

A NEW DIRECTION IN AIDS THERAPY

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ABSTRACT

Proline-Rich Polypeptides (PRP) are known to have the ability to both up-regulate and down-regulate the immune system. As PRP is available in abundant quantities from bovine colostrum and at low cost, a research trial using a PRP spray was designed to test its effectiveness for AIDS patients, particularly those in areas of the world where expensive pharmaceutical treatments are unavailable. Preliminary phase I studies showed promise in increasing CD4+ T-cell counts in AIDS patients, so phase II studies were carried out in Kenya and Nigeria with more patients. Results of the studies showed increases in CD4+ T-cell counts to normal or near normal levels and the remission of AIDS symptoms in most patients, as well as significant weight gains. This suggests that PRP spray may prove to be a useful, low cost, easy to use, and side effect-free adjunct therapy in the treatment of AIDS.

Background

The appearance of AIDS in the early 1980s forced medical science to reevaluate itself in regard to infectious disease in general and viral infections in particular. Prior to this time it appeared that modern medicine had infectious disease on the run. Age-old killers like polio and smallpox had been all but eliminated. There was a general feeling that all infectious diseases would be conquered in time.

AIDS, however, graphically demonstrated that our knowledge of viruses and how to treat viral infections was horribly lacking. Our ignorance of the retrovirus that finally was linked to the disease and our futile attempts to control it compelled new approaches and ways of thinking.

In the quarter century since AIDS first appeared, a great deal has been learned about viruses in general and retroviruses in particular. Many new treatments have emerged for both HIV

infection itself and the opportunistic diseases which take advantage of the compromised immune systems of AIDS sufferers. Yet still the cure for the disease eludes us, as does an effective vaccine. According to the Joint United Nations Programme on AIDS and the World Health Organization (WHO), some 25 million people have died of AIDS in those 25 years (that's a million a year), and an estimated 38.6 million are infected with the virus, making it one of the most lethal epidemics in history. In 2005, AIDS claimed 2.4-3.3 million lives, including over 570,000 children¹.

While sub-Saharan Africa has been hardest hit, AIDS is a major problem globally. Over one million are reported to be infected in China and six million in India. More than a half million have died from AIDS in the US, and over a million are infected². In Botswana, roughly one in three people in the entire country are infected, and life expectancy has declined from 65 pre-AIDS to only 40 today³.

Efforts continue to find both a cure for AIDS and an effective vaccine to prevent further AIDS infections. Yet the very nature of retroviruses make them an exceedingly difficult target.

HIV is a single-stranded, positive-sense, enveloped RNA virus. When the virus infects a cell, its RNA is encoded into a double-stranded DNA molecule by a virally encoded reverse transcriptase molecule present in the viral particle. The viral DNA is then integrated into the cellular DNA by a virally encoded integrase enzyme. Often the virus will become latent at this stage, making any antiviral treatment impossible until it once again becomes active. This latency period can last for years. When the virus becomes active, it replicates and produces large numbers of viral particles that are then released to infect other cells.

What is particularly lethal about HIV is that it primarily infects the very cells in the immune system that would normally keep it in check – CD4+ T cells, macrophages and dendritic cells. Infection of CD4+ cells kills in three different ways: direct viral killing of the cells; increased rates of apoptosis (programmed cell death) in infected cells; and targeting of CD4+ cells by CD8 cytotoxic lymphocytes that recognize infected cells and destroy them. This loss of CD4+ cells is cumulative, and eventually the numbers of CD4+ cells decline below critical levels to where cell-mediated immune function is lost. This leaves the body open to opportunistic infections like *Pneumocystis pneumonia* and Kaposi's sarcoma, which are what actually kill victims. By robbing the body of its own defenses, HIV ultimately kills its host, though at times over a period of years. The virus also mutates rapidly making it difficult to produce an effective vaccine.

The main strategy that the scientific community has used in its attempts to attack HIV reflect the trends used against other pathogens, namely a pharmaceutical strategy to directly attack the virus. As such the antiviral drugs that have been developed to combat HIV have many of the same limitations as previous pharmaceutical drugs developed to combat viral infections. First, they target the infected cells directly, usually by disrupting their ability to replicate the virus. Unfortunately, many uninfected cells in the area of the infected cells are collaterally affected and killed. These drugs are also not effective in all patients.

