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Open study of AL-721 treatment of HIV-infected subjects with generalized lymphadenopathy syndrome: An eight week open trial and follow-up

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Summary

AL-721 is a lipid compound composed of neutral lipids, phosphatidylcholine and phosphatidylethanolamine in a 7:2:1 ratio. The objective of this open study was to evaluate the effects of AL-721 in vivo in an 8-week open trial in which 10 g twice daily was administered on a low fat diet to eight HIV-infected subjects with lymphadenopathy syndrome (LAS).

Serial lymphocyte cocultivation studies in 7 patients with initial culture positivity appeared to demonstrate reduction of reverse transcriptase peak counts in 5 with the trough noted in 4 at 8 weeks and in one at 4 weeks following termination of therapy. The mean values for all 7 patients revealed a baseline value of 73 419 with decrease to a low of 27 418 at 8 weeks.

Mean levels of total lymphocytes, T-4, T-8 and T-11 cells were not altered but lymphoproliferative responses to concanavalin A and pokeweed mitogens appeared to be augmented in 4 of the 8 subjects in association with AL-721 treatment. No side effects were noted.

In a subsequent follow-up study using a normal diet in the same subjects lymphocyte cocultivation and mitogen-induced responses were less consistently affected when 15 g twice daily AL-721 was readministered. In addition, serum HIV

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p24 antigen and CD4 levels were not altered during both the 8-week open and subsequent AL-721 readministration. Four of the 8 patients have progressed to AIDS over the subsequent 14 months.

AL-721; Anti-HIV; Immunostimulating effects

Introduction

Antibodies to HIV (human immune deficiency virus, HTLV-III/LAV) have been demonstrated in over 90% of homosexual men with generalized lymphadenopathy syndrome (LAS) (Sarngadharan et al., 1984; Laurence et al., 1984). While the clinical outcome for individuals with generalized lymphadenopathy remains unclear, a significant percent of cases prospectively followed have developed CDC-defined acquired immune deficiency syndrome. Studies on the molecular biology of HIV replication suggested the use of viral reverse transcriptase as a target for antiviral agents (Hirsch and Kaplan, 1985). Azidothymidine, a reverse transcriptase inhibitor, recently has been established as an effective agent capable of decreasing mortality and frequency of opportunistic infections in a selected group of subjects with AIDS and ARC (Fischl et al., 1987) but bone marrow toxicity has been a limiting factor (Richman et al., 1987). There is a continued need to identify effective anti-retroviral agents that are capable of inhibiting HIV function and replication and that are also relatively nontoxic.

Other potential sites of action for anti-retroviral agents are being identified and include potential interference with viral-host cell adherence. AL-721 is a lipid compound composed of neutral lipids, phosphatidylcholine and phosphatidylethanolamine in a 7:2:1 ratio (Lyte and Shinitzky, 1985; Shinitzky, 1984; Rivnay et al., 1979; Rivnay et al., 1980, Shinitzky et al., 1983) that has demonstrated ability to extract cholesterol from cellular membranes both in vitro and in vivo. Sarin et al. (1985) reported that AL-721 inhibited in vitro HIV infection of human peripheral blood lymphocytes or an immortalized H9 helper T-cell line as measured by absence of lymphocyte cytopathogenicity, low virus-specific reverse transcriptase activity and reduction of p15 and p24 antigen synthesis. Because AL-721 was co-administered to the host cells along with HIV, it was not possible in the latter experiments to determine whether the antiviral effects were due to actions on retroviral envelope or host cell membrane or both.

Previous in vivo trials in human subjects (Shinitzky, 1984; Shinitzky et al., 1983) involved administration of 10–15 g of AL-721 each morning for 3 weeks to 16 normal elderly patients over 75 years of age which restored towards normal the previously diminished phytohemagglutinin-induced lymphocyte proliferative responses with an effect comparable to that produced by an in vitro concentration above 1000 μg per milliliter. The role of AL-721 in producing this change was suggested by the fact that lymphocyte responsiveness declined towards the baseline level once AL-721 was discontinued.

