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Polymerase chain reaction evidence for human immunodeficiency virus 1 neutralization by passive immunization in patients with AIDS and AIDS-related complex

(immunotherapy)

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ABSTRACT **We tried to assess the long-term safety and potential efficacy of passive immunization in AIDS-related-complex (ARC) and AIDS patients. We also wanted to establish whether hyperimmune plasma from healthy human immunodeficiency virus 1 (HIV-1)-infected individuals clears the cell-free virus from circulation. Using the polymerase chain reaction (PCR), we were able to provide conclusive evidence that hyperimmune plasma is effective and maintains long-term neutralization of viremia. Using the cell test, we found that in most patients the total antibody level was maintained; in one of the ARC patients, it actually increased 8-fold and has remained at that level for nearly 2 years. The CD4$^+$ cell count decreased in the AIDS patients but was stable in the ARC patient. Clinically, there was an initial improvement in all patients, but five of six of the advanced/terminal AIDS patients had died by month 17. Our studies suggest that passive immunization may be safe in ARC and AIDS patients. It reduces HIV-1 viremia to levels undetectable even by PCR. To advanced/terminal patients, the benefit is of limited duration, while to ARC patients it may be long-term. Therefore, passive immunization should start early in the disease.**

Infection by the human immunodeficiency virus (HIV) elicits an efficient immune response (1–3), but this confers only temporary protection, averaging about 9.8 years (4), against the development of disease, as most infected individuals develop the acquired immunodeficiency syndrome (AIDS) or the AIDS-related complex (ARC). HIV infects helper T cells (5) and depletes the CD4$^+$ cells, which are essential for the generation of antibodies and cell-mediated immunity. Without them, a severe deficiency in immune response develops.

The development of an effective vaccine against HIV appears to be difficult. In experimental work, vaccinated chimpanzees developed and maintained high levels of antibodies, yet when challenged by live HIV-1 they became infected (6, 7). Similarly, passive immunization of chimpanzees with human immune globulin failed to protect the animals against experimental challenge with HIV (8). On the other hand protection against infection was conferred on eight of nine rhesus monkeys that were immunized with formalin-inactivated whole simian immunodeficiency virus (SIV) vaccine (9).

We and others (2, 10, 11) have found that healthy HIV-1-infected individuals have neutralizing antibodies to the virus, whereas AIDS patients lack them, while other groups could not find a clear difference in the pattern of neutralization response between the healthy HIV-infected individuals and AIDS patients (12, 13). Neutralizing