Survival and Quality of Life in HIV - Positive Patients Treated with a Polyantigenic Immunomodulator

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ABSTRACT. A polyantigenic immunomodulator (PAI), previously known as polyantigenic vaccine, which consists of a mixture of antigens of inactivated bacteria with antigens of influenza virus in a peanut-oil-arlacel-A-aluminium monoesterate emulsion, increased tumor resistance and induced tumor regression in tumor bearing mice. This report presents clinical and laboratory data that demonstrate the effect of PAI in long term prolongation of disease free state in HIV positive patients. A total of 40 patients, 35 males and 5 females, with a mean age of 41.1 ± 10.5 years, ranging from 28 to 68 years, HIV positive by (ELISA and Western Blot), with no restriction on the CD4 + T lymphocytes counts, were included in this open study. The PAI regimen was one subcutaneous injection per week for patients with < 400 CD4 + lymphocytes and one monthly injection for patients with CD4 + count > 400. All patients were monitored at different intervals for lymphocyte counts, clinical condition and treatment toxicity. After a follow up of eight years 81% of the patients were alive and 47% were free of disease. In patients without AIDS, the weight was 153.9 ± 28 pounds pre-PAI and 164.6 ± 27 ($P = 1.2 \times 10^{-4}$); the CD4 + lymphocyte count was 795 ± 421 pre-PAI and 585 ± 279 post PAI (P = 0.08). In patients alive with AIDS, the weight was 159.5 ± 32 pre-PAI and 163.9 ± 32 pounds post-PAI (P = 0.8); the CD4 + lymphocyte counts was 491 ± 255 pre-PAI and 298 ± 142 post-PAI (P = 0.08); and five AIDS-related infections occurred in five patients. In patients who died the weight was 157.7 ± 23 pre and 146.8 ± 30 post (P = 0.10); and the CD_4 count was 340.7 ± 149 pre and 103.4 ± 88 post (P = 0.0057). All died with infection. No toxicity with the use of PAI was reported. PAI improves disease free survival and quality of life in HIV + patients. *Key Words: AIDS, HIV, Immunopotentiation, Quality-of-Life.*

A polyantigenic immunomodulator (PAI), previously known as polyantigenic vaccine, which consists of a mixture of antigens of inactivated bacteria with antigens of influenza virus in a peanut-oil-arlacel-A-aluminium monoesterate emulsion, increased tumor resistance and induced tumor regression in tumor bearing mice (1, 2). In studies with humans this immunomodulator stimulated the phytohemagglutin (PHA) blast-transformation in cancer patients and patient with viral infections (3). The administration of PAI to HIV infected patients led to a sustained augmentation of the lymphoproliferative response to PHA, normalization of the T-helper-suppressor ratio with the disappearance of clinical symptoms and a reduction of lymph node size, weight gain and inhibition of further development of infectious processes in some AIDS patients (4). This report presents clinical and laboratory data demonstrating the effect of PAI in the long term prolongation of disease-free state in HIV positive patients.

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Patients and Methods

Patient Selection. Since AIDS has become a significant problem in Puerto Rico, awareness in the population is

relatively high. Therefore, referral to specialists who take care of HIV positive or AIDS patients is common throughout the Island. Services available include: adult and child care specialists, as well as, prenatal care for infected women and the newborn. Consequently, all patients in this study were referred to either of the two senior authors by primary care physicians or by other patients under their care.

All patients were accepted regardless of the state of the disease or the number of previous complications. However, for this study only HIV positive patients who had not developed complications or progressed to AIDS were included. There were no other exclusion criterion, with the exception that in the statistical analysis three additional patients were excluded from further analysis because they developed AIDS within the first three months of treatment with polyantigenic immunomodulator (PAI). A total of 40 patients were included in this analysis.

All patients underwent an initial evaluation that included the following: general history and physical examination; chest X-ray; CBC; platelets count; urinalysis; chemistry profile (SMAC-23); and CD_4/CD_8 subsets.

All patients referred to the clinics previously had an HIV test determination, and all had positive ELISA tests (several commercial companies) confirmed by Western Blot. If the initial report by the referring physician was doubtful, serology and Western blot were repeated.

The option of treating the patient with the polyantigenic immunomodulator was discussed amply with the patient and family. Our previous experience with PAI in the management of cancer and HIV positive patients was discussed, as was the schedule of administration, and the possible complications (3, 4). The option of no treatment, as well as, starting the patients on other existing protocols with antiviral chemotherapy was also discussed with the patients. The nature of the complications, the option of discontinuing treatment at any stage, and the results of the previous studies were fully explained to the patients. Particular emphasis was given to obtaining a history of allergy.