Secondly, all of the antivirals developed to fight HIV have serious side effects, including nausea, diarrhea, vomiting, anemia, and others. Finally, these drugs are very expensive and thus not available to those who have no insurance coverage or other means of paying for them. This is a major problem in Africa where nearly all AIDS victims have no means to pay for expensive antiretroviral therapies (ART). Combination therapy, which is currently the treatment of choice, costs about \$950 a month. Drug companies have lowered their prices in some African countries to about \$500 a month, but this is still far beyond most people's ability to pay. The average monthly salary among middle class wage earners in Uganda, for example, is only about \$400 a month⁴.

Currently the FDA has approved 29 pharmaceutical drugs for use in the treatment of HIV infection⁵. Nearly all inhibit viral replication and include reverse transcriptase inhibitors and protease inhibitors. One, Fuzeon, blocks viral fusion to target cells. HIV has responded by developing resistant

strains that are not affected by the drugs, even combinations of them. The future outlook for AIDS treatment from a pharmaceutical perspective remains bleak despite significant gains in understanding the virus.

This situation has forced scientists to look elsewhere for effective solutions. ART focuses primarily on attacking infected cells directly. A more effective method would be to stimulate the body's own defenses to attack the virus as well as infected cells. This would make it much more difficult for the HIV to avoid attack through mutation as the immune system has the ability to adapt to the new strains rapidly.

One such area of investigation is based on an old remedy, colostrum, the first milk produced by a mammal following the birth of a newborn, which was widely investigated as an antibiotic before modern antibiotics were developed. Colostrum and derivatives of it have already demonstrated their usefulness in treating opportunistic infections associated with AIDS, such as diarrhea caused by *Clostridium*, *Campylobacter* and *Amoeba* spp⁶. Specifically one of the components of colostrum, called alternatively PRP (proline-rich polypeptide), transfer factor, dialyzable leukocyte extract (DLE), infopeptides, or colostrinin, has shown great promise. This unique polypeptide (actually a peptide fraction of whole colostrum) has been shown to have immunomodulatory abilities as well as antiviral activity⁷.

The principal immunomodulatory action of PRP is to stimulate the maturation of immature thymocytes into either helper or suppressor (also called regulatory) T cells^{8,9}, depending on the need of the body at the time. Helper T cells present antigens (such as a viral protein) to B lymphocytes, which then

produce antibodies to that antigen¹⁰. Helper T cells also help produce memory T cells which retain the "memory" of an antigen in order to expedite the production of antibodies in the event the antigen is reencountered in the future¹¹. Suppressor T cells, on the other hand, deactivate other lymphocytes after an infection has been cleared to avoid damage to healthy tissues¹². PRP also promotes the growth and differentiation of B cells in response to an infection¹³ and the differentiation and maturation of macrophages and monocytes¹⁴. The activity of Natural Killer (NK) cells, cytotoxic cells of the innate immune system, was increased up to 5 times by PRP^{15,16,17}.

PRP modulates the cytokine system as well. It stimulates the production of a wide range of cytokines, including the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF- α), which initiates the inflammatory cascade of cytokine production, and interferon-gamma (INF- γ), and the anti-inflammatory cytokines interleukins-6 and -10 (IL-6 and IL-10)¹⁸.

PRP functions as a molecular signaling device which works through receptors on target cell surfaces¹⁹ to initiate or suppress the production of specific proteins. It is not species specific; PRP from bovine colostrum works as effectively in humans as PRP from human colostrum²⁰. As it is a natural product, there are no known side effects or drug interactions, and it can be taken safely by all ages.

Preliminary experimental and clinical studies have shown that PRP holds great promise in combating AIDS. In an experimental *in vitro* system, PRP blocked HIV infection of cells²¹. PRP in combination with zidovudine (ZDV), an anti-retroviral drug, is known to be

effective in patients suffering from AIDS-Related Complex (ARC), increasing levels of white blood cells, CD8 lymphocytes and IL-2²². A preliminary study on 25 men with AIDS resulted in clinical improvement or a stabilized clinical condition in 20 of the 25. 12 of 14 anergic (unresponsive to antigenic stimulation) patients demonstrated restored delayed type hypersensitivity to recall antigens within 60 days²³.

Recent research has found that while HIV targets both helper CD4+ and suppressor (or regulatory) CD4+ T cells, they are not suppressed at the same rate. In fact, regulatory T cells decline at a slower rate than helper T cells. As regulatory T cells actively down-regulate the immune response, the disparity between regulatory T cells and helper T cells tends to accelerate the course of the disease and is a strong clinical predictor of CD4+ depletion and death²⁴. The immunomodulatory effect of PRP could potentially help restore the balance of helper and regulatory T cells.

