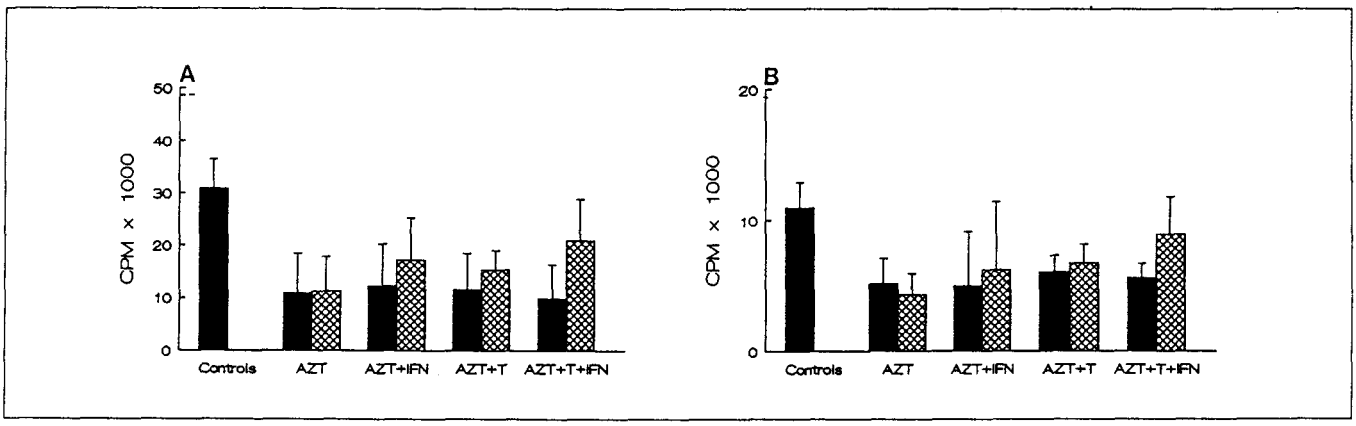

Fig. 2. Proliferative responses of peripheral blood mononuclear cells to cell wall extract from **A** *Candida albicans* or **B** tetanus toxoid from healthy human immunodeficiency virus (HIV) seronegative volunteers (controls) or HIV seropositive patients at enrolment (■) or after 12 months of treatment (▨) with AZT alone, AZT plus IFN-α, AZT plus Tα$_1$ or AZT plus Tα$_1$ and IFN-α. Results represent mean values obtained from all evaluable patients in the study + SD

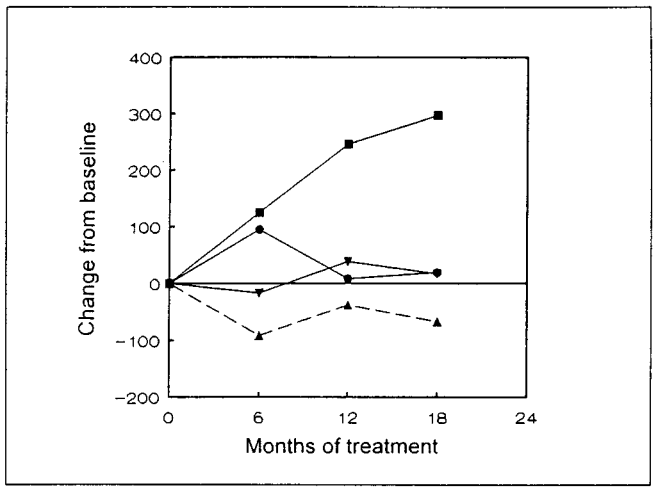

Fig. 3. Combination therapy with AZT + $T\alpha_1$ + IFN-α: CD4+ counts over 18 months from patients for whom data were available. *Solid lines*, mean values obtained in patients treated with AZT + IFN-α (–●–), AZT + $T\alpha_1$ (–▼–) or AZT + $T\alpha_1$ + IFN-α (–■–); *dashed line*, mean values obtained in patients treated with AZT alone

216 at 12 months to 633 ± 281 at 18 months in the group treated with AZT plus $T\alpha_1$ and IFN-α.