The objective of this open study, initiated in the summer of 1986, was to evaluate AL-721 in HIV-infected subjects with LAS as determined by its effect on reverse transcriptase activity, T-lymphocyte subset numbers and mitogen-induced lymphoproliferative responses during an 8-week trial. Following a drug-free interval of 3–4 months, the drug was readministered and observations were made regarding clinical course. In addition, circulating levels of serum HIV p24 antigen were subsequently assayed after diagnostic reagents became available.

Materials and Methods

Subjects

Eight male homosexual subjects were enrolled with a diagnosis of persistent generalized lymphadenopathy (PGL) as defined by the CDC (MMWR, 1982). The mean duration since initial diagnosis of PGL was 27.8 ± 9.0 (SD) with a range of 16 to 41 months. Entry criteria included evidence of T-cell dysfunction as shown by either reversal of CD4/CD8 cell ratio to less than 0.7 or CD4 cell counts below 500/cmm, positive ELISA assay for HIV antibody and positive lymphocyte cocultivation assay for reverse transcriptase activity during pretreatment evaluation.

Individuals were excluded if there was evidence of a wasting syndrome, intermittent or persistent fever, prior antiviral or immunomodulator therapy within 3 months or symptomatic mucosal candidiasis.

Treatment protocol

Each patient received AL-721 for 8 weeks, 10 grams orally twice daily packaged in polyethylene-lined aluminium foil sachets dispensed and stored in a frozen state and reconstituted in approximately 10 fluid ounces of chilled orange juice. Subjects were instructed to take the first dose 1 h after a fat-free breakfast and the second at least 3 h after a low fat dinner. In addition they were examined at 4 and 8 weeks following discontinuation of treatment for a total observation period of at least 16 weeks. Each patient was examined and blood obtained for complete cell counts, serum chemistry, immunologic and viral studies before, at baseline and again at 4 and 8 weeks of therapy and then at 4 and 8–12 weeks following cessation of AL-721. The drug was supplied by Matrix Research Laboratories Inc., New York, N.Y., a subsidiary of the Ethigen Corporation (California). Subsequently AL-721 was readministered to seven of the subjects at a dosage of 15 g twice daily after a 4 month period without the drug. Since this was an open study, the virology and immunology laboratories were aware of the treatment protocols and were not blinded.

Reverse transcriptase activity

Lymphocytes were separated from the peripheral blood of each subject using Ficoll-Hypaque and resuspended at a concentration of one million cells per ml in growth medium containing phytohemagglutinin (PHA), 10 percent inactivated fetal calf serum, interleukin-2, anti-alpha interferon, gentamicin, L-glutamine, po-

lybrene, beta-mercaptoethanol and RPMI 1640. After 4–5 days, each patient's lymphocytes were cocultivated with PHA-stimulated cells obtained from an HIV-seronegative, culture-negative donor in a ratio of 1:3. A single donor was used for the entire study. Supernatants were obtained for assay of reverse transcriptase (RT) activity every 3 to 5 days for 4 weeks and the cells resuspended each time in growth medium.

RT was assayed as previously described (Buimovici-Klein et al., 1986). Supernatants were spun at 45K rpm for 30 min, the resulting pellet treated with buffer containing 0.1% Triton and incubated at 37°C for 60 min in a solution containing Tris 1 M at pH 7.8, dithiothreitol 20 mM, potassium chloride 1 M, magnesium chloride 0.5 M, poly rA-oligo dT and tritiated dT triphosphate. Specimens were filtered, oven-dried, placed in scintillation vials with xylene OCS scintillation solution and read in a beta scintillation counter for one minute. Positive and negative control supernatants were used for each run of the RT assay with mean ± S.D. values of 162626 ± 40395 and 1508 ± 697 respectively while the mean of normal donor lymphocyte supernatant RT was 2404 ± 1706 cpm. Supernatant RT cpm above 10000 and five-fold or greater than the concurrent donor supernatant value was considered as positive for entry into the study.