Material and Methods

Study Design

With this alternative treatment approach in mind, clinical trials were developed to test a new oral spray product containing colostrum-derived PRP as well as other growth and immune factors, including trypsin inhibitors, glycoconjugates, orotic acid, lysozyme, and others. This was an open-label intervention/prospective trial to determine the effect of Cytolox[®] on AIDS patients.

Inclusion Criteria

Patients were eligible to participate in the study if they met the following criteria:

- 1) HIV seropositive
- 2) Symptomatic or asymptomatic
- 3) 18 years or older
- 4) Willing to maintain a proper diet and drink at least 5-8 8 oz. glasses of water a day
- 5) Be willing and able to take the Cytolox[®] PRP spray regularly, three sprays every four hours around the clock
- 6) Report for weekly follow-up visits to a physician for the duration of the study (30 days)

Potential participants were excluded if they had been treated with any other investigational drug(s) within the 30 day period prior to the onset of the study. Women who were or thought they might become pregnant during the study were also excluded.

Dosage

Three sprays (2 ml total) of Cytolox[®] were given every four hours.

Measurements

Patients received an initial evaluation to assess the severity of the disease, have their weight measured, and blood drawn to measure baseline CD4+ counts. A Symptom Assessment Form was completed to evaluate clinical symptoms, including diarrhea, nausea, vomiting, fever, cough, tuberculosis, fatigue/malaise, paresthesia, and any other symptoms. Patients then returned to the clinic weekly for follow-up assessments and measurements, including the Symptom Assessment Form. Physical findings included weight, chest exam, liver or spleen enlargement, lymphadenopathy, hair changes, and the presence or absence of skin rash or herpes zoster. Lab tests were performed for CD4+ lymphocyte

counts, white and red blood cell counts, hemoglobin, hematocrit, sed rate, and chest X-ray (if deemed necessary). A record was also kept of other medications the patient was receiving. Patients were also requested to report any adverse reactions they might experience. Patients were also requested to sign an informed consent form.

At the end of the trial period, patients were allowed to continue or discontinue use of the spray at their or their physician's discretion. Patients were discontinued from the study if they failed to comply with taking the test product, they had an adverse reaction that was unacceptable to them or their physician, if their physician decided it was in the patient's best interest, or if the patient wished to discontinue for any reason.

Safety

As Cytolox[®] is a food substance derived from colostrum rather than a drug, it is entirely safe for human consumption. There are no known side effects nor drug interactions. While those with lactose intolerance could potentially suffer an allergic reaction, such occurrences are exceedingly rare.

Results

Phase I trials were conducted at the Infectious Disease Clinic in Dayton, Ohio, from February to April, 1996. Phase II trials were conducted at the University of Nairobi, Nairobi, Kenya, and in Nigeria from March to August 2000. A total of 39 patients took part in the two studies. Results of the Nigerian study are summarized in Tables 1-4.

	Initial	30 Day	60 Day	90 Day
Total Patient Reports	35	31	20	17
Score	6.1	1.8	1.2	1.3
Percent Reduction		69	80	79
Expected Phase III % Reduction		50-70	60-80	75-85

Table1. Clinical Symptoms Score.

	Initial	30 Day	60 Day	90 Day
Total Patient Reports	30	27	13	13
Score	4.0	2.5	2.1	1.6
Percent Reduction		38	49	60
Expected Phase III % Reduction		30-50	40-60	50-70

Table 2. Physical Findings Score.

	Initial Total	30 Day Total	60 Day Total
	92,448	9,755	445
	28,049	625	n/a
	33,093	239	n/a
	439	n/a	175
	59,821	n/a	320
	40,381	180	n/a
Expected Phase III % Reduction		<1,000	<500 (<250 at 90 days)