Preparation of the Immunomodulator. Polyantigenic immunomodulator (developed by one of us [A.M.M.] and previously known as polyantigenic vaccine) consists of a mixture of antigens from inactivated bacteria and influenza virus in a peanut-oil-arlacel-A-aluminum-monoesterate emulsion. The bacterial antigen mixture was obtained from Miles Laboratory, influenza virus antigen (Flu-vaccine whole virion) was obtained from Connaught Laboratory, and peanut oil and arlacel-A were obtained from Sigma Company. The mixture was prepared by the P.I. in our laboratory through mixing of the components until emulsification was obtained. This method has been previously reported (2).

Administration. The emulsion was prepared in small quantities and administered within 15 days of preparation. The treatment consisted of 0.3ml administered subcutaneously either in the buttock or upper arm. Care was taken to avoid injecting into a blood vessel or intramuscularly. Injection sites were routinely rotated. The schedule of administration depended on the initial CD_4 count: if the CD_4 count was greater than 400 cells per ml, PAI was injected once a month; and if less than 400, but greater than 200, the injections were administered every two weeks. For initial count less than 200, injections were given weekly. Treatment was provided indefinitely.

Follow-up and monitoring was done as outpatients in the office with the frequency depending on the number of injections to be given. During follow-up visits, in addition to the injection, a brief history of complications was obtained, as well as, general health assessment. All events, hospitalizations or changes in patient status, were documented and analyzed. There were no instances of patients non-compliance or discontinuation of therapy.

Parameters. Patients were monitored for infection, skin changes, complications of administration, changes in weight or appetite, or any other symptom or event during every visit. CD_4 and CD_8 cell sub-set determination was performed in peripheral blood using the EPICS Profile Analyzer (Coulter Corp.). In addition, the following elements of performance were noted by direct observation and interview: activity at home and with the family; work; well-being; self image; symptoms (5).

Statistical Analysis. Patient and Disease-Free Survival was analyzed using standard life table techniques. Demographic data, weight changes, CD_4 changes, and infection incidence were analyzed using both parametric (paired t-Test) and Analysis of Variance, and non-parametric statistics (Chi square and Sign Test).

Results

Demography. There was a thirty five male-to-five female disproportion (Chi square; $P = .000058 \times 10^{-5}$). The age at the time of diagnosis ranged from 28 to 68 years with a mean of 41.1 ± 10.46 (mean $\pm$ standard deviation). The definition of race in Puerto Rico is very difficult, and no attempt was made to identify this factor. AZT use was the same for both groups ($P = 0.52$).

Patient Survival. The overall patient and disease-free survival is shown in Table I. There was gradual attrition of some of the patients who developed one or more of the criteria of AIDS signifying disease progression. Eight year disease-free survival was 47%, while overall 8 year patient survival was 81%.

Table 1. Patient and Disease-Free Survival[1]

YEAR	NUMBER RISK	PATIENT SURVIVAL (%)	DISEASE-FREE SURVIVAL (%)
1	40	100	100
2	40	100	100
3	39	97	97
4	37	97	97
5	31	88	88
6	27	85	78
7	20	81	65
8	9	81	47

(1) Life-Table

Weight. We made a distinction between the patients who had not developed AIDS to date, the patients who had progression into AIDS and patients who died. As shown in Table II, the initial weight of the three groups was comparable and the difference was not statistically significant. However, the most recent weight in each group showed that patients without progression gained weight from 155.8 $\pm$ 28.5 to 164.2 $\pm$ 26.9, which was highly significant. Weight stability is also seen in

Table 2. Weight Changes[1]

	PRE-PAI	POST-PAI	
AIDS-free	153.9 ± 28	164.6 ± 27	$P = 1.2 \times 10^{-4(3)}$
AIDS	159.5 ± 32 P = NS[2]	163.9 ± 32 P = NS[2]	P = 0.8 [2]
Died	157.7 ± 23 P = NS[2]	146.8 ± 30 P = NS[2]	P = 0.10[3] P = 0.11[4]

(1) Mean in Pounds, +/- S.D.; (2) ANOVA; (3) t-test paired values; (4) sign test.

survivors with AIDS. Conversely, patients who developed AIDS and died, showed a consistent and gradual decrease in weight from 157.7 $\pm$ 23 to 146.8 $\pm$ 30, which was also statistically significant, although this was obtained with non-parametric analysis due to the size of the sample.