T-cell subsets

Direct immunofluorescence staining of whole blood using an EPICS C flow cytometer was used to determine lymphocyte surface markers utilizing the Coulter whole blood procedure. Hundred-μl aliquots of peripheral blood collected in EDTA tubes were mixed with monoclonal antibodies CD2, CD4 and CD8 FITC (Coulter Corp., Hialeah, FL), incubated for 45 min at room temperature, red cells lysed, fixed, washed, suspended in PBS and analyzed using a Quartz 3 flow-cell biohazard assembly and confocal lens.

Lymphoproliferative responses

Responses to mitogens were determined in triplicate lymphocyte cultures with cells suspended in 0.2 ml minimum essential medium (GIBCO) supplemented with 10% heat-inactivated fetal calf serum, 0.25 mM hepes buffer, 2 mM L-glutamine and 100 units/ml penicillin and 100 μg/ml streptomycin (GIBCO) in flat-bottomed wells of microtiter plates (Flow Labs., Inc., McLean, VA) without mitogen as well as with 2 concentrations of each mitogen (concanavalin A, Con A; pokeweed, PWM; and PHA). Cells were incubated 4 days with PHA and Con A and 7 days with PWM in a 5% CO_2 humidified incubator at 37°C. Tritium-labeled thymidine (New England Nuclear, Boston, MA) was added one day prior to harvesting, cells were collected with an automatic harvester (Adaps, Inc., Dedham, MA) and counted in a Beckman liquid scintillation counter with results expressed as counts per min. Final solutions of PWM were 1:50 and 1:100 while Con A was present at concentrations of 10 and 5 μg/ml and PHA at 5 and 2.5 μg/ml. Results were analyzed by averaging the counts per min for each of two concentrations for each mitogen after subtraction of background values. A study of serial determination over 7 months in a normal subject disclosed the coefficient of variation to be less than

15% except with PHA at a concentration of 5 μg/ml. Peripheral blood from a normal subject was used with each assay as a control but normal controls were not consistently matched for each patient.

Antigenemia and antibody titers to core antigens

Sera from all subjects in the open two-month trial were analyzed for HIV p24 antigen and antibody at Abbott Laboratories through the assistance of George G. Jackson, M.D., University of Illinois at Chicago. Serum samples were assayed for HIV p24 antigen in a solid phase immunoassay and for antibody to p24 using a competitive enzyme immunoassay (Abbot Laboratories, North Chicago, IL). This assay was performed at SLRHC with later serum specimens using reagents obtained from Abbott Laboratories.

Results

Initial AL-721 administration

The mean weight of patients at entry was 170 pounds ± 10 (SEM) and this decreased to a mean of 164 ± 8 at 7 weeks with a return to 167 ± 18 at 12 weeks. Weight decreased during the 8 week period in 7 of 8 subjects with a range of 2–23 pounds. The decrease in weight may have resulted from the dietary changes that were recommended to potentially facilitate AL-721 absorption by utilizing a fat-free breakfast and low fat dinner prior to administration of oral AL-721. Fasting serum levels of total phospholipids, HDL-and LDL-cholesterol and triglycerides were not modified in any consistent fashion during the 8-week treatment period or following discontinuation of the drug and modified diet. No side effects were noted in association with AL-721 therapy.

Reverse transcriptase activity results are summarized in Table 1 and Fig. 1. One of the eight patients (No.8) was excluded from this analysis because the screening lymphocyte culture performed at an outside laboratory was borderline and the 0 week peak RT value in our laboratory was similar to the donor control. In 5 of the remaining 7 subjects, peak RT counts decreased to a value of 5325 or below during AL-721 treatment with the trough noted in 4–8 weeks (no. 2,3,4,7) and one (No.1) at 1 month following treatment. The RT values for all 7 patients revealed a mean baseline value of 73419 which decreased to a low of 27418 at 8 weeks (P=0.024, one-tailed Student's t-test) with variable return towards baseline by 8–12 weeks following discontinuation of drug treatment. The mean patient to donor RT ratio declined from 26.4 to 10.4 at 4 weeks following treatment while RT values declined to a mean 41.8% of baseline values at 8 weeks. In addition the mean day of peak RT count increased from 9.7 days at baseline to 17.1 days at 12 weeks or 4 weeks post-therapy.