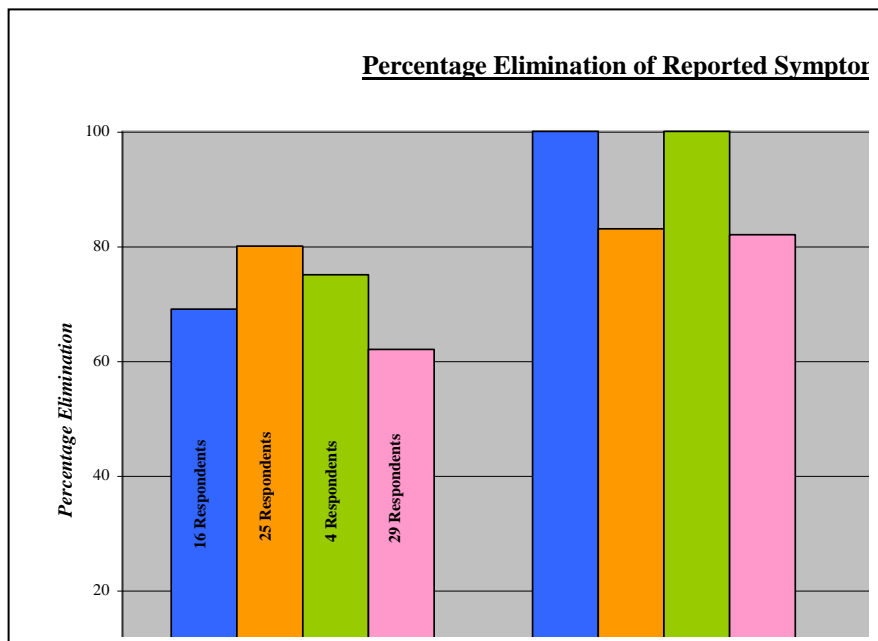
Table 3. Viral Load. Viral load counts in six patients from the Phase II Trial.

	Initial Total	30 Day Total	60 Day Total
	74	153	121
	274	282	n/a
	245	301	n/a
	60	47	n/a
	101	n/a	117
	211	n/a	291
	249	271	n/a
Expected Phase III % Reduction		>250	>250 (>500 at 90 days)

Table 4. CD4+ Count. Only available for seven patients from the Phase II Trial.

The status of specific clinical conditions during the two studies. Results are in the patients was also monitored shown in Tables 5 and 6.

Table 5. Relief of diarrhea, nausea, vomiting, and fatigue/malaise in trial participants over the 90 days of the study