T Cell Changes. CD_4 changes were analyzed separately for the patients who developed AIDS, those who died and those who are alive and healthy. As shown in Table III, initial CD_4 appears to be a predictor of subsequent outcome. Patients who did not develop AIDS presented higher CD_4 levels than those who subsequently progressed into AIDS, and those who died. Although the variability was very high, the differences were significant. Also, although the groups showed a gradual decline in CD_4 cell counts, the slope of the decrease in the patients who remained disease-free was very gradual (and not statistically significant), while the drop of CD_4 cells in patients, who succumbed to the disease, was more pronounced and statistically significant.

Infections. Individuals in both groups (AIDS and HIV positive) developed infections; however, there was a

Table 3. CD_4 Changes[1]

	PRE-PAI	POST-PAI	
AIDS-free	795 ± 421	585 ± 279	P = 0.08[2]
Alive and AIDS	491 ± 255	298 ± 142	P = 0.06[3] = 0.08[2]
	P = 0.06(2)	P = 0.0097	
Died	340 ± 149 P = 0.01[2]	103 ± 88 $P = 2.5 \times 10^{-4(2)}$	P = 0.0057[2]

(1) Mean CD_4 Count +/- S.D.; (2) ANOVA; (3) T-test for paired values.

remarkable and significant difference in the number of patients who developed infections in each group. All AIDS patients developed infections, while only eight of thirty five non AIDS HIV positive patients, developed infections.

All infections were treated by one of the senior authors (G.E.) on an ambulatory basis. Occasionally, more seriously ill patients were hospitalized for closer observation and intravenous antimicrobial treatment, while patients who developed severe Pneumocystis carinii pneumonia with respiratory failure were referred to a tertiary hospital for critical care. PCP pneumonia was treated with Septra orally or intravenously, and occasionally with pentamidine. Cutaneous H. zoster was treated with oral Acyclovir and occasionally with Acyclovir intravenously, while Kaposi sarcoma was treated with Interferon. An occasional febrile sensation (fever not confirmed) was reported by some patients one or two days after the injection of PAI.

Induration (sterile abscess) at the site of injection was occasionally observed. Allergic reactions, local infection or ulceration of the injection site were not observed.

Quality of life. Was reported as excellent in all AIDS-free patients and on 90% living AIDS patients ($P = 10^{-4}$, Chi Square) using an index similar to the Karnovski Index[6]. Thirty one patients were employed full-time, six retired and worked at home, and two worked part-time.

Discussion

Intensive worldwide AIDS research has clarified a number of important aspects regarding its epidemiology, clinical course and pathophysiology[7], but in relation to treatment so far advances have not been uniformly

successful. We know the route of transmission, the role of CD_4 cells in its pathophysiology, the relationship between viremia and CD_4 levels, the types of antibodies produced against the different surface and core proteins and the progression into clinically evident disease[8-10]. Nevertheless, this knowledge has not traslated into benefits for the patient.

Routine diagnostic testing has made obvious that the so-called "clinical latency period" may be as long as ten years[11]. However, other studies recognizing the complex and variable interplay of a multitude of viral and host factors and their interactions, suggest that such long term survival, may be the exception rather than the rule. Studies have been recently published on factors responsible for the exceptional survival of these long-term disease-free patients[12]. On the other hand, once the AIDS related symptoms appear, the survival curve slopes rapidly downward with less than two years survival in most cases. Clearance of HIV infection is currently unclear[13, 14].

It is not clear in our study population at exactly what stage our cases were being referred to us. These patients, as a rule, sought medical advice from the referring physician because of suspicious symptoms or probability of contagion by having high risk behavior. We believe that our population is mixed and includes both patients early in the clinical latency period and patients in more advanced stages. The wide range of CD_4 levels in our study strongly suggests that this indeed was the situation. Furthermore, the patients who eventually died may be considered by recent criteria[15], to be in the severe suppression category suggesting imminent development of symptoms. The CD_4 levels were markedly suppressed suggesting an overwhelming viral load[16, 17].

Our patients did not accept the option of being placed in a no-treatment group because of the serious nature of their disease. They believed urgent treatment was needed due to the dismal outcome awaiting them if treatment was given. This patient cohort was not seeking to be involved in a study, but rather they wanted to receive immunomodulating experimental treatment. Thus, the groups for comparison emerged from the outcomes. Puerto Rico has one of the highest rates of HIV infection within the United States and its territories, especially in women and children (18).