The baseline mean ± SEM total lymphocyte count was 1474 ± 237/cmm for the 8 subjects, CD2 1059 ± 209, CD4 331 ± 68, and CD8 819 ± 145/cmm while the CD4/CD8 ratio was 0.43 ± 0.10. There were no significant trends with any of these parameters during and following the treatment period. Absolute values for CD4 and CD8 lymphocytes are summarized in Table 2.

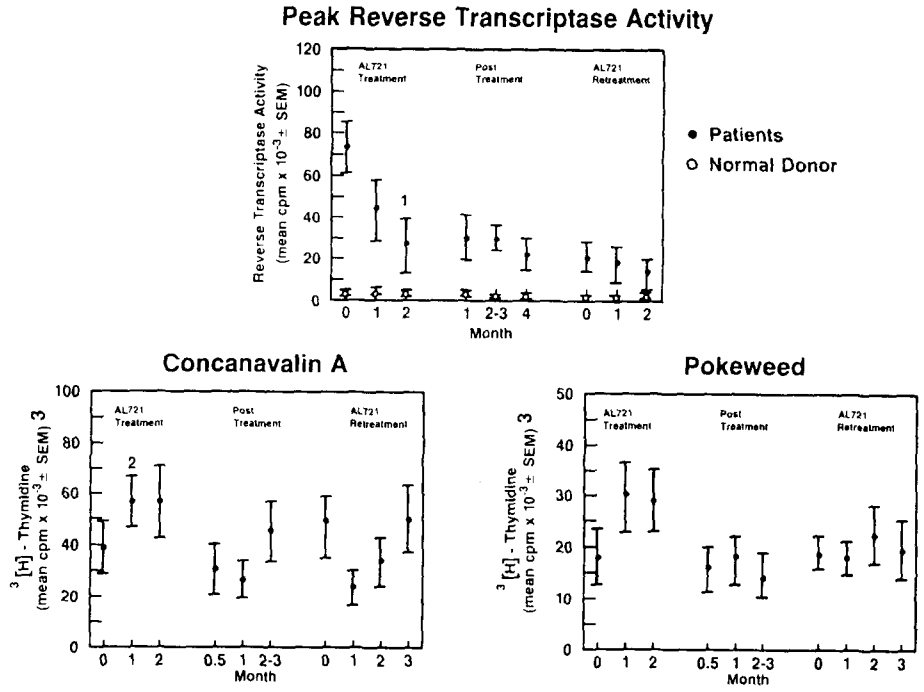
TABLE 1

Serial lymphocyte co-cultivation studies with peak reverse transcriptase assays with LAS (Mean ± SEM) at the monthly periods noted