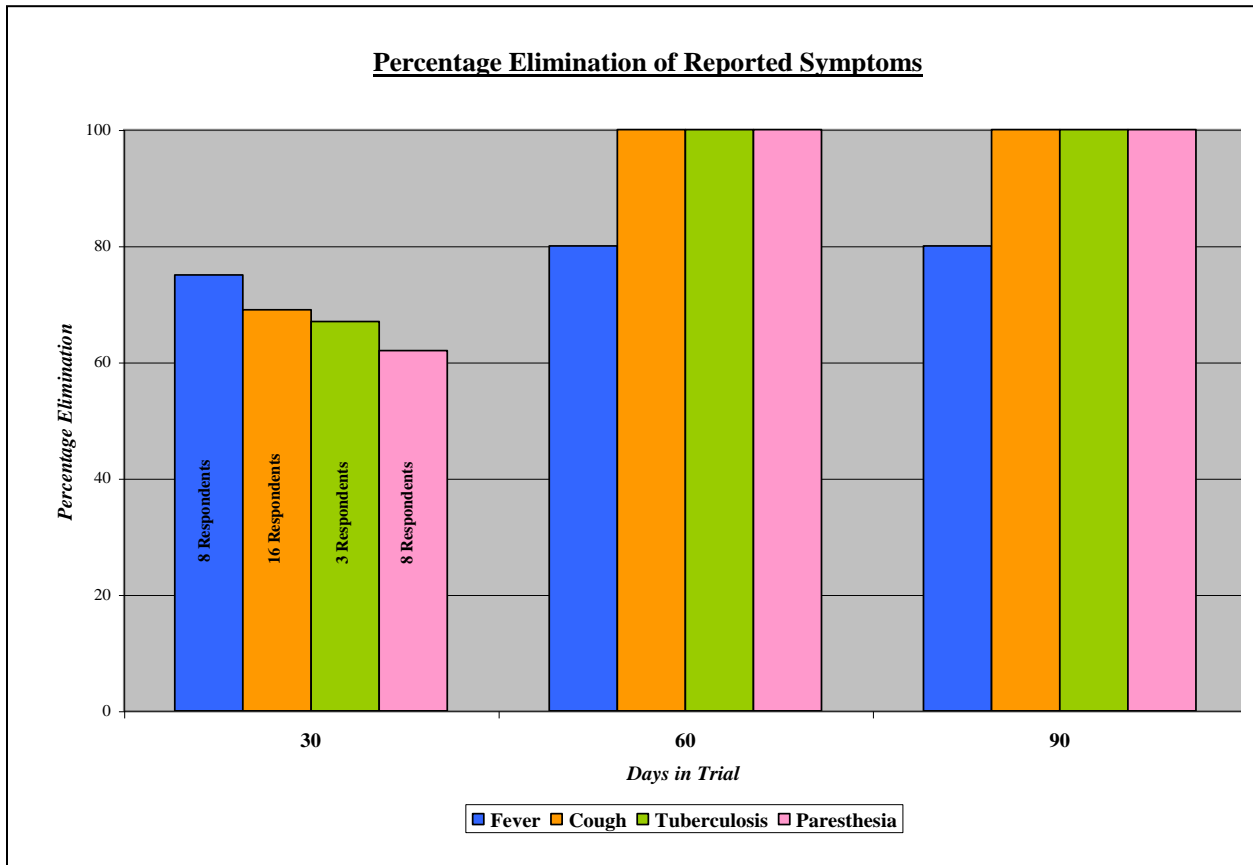


Table 6. Relief of fever, cough, tuberculosis and paresthesia in trial participants over the 90 days of the study.

A comment on the CD4+ results. CD4+ counts are a valid marker of the progression of the HIV infection. However, CD4+ levels are only one measure of wellness. With PRP treatment, CD4+ levels are likely to normalize more slowly than other measures of wellness. Viral load levels may actually increase in the peripheral blood after initiation of treatment as the virus is prevented from entering T cells, particularly in the lymph nodes. This increase in blood levels of HIV causes a temporary drop in CD4+ levels in the peripheral blood. CD4+ levels do increase over time with continued treatment. Normal CD4+ counts for adults range from 500-1500 cells/mm³.

In the Nigerian study, PRP oral spray products were shown to boost T-cell (CD4+) levels to normal or near-normal levels (median 502, none less than 300) in AIDS patients whose T-cell levels prior to treatment were well below normal (median 275) (see Tables 13 and 14). Along with the increase in T-cells came a remission of AIDS symptoms within two days of start of treatment, including nausea, vomiting and diarrhea. Weight gains of up to 5% were recorded (Table 15). Patients taking the PRP spray fared much better in terms of quality of life than did patients on anti-retroviral drugs.

	Before		After	
	No.	%	No.	%
150-200	17	29%	0	0%
201-250	10	17%	0	0%
251-300	21	36%	0	0%
301-400	4	7%	8	14%
401-500	4	7%	19	33%
501-600	2	3%	12	21%
601-1000	0	0%	19	33%
Totals	58		58	

Table 7 . CD4+ counts in 58 experimental subjects before and after application of oral PRP spray.

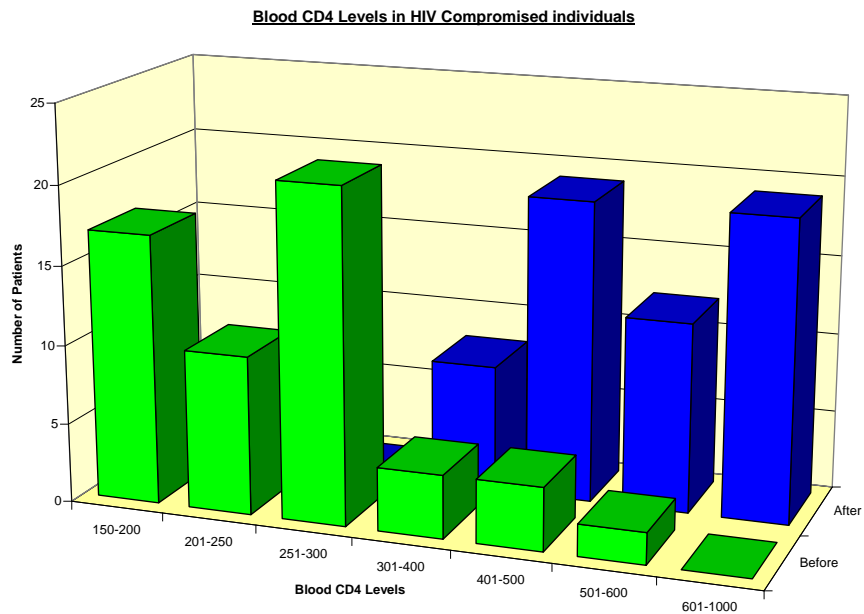


Table 8. CD4+ lymphocyte levels in HIV compromised individuals before and after treatment with PRP oral spray. This bar graph clearly illustrates the marked increase in CD4+ lymphocyte counts in patients with long-term AIDS and severely depleted CD4+ counts after administration of oral PRP spray. Results from Trial 1 held in Nigeria.