Since these results compare favorably to those reported in the literature we suspect that PAI improves survival. Furthermore, the statistically significant weight gain, as well as, an improved quality of life suggests that PAI exerts a beneficial effect in the control of the disease. Larger scale studies are mandatory to confirm this positive experience (19).

Previous studies in our laboratory indicate that PAI is able to successfully limit the development of a number of very aggressive tumors in the mouse model (1, 2). Additional unpublished studies have shown that the effect of the adjuvant therapy is highly protective in the development of peritonitis and sepsis, and that this effect is further enhanced with the addition of antigen components (Santiago-Delpín, EA, unpublished results). In human studies, this immunomodulator has been shown to stimulate the phytohemagglutin blast-transformation in cancer patients and patients with viral infections (3, 4). The administration of PAI to patients with acquired immunodeficiencies leads to a sustained augmentation of the proliferative response to PHA, normalization of the helper/suppressor ratio, disappearance of clinical symptoms with a decrease in lymph node size, weight gain and a decrease in the incidence of infectious processes (4).

A number of "therapeutic vaccines" are currently being suggested as an approach in the management of HIV infection (20-23). This follows the model of other immunotherapeutic approaches for cancer (24-25). Although not strictly a vaccine approach, the immunostimulatory properties of the PAI mixture enhances the immune response and probably is the mechanism of action in the currently observed effect, with an important adjuvant contribution (29), although a direct effect could operate also (30), or indirectly, by stimulating lymphokines (31). However, in-vitro studies where lymphocytes from normal and HIV infected patients were cultured with and without PAI, showed marked inhibition of viral particle shedding (32). These studies suggest that other immunomodulatory possibilities may be operant as well. Perhaps with improvements in antiviral chemotherapy (33), combined modalities can be conceived.

From the diagnostic-prognostic point of view, this finding may help in predicting the risk of disease progression in the individual patient. Combined sequential protocols with the new proteases inhibitors to decrease viral load should be contemplated, especially in view of gene polymorphism and/or accumulated mutations showing resistance to the latter (34).

Resumen

Observaciones clínicas y resultados de laboratorios demuestran que el inmunoregulador poliantigénico (IPA) tiene el efecto de prolongar la vida libre de síntomas a pacientes infectados con el virus de la inmunodeficiencia humana (VIH). IPA consiste de una mezcla de antígenos de bacterias inactivadas con antígenos del virus de influenza inactivado en una emulsión de aceite de mani-

arlacel A - y monoesterato de aluminio. En ratones con tumores malignos el IPA aumenta la resistencia a tumores e induce regresión de los mismos.

Cuarenta pacientes positivos al VIH cuya edad media era de 41.1 ± 10.5 años y sin limitación alguna en cuanto al contaje de linfocitos TCD_4+ fueron incluidos en este estudio. El tratamiento con IPA fue una inyección subcutánea semanal a pacientes con linfocitos CD_4+ < 400 y para pacientes con CD_4+ > 400 una inyección mensual. Todos los pacientes fueron evaluados a diferentes intervalos para contajes de CD_4+, condición clínica y para toxicidad del tratamiento. Al cabo de ocho años el 81% de dichos pacientes que aún estaban con vida y el 47% permanecían libre de enfermedad. En pacientes que no han desarrollado SIDA el peso era de 153.9 ± 28 lbs antes de recibir el IPA y 164.6 ± 27 ($P = 1.2 \times 10^{-4}$) después de recibirlo. El contaje de CD_4 fue 795 ± 421 previo al IPA y 585 ± 279 post IPA (P = 0.08). Pacientes vivos con SIDA: su peso era de 159.5 ± 32 lbs previo al IPA y 163.9 ± 32 lbs post IPA (P = 0.8); el contaje de CD_4+ era de 491 ± 255 previo al IPA y 298 ± 142 post IPA (P = 0.08). Cinco pacientes contrajeron enfermedades relacionadas con el SIDA. El peso de pacientes que murieron era de 157.7 ± 23 previo al IPA y 146.8 ± 30 post IPA (P = 0.10) y el contaje de CD_4+ fue de 340.7 ± 149 previo al IPA y 103 ± 88 post IPA (P = 0.0057). Todos murieron de infecciones relacionadas con el SIDA, No se reportó toxicidad alguna atribuible al uso de IPA. El IPA mejora la calidad de vida y prolonga la supervivencia libre de enfermedad en los pacientes infectados con VIH.

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