Patient	AL-721 Treatment			Post treatment			AL-721 Retreatment		
	0	1 Mo.	2 Mo.	1 Mo.	2-3 Mo.	4 Mo.	0	1 Mo.	2 Mo.
1. (T.J.)	74,783	67,350	12,896	4,160	20,412	74,328	67,621	78,277	35,508
2. (A.E.)	69,491	91,001	4,869	31,272	28,728	....	....	....	....
3. (O.D.)	76,772	13,349	5,325	79,998	26,192	14,800	13,374	5,511	3,961
4. (B.W.)	126,070	12,064	3,895	3,986	27,908	3,971	3,136	3,807	4,821
5. (F.A.)	41,723	17,733	69,949	16,792	31,884	16,404	19,316	2,950	4,473
6. (M.T.)	101,372	100,325	90,015	36,863	30,750	16,168	17,950	28,007	20,175
7. (M.W.)	23,725	10,752	4,975	38,483	42,144	17,418	11,550	4,499	25,746
8. (S.S.)	....	....	....	....	....	11,006	10,170	5,094	3,775
Patient mean peak counts	73,419± 12,962	44,653± 15,180	27,418±[b] 13,973	30,219± 9,929	29,717± 2,504	22,025± 8,233	20,445± 7,515	18,306± 9,758	14,065± 4,560
Donor control counts	2,765±109	2,965±8	2,572±135	2,835±134	1977±87	1818±206	1739±241	1540±101	2027±150
Patient/donor ratio	26.4±4.3	15.5±5.1	11.2±6.0	10.4±3.2	14.0±1.2	12.1±4.5	12.6±4.3	11.9±6.3	7.0±2.2
Patient % baseline RT[a]	100%	59.5±5.1%	41.8±24.4%	56.0±23.9%	59.4±23.2%	.....	.....	.....	....
Day of peak	9.7 ± 1.3	14.7 ± 1.7	13.3 ± 1.7	17.1 ± 2.5	9.4 ± 0.4	12.9 ± 0.9	14.7 ± 1.5	16.0 ± 2.6	17.6 ± 1.9

[a] Calculated as (peak count at visit minus donor control at visit)/(peak count at week 0 minus donor control at week 0) times 100.

[b] $P = 0.024$, one-tailed Student's t-test comparing 0 and 2 mo. Al-721 treatment.



1 p = 0.024, one-tailed Student's t-test comparing 0 and 2 months AL721 administration
2 p = 0.043, one-tailed Student's t-test comparing 0 and 1 month AL721 administration
3 cpm as the mean of both concentrations minus baseline values

Fig. 1. The mean and SEM are displayed for peak reverse transcriptase activity, concanavalin A and pokeweed lymphoproliferative responses during the periods of AL-271 administration, post-treatment and AL-721 readministration.

Lymphoproliferative responses in the 8 subjects are summarized in Table 3 for Con A and Table 4 for PWM. Mean PHA baseline counts were comparable to the donors and did not appear to change during the course of the study. In contrast, augmentation of Con A responses was noted with a rise of mean cpm from 38771 at baseline to 56709 at 4 weeks and 56721 at 8 weeks (P=0.043 one-tailed Student's t-test) and subsequent decline to 30567 and 26351 at the 2nd and 4th post-therapy weeks. Even more pronounced responses were noted with pokeweed resulting in a rise from a mean baseline count of 17939 (mean donor count 53321) to 30305 and 26923 at 4 and 8 weeks respectively followed by decline to 16046 at 2 weeks, 18223 at 4 weeks and 14138 at 8–12 weeks following therapy. The results with PWM did not achieve statistical significance with the small sample studied. Augmentation of proliferative responses to Con A on 2 consecutive studies was noted in 4 of the 8 subjects (No. 5,6,7, and 8) and increased PWM-induced lymphoproliferation was observed as well in 4 (No. 1,4,5,8) with dual augmentation in 2 of the 4.

TABLE 2

Peripheral Blood CD4 and CD8 Counts Per Cmm

Patient	AL-721 Administration---CD4 Counts					
	Before	Baseline	2-weeks	4-weeks	6-weeks	8-weeks
TJ	550	481	----	419	384	300
AE	24	6	8	19	9	23
OD	176	220	249	317	209	152
BW	333	599	617	699	349	----
FA	105	233	----	184	184	147
MT	232	270	400	325	261	336
MW	349	500	233	439	408	362
SS	149	341	----	247	360	220
Mean	240	331	299	331	258	222
S.D.	167	191	226	200	126	77

Patient	AL-721 Administration---CD8 Counts					
	Before	Baseline	2-weeks	4-weeks	6-weeks	8-weeks
TJ	1451	795	----	960	568	687
AE	510	201	455	562	198	514
OD	844	947	1073	1629	662	700
BW	832	1220	805	953	569	----
FA	632	625	----	722	368	781
MT	646	319	516	573	269	476
MW	1048	1198	548	1118	1021	833
SS	1368	1252	----	981	973	1035
Mean	916	819	689	937	579	834
S.D.	346	410	250	345	303	180