Blood CD4 Levels in HIV Compromised Individuals
Trial 2

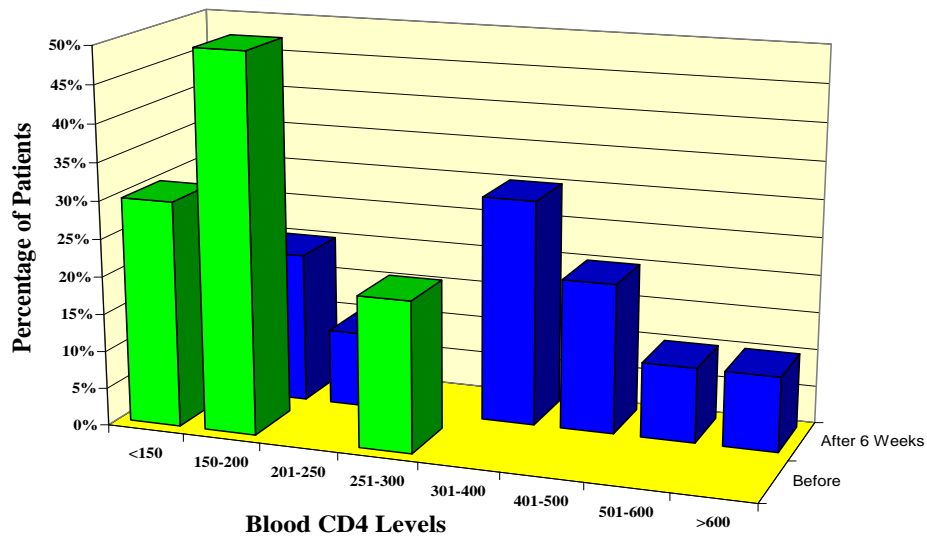


Table 9. Cd4+ lymphocyte levels in HIV compromised individuals before and after treatment with PRP oral spray. These results are from Trial 2 held in Kenya.

Change In CD4 Levels By Patient

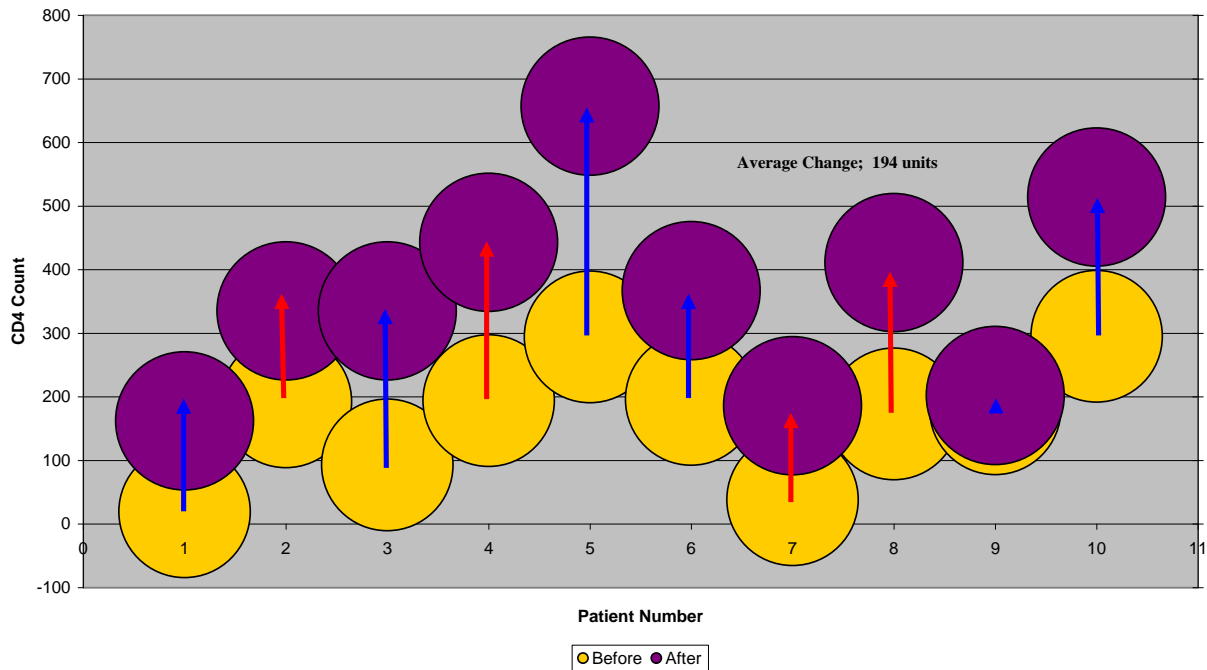


Table 10. A graphical representation of changes in CD4+ lymphocyte levels in patients participating in Trial 2. While levels for some increased over 100% in some cases, what is particularly significant is that levels increased for all participants in the study.