Discussion

This study was carried out in order to investigate the use of combination therapy with $T\alpha_1$, IFN-α and AZT in HIV positive subjects. The rationale of this new combination therapy is based on our previous studies which showed that treatment with $T\alpha_1$ followed by low doses of IFN-α was highly effective in restoring cytotoxic activities in animal experimental models of immunosuppression induced by tumors and/or cytostatic drugs [6–8, 10–12]. Higher doses of both $T\alpha_1$ and IFN-α did not improve the results and the effect was inferior when high doses of INF-α were utilized. The possibility of using a similar protocol in HIV-infected patients was then investigated.

This study demonstrated that, as in the murine system, a synergistic effect on cytotoxicity was observed in PBMC from HIV-infected patients. Moreover, the antiviral activity of AZT in vitro was not affected by the addition of $T\alpha_1$ or IFN-α to the cultures. Neither $T\alpha_1$ or IFN-α showed major side effects or toxicities in vivo when administered either as single agents with AZT or in combination. We observed only minor side effects, consisting of moderate fever and minimal flu-like symptoms, related to the administration of IFN-α, as reported by other authors [15]. The combination of AZT, $T\alpha_1$ and IFN was more effective than AZT or AZT with either agent alone in increasing the number of circulating CD4+ lymphocytes. During the first 6 months of treatment with AZT and IFN-α, there was an increase in CD4+ cells, but this declined over the following 6 months. This temporary effectiveness appears to coincide with the efficacy of AZT and decreases with the loss of AZT activity. A number of other researchers have also observed that the duration of the beneficial effects of AZT therapy starts to decrease after 6 months and seems linked to the emergence of strains of HIV-1 resistant to this antiretroviral drug [3, 16, 17]. Moreover, since progression to AIDS seems to correlate not only with the reduction in the number but also the function of CD4+ cells, the increased proliferative response to recall antigens of PBMC from patients treated with AZT, $T\alpha_1$ and IFN is of particular interest. Combination therapy with both cytokines appears to have a long-term beneficial effect, as indicated by the number of CD4+ cells in the group receiving AZT, $T\alpha_1$ and IFN-α after 18 months of treatment.

The mechanism of action of combination immunotherapy appears to be indirect, since $T\alpha_1$, IFN-α or the combination of $T\alpha_1$ plus IFN-α had no antiviral activity. $T\alpha_1$ may induce thymic maturation of the CD4+ population from more immature T cell populations and may increase IL-2 and IFN-γ production from CD4+ mature cells. Alternatively, $T\alpha_1$ could protect CD4+ cells from damage caused by mechanisms other than the direct cytopathic action of HIV, which are at present poorly defined. Moreover, combination treatment could reduce CD4+ infected cells by increasing cytotoxic activities. In this respect the significant difference after 1 year in the number of viral copies between patients treated with AZT alone and those receiving combination treatment is of interest. These results could also explain why the increase in the number of CD4+ cells in patients treated with AZT plus $T\alpha_1$ and IFN-α is significant after 12 months of therapy. A partial reconstitution of the immune system, at least in the few patients ($n = 5$) observed, could thus follow the reduction in viral burden during the 1st year of therapy. However, any conclusion is premature, since only a very small number of patients continued to be treated and controlled.

At present we are unable to evaluate the effect of combination therapies versus AZT alone on disease progression, since none of the patients in any of the groups has yet progressed to AIDS. The increase in the CD4+ cell number and the restoration of some immune functions in the group treated with AZT, $T\alpha_1$ and IFN-α is encouraging and suggests a possible impact on the disease. However, the small number of patients enrolled in this study does not allow any definitive conclusion on the efficacy of this novel combination therapy in HIV infection.

In conclusion, our results, although preliminary, suggest that the combination of AZT, $T\alpha_1$ and IFN-α was well tolerated in this cohort of patients and was associated with a substantial and sustained increase in the number and function of CD4+ cells. These data indicate the need for a controlled, double-blinded clinical trial in a larger cohort of patients to assess the potential advantages and efficacy of the combination treatment in comparison with groups of patients treated with AZT alone or with single agents. Such a multicenter trial has recently been initiated with the approval and the support of the Ministry of Health of Italy.

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