Readministration of AL-721

During the readministration of AL-721, the low fat diet was not continued. RT values are summarized in Table 1 and Fig. 1 for AL-721 retreatment. RT activity decreased from baseline in patients 1,3,5 and 8 at either or both of the one and two month periods but there were no significant changes in the mean values for the 7 patients studied. Effects on Con A and PWM lymphoproliferative responses were variable. In addition, several patients developed clinical manifestations that required other drug therapies.

Serum HIV p24 antigen and antibody assays

Results of serum p24 antigen and antibody assay are summarized in Table 5. Five subjects were p24 antigen-positive initially while 3 were antigen-negative, antibody-positive. One antigen-positive subject (No. 5) with a level of 88 μg/ml showed a reduction to 78 and 20 at 4 and 8 weeks of AL-721 and to 0 on follow-up examination. This was accompanied by p24 antibody conversion from negative to positive. The patient had remained positive by RT assay but appeared to have augmented lymphoproliferative responses to Con A and PWM. The remaining 4

TABLE 3

Serial lymphoproliferative responses (CPM)[a] With Con A in 8 Subjects with LAS (Mean ± SEM at the time periods noted

Patient	Baseline		AL-721 Treatment			Post-treatment			AL-721 Retreatment			
	CD4/cmm	CD8/cmm	0	1 Mo.	2 Mo.	2 Wks.	1 Mo.	2-3 Mo.	0	1 Mo.	2 Mo.	3 Mo.
1. (T.J.)	481	795	27,730	22,822	16,402	17,544	36,571	42,029	20,774	48,242	33,340	36,757
2. (A.E.)	6	201	7,983	1,390	4,008	1,394	1,459	1,405	....	....	....	....
3. (O.D.)	220	947	52,691	74,646	22,001	44,984	17,913	41,899	57,549	34,537	31,596	68,823
4. (B.W.)	599	1,220	96,749	79,325	112,319	52,734	29,621	117,678	33,606	26,477	64,722	105,459
5. (F.A.)	233	625	26,543	44,284	69,803	10,161	23,668	16,140	18,686	6,941	12,836	19,778
6. (M.T.)	270	319	46,033	83,632	86,255	79,980	65,906	87,258	28,864	16,490	61,870	----
7. (M.W.)	500	1,195	39,009	86,142	68,867	28,033	11,904	46,622	110,794	22,668	33,912	....
8. (S.S.)	341	1,252	13,432	61,433	74,110	9,750	23,765	9,844	73,949	12,103	12,768	17,888
Mean ± SEM	331± 68	819± 145	38,771± 9,883	56,709± 11,027[b]	56,721± 13,492	30,567± 9,468	26,351± 6,805	45,359± 14,042	49,175± 12,790	23,923± 5,335	33,863± 9,360	49,740± 16,658
Patient % baseline			100	169±47	181±60	72±16	82±22	102±19	100	72±28	97±34	148±48

[a] Counts per minute (CMP) given as mean of both concentrations minus background value.

[b] $P=0.043$ one-tailed Student's t-test comparing 0 and 1 Mo. Al-721 administration.

TABLE 4

Serial lymphoproliferative responses (CPM)[a] with PWM in 8 subjects with LAS (Mean ± SEM) at the time periods noted