Observed Weight Gain / Loss with Treatment

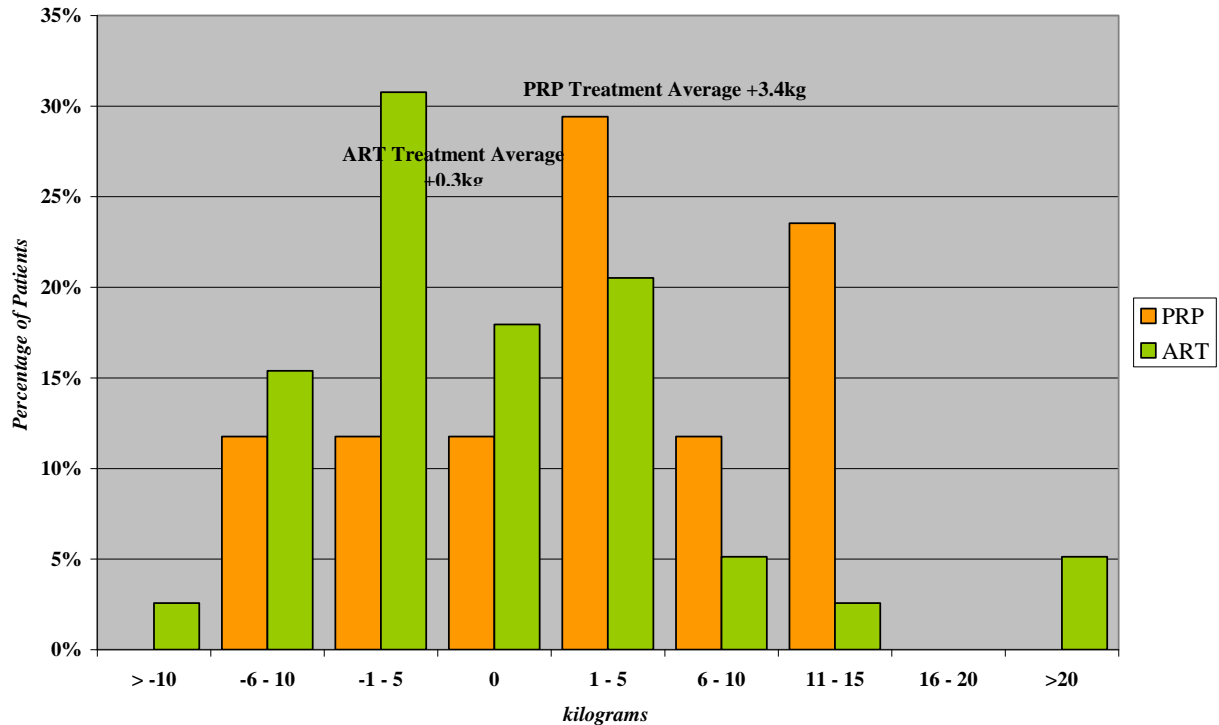


Table 11. Weight loss/gain for patients on oral PRP or anti-retrovirus therapy.

Conclusions

The results of the African trials confirm the earlier results that an oral PRP spray treatment can be an important alternative or adjunct therapy for AIDS patients. Further studies will be needed to study the long-term effects of the therapy and whether treatment over a longer period can eliminate the virus from the body. Phase III trials are currently underway in India under the

auspices of WHO, and results should soon be available.

In addition to Cytolox[®], there are two similar products on the market, Immulox[®] (which is similar to Cytolox[®]) and Viralox[®]. Viralox[®] includes lactoferrin along with the PRP. Comparison trials with Viralox[®] and Cytolox[®] are scheduled in the near future in Africa.

¹ Figures cited in Wikipedia article on AIDS, <http://en.wikipedia.org/wiki/AIDS>

² The Henry J. Kaiser Family Foundation: **HIV/AIDS Policy Fact Sheet** (November 2005).

³ United Nations Department of Economic and Social Affairs/Population Division report: **The Impact of AIDS** (2004). <http://www.un.org/esa/population/publications/AIDSimpact/AIDSWebAnnounce.htm>

⁴ BBC News, 24 October 2000, **AIDS Drug Factfile**
http://news.bbc.co.uk/1/hi/health/background_briefings/aids/988245.stm

⁵ U.S. Food and Drug Administration, "Drugs used in the treatment of HIV infection" (August 2006).
<http://www.fda.gov/oashi/aids/virals.html>

⁶ Floren, CH, Chinenye, S, Elfstrand, L, Hagman, C, Ihse, I. **ColoPlus, a new product based on bovine colostrum, alleviates HIV-associated diarrhea.** *Scandinavian Journal of Gastroenterology* 41(6):682-686 (2006). HIV-associated diarrhea is common in AIDS patients in developing countries particularly. It is a major contributory factor in the decrease in the helper CD4+ T-cell population. ColoPlus is a product derived from bovine colostrum which is rich in immunoglobulins, growth factors, antibacterial peptides and nutrients. It is specially designed for slow passage through the gastrointestinal tract and high nutritional value. The study was undertaken to see if oral doses of ColoPlus could influence HIV-associated diarrhea. The study was done in Nigeria and included 30 patients with HIV-associated diarrhea. The patients were treated for 4 weeks in an open-labeled, non-randomized trial. Results of the study showed a marked reduction in stool evacuations per day from 7 ± 2.7 to 1.3 ± 0.5 , a decrease in self-estimated fatigue of 81%, an increase in body weight of 7.3 kg per patient, and a 125% increase in CD4+ count.

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⁹ Wieczorek, Z, Zimecki, M, Spiegel, K, Lisowski, J, Janusz, M. **Differentiation of T cells into helper cells from immature precursors: identification of a target cell for a proline-rich polypeptide (PRP).** *Archivum immunologiae et therapiae experimentalis (Warszawa)* 37(3-4):313-322 (1989). The precursors of helper T cells belong to a minor thymocyte subset bearing the Thy-1 +/-, H-2+, L3T4-, Iy2-, CD3- phenotype. PRP induced the production of antigens consistent with mature helper T cells

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¹⁶ **Effects of Oral Dietary Supplementation with Ai/E¹⁰® Upon Natural Killer (NK) Cell Activity in a Healthy Human Population.** Quantum Research, Inc., Scottsdale, Arizona (2001). Dialyzable Leukocyte Extract (DLE) was administered to 12 healthy male and female subjects aged 24-63. Natural Killer (NK) cell activity was prior to initiation of the study and after completion of the study. NK cell activity averaged 30 lytic units (LU) prior to the study and 101 LU following the study for an average increase of 207%.

¹⁷ **An Examination of Immune Response Modulation in Humans by Ai/E¹⁰® Utilizing A Double Blind Study.** Immune Consultants, Inc., Tucson, Arizona (2001). 20 subjects, 10 men and 10 women, ranging in age from 32-61 participated in a double blind study in which 10 received DLE and the other 10 received placebo. 7 of the 10 receiving the DLE had a significant increase in three major immune markers: NK cell activity, TNF- α levels, and phagocytic index (PI), an indicator of macrophage activity. Those receiving placebo had mixed results.

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²² Raise, E, Guerra, L, Viza, D, Pizza, G, De Vinci, C, Schiattone, ML, Rocaccio, L, Cicognani, M, Gritti, F. **Preliminary results in HIV-1-infected patients treated with transfer factor (TF) and zidovudine (ZDV).** *Biotherapy* 9(1-3):49-54 (1996). HIV-1 specific transfer factor (an alternative name for PRP) plus zidovudine (ZDV) was tested for efficacy in patients with AIDS-related complex (ARC). Patients receiving both transfer factor and ZDV experienced an increase in white blood cells, CD8+ lymphocytes and IL-2 levels over those receiving ZDV alone.

²³ Pizza, G, Chiodo, F, Colangeli, V, Gritti, F, Raise, E, Fudenberg, HH, De Vinci, C, Viza, D. **Preliminary observations using HIV-specific transfer factor in AIDS.** *Biotherapy* 9(1-3):4-47 (1996). 25 HIV infected patients at various stages (CDC stages II-IV) were treated with HIV-specific transfer factor (PRP) for periods of 60-1870 days. All patients were receiving antiviral treatment as well. Clinical improvement or a stabilized clinical condition was observed in 20 of the 25, and 12 of 14 anergic patients showed restored delayed hypersensitivity reactions to recall antigens within 60 days. Treatment was well-tolerated and appears beneficial to AIDS patients.

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helper ratio in many patients with advanced disease. This skewing may contribute to T cell effector dysfunction.