Patient	AL-721 Treatment			Post-treatment			AL-721 Retreatment			
	0	1 Mo.	2 Mo.	2 Wks.	1 Mo.	2-3 Mo.	0	1 Mo.	2 Mo.	3 Mo.
1. (T.J.)	4,535	11,836	9,355	26,819	10,282	5,610	12,349	8,262	7,906	14,863
2. (A.E.)	2,530	1,086	10,991	2,354	109	228	....	....	....	....
3. (O.D.)	22,031	27,168	10,367	14,952	11,528	16,082	29,812	15,836	13,578	15,675
4. (B.W.)	29,963	41,471	47,148	31,341	36,028	37,620	36,873	24,793	18,894	42,802
5. (F.A.)	3,649	6,465	...	7,220	9,754	2,300	8,221	5,137	4,282	6,042
6. (M.T.)	36,741	42,322	36,078	15,794	34,278	18,401	16,522	24,620	31,935	....
7. (M.W.)	38,414	40,703	36,798	27,185	20,884	28,532	19,929	35,786	62,704	....
8. (S.S.)	5,650	71,391	51,725	2,669	22,917	4,333	6,502	11,637	17,059	17,699
Mean ± SEM	17,939± 5,520	30,305± 8,279	28,923± 6,925	16,046± 4,501	18,223± 4,455	14,138± 4,787	18,613± 4,245	18,010± 4,116	22,337± 7,515	19,416± 6,178
Patient % baseline	100	278±143	279±116	152±65	153±28	75±13	100	107±22	140±43	126±38

[a]Counts per minute (CPM) given as mean of both concentrations minus background value.

TABLE 5

Circulating P24 antigen[a] and antibody levels during initial 8-week trial of AL-721

	AL-721 Treatment						Follow-up				AL-721 Readministration			
	0		1 Mo.		2 Mo.		1 Mo.		2-3 Mo.		0 Mo.	1 Mo.	2 Mo.	3 Mo.
Patient	Ag	Ab	Ag	Ab	Ag	Ab	Ag	Ab	Ag	Ab	Antigen alone			
1. (T.J.)	0	Positive	0	Positive	0	Positive	0	Positive	0	Positive	0	0	0	0
2. (A.E.)	150	Negative	163	Negative	215	Negative	...	....	39	Negative	..	..	..	..
3. (O.D.)	0	Positive	0	Positive	0	Positive	0	Positive	0	Positive	0	0	0	0
4. (B.W.)	...	.....	0	Positive	0	Positive	0	Positive	0	Positive	0	0	0	0
5. (F.A.)	88	Negative	78	Negative	20	Negative	0	Positive	0	Positive	0	0	0	0
6. (M.T.)	102	Negative	135	Negative	151	Negative	105	Negative	144	Negative	225	225	63	120
7. (M.W.)	166	Negative	102	Negative	109	Negative	...	....	269	Negative	63	238	238	242
8. (S.S.)	...	....	55	Positive	...	.....	133	Negative	351	Negative	437	63	120	500

[a]Circulating antigen value expressed as pg/ml.

HIV p24 antigen-positive patients had persistently elevated serum levels during the 8-week open trial subsequent follow-up.

Clinical progression to AIDS

There were no significant clinical events during the 8 weeks of the study. However, during the 3–4 month drug-free period 3 patients progressed to AIDS and following subsequent AL-721 readministration, one other patient also developed AIDS. One patient (No.2) with CD4 counts in the range of 6–33 per cmm throughout the 8-week period developed cytomegalovirus and *Toxoplasma* infections during the month following therapy and subsequently expired. A second (No. 7) developed Burkitt's lymphoma within 2 months following treatment. He had noted a large left axillary lymph node which was biopsied. He has continued on AL-721 and received 6 cycles of chemotherapy. At the present time the lymphoma appears to be in remission and he is taking AL-721, cotrimoxazole and coumadin. A third patient (No.3) developed *Pneumocystis carinii* pneumonia in January 1987 and Kaposi's sarcoma in March 1987. He remains stable clinically on AL-721 and aerosol pentamidine. A fourth patient (No.6) has had progressive deterioration with depression, weight loss, dyspnea, exaccerbation of psoriases and then expired during November 1987 with *Pneumocystis carinii* pneumonia. Thus, AIDS has developed in one of three p24 antigen-negative and in three of the five antigen-positive patients. A fifth patient (No.5) developed herpes zoster, depression, weight loss and progressive CD-4 lymphopenia down to 50 per cmm. In August 1987 he was placed on AZT, continued AL-721 and manifested improvement with weight gain and rise of CD4 values to 133 per cmm. A sixth patient, No.1, has had intermittent fever and diarrhea and has continued on AL-721. The remaining two patients were essentially asymptomatic as of November 1987.

Side effects

No significant side effects were noted. Several patients noted nausea following drug administration but hematologic, hepatic or renal toxicity was not noted.

Discussion

This open study in a small group of patients with LAS included selected subjects with initial HIV cultures positivity to ascertain whether AL-721 administered orally might affect RT levels. HIV has been reported to be isolated from approximately 50% of AIDS patients, 85% of ARC patients and 30% of healthy individuals at risk for AIDS (Kaplan et al., 1985). There is some evidence that favors the concept that culture positivity is associated with reduced CD4 lymphocyte numbers in the peripheral blood and the presence of constitutional symptoms. Kaplan et al. (1985) reported that the helper T-cell count was less than 408/cmm in the 16 culture-positive homosexual men in contrast to only 13% of 8 culture-negative subjects. The 7 high RT level culture-positive subjects in this study were screened from over 24 subjects with asymptomatic LAS and probably reflected the rela-

tively lower frequency of high RT level culture positivity in this subset of patients. Thus, we have selected a subset of LAS which probably differs in several aspects from the larger subset of culture-negative or low level RT-positive patients.

In this selected group with LAS there appeared to be a detectable effect on culture-positivity during the initial 8-week trial as reflected by both delay and frequency of the detection of reverse transcriptase by cocultivation utilizing adult peripheral blood lymphocytes. In the interpretation of these results one must take into account that specific RT determination in stimulated lymphocyte cultures represents an amplification technique which has not been standardized and is not a direct measure of viral load. Variables include the use of donor adult, fetal or cloned lymphocyte lines, different assays for detection of reverse transcriptase or HIV core antigens performed at variable intervals over inconsistent periods of time and a multiplicity of methodologic details. Despite these drawbacks, the decreasing RT values observed in the study may be suggestive of diminished HIV recoverability in 5 of the 7 subjects that could be evaluated. The delay in the appearance of peak RT values (from a mean of 9.7 days at baseline to 17.1 days at 4 weeks following AL-721 treatment) could also be suggestive of the possibility that viral load may have been reduced.

While there were no evident effects on absolute numbers of lymphocyte subsets, augmentation of Con A and PWM-induced lymphoproliferative responses was suggested with each mitogen in 4 of 8 subjects, mainly in those with significant depression of responses prior to therapy.

The authors believe that the data suggest that during the initial 8-week trial, the administration of AL-721 with a low fat diet was associated with decreased viral RT expression upon cocultivation and augmented mitogen-induced lymphoproliferative responses. However, the failure to observe augmented CD4 counts is of significant concern and do not support substantial anti-viral effect. Subsequent studies have failed to reveal a consistent reduction of circulating p24 antigen during either AL-721 administration or readministration. One of 5 antigen-positive subjects converted to antigen-negativity and antibody-positivity but this may or may not have been drug related. Previous investigations have noted that virus isolation and antigen detection may give discordant results with HIV infection (Gaines et al., 1987; Carter et al., 1987) and that neither is clearly superior in evaluating antiretroviral activity of therapeutic agents. In this open study the follow-up observations have revealed progression to AIDS in 50%.

This study does provide some evidence that AL-721 might reduce the extent of HIV proliferation during cocultivation as well as improve mitogen-induced lymphoproliferative responses. In addition, the doses of AL-721 used in these studies were well tolerated with no apparent toxicity. However, the lack of apparent effect upon CD4 lymphocyte counts and circulating serum HIV p24 antigen values weigh against the potential of this drug. The description of the clinical course in such a small group of patients must be considered anecdotal. Only a controlled clinical trial would be able to assess efficacy. It remains possible that interruption of therapy and the readministration of AL-721 with a normal diet may have affected the follow-up study results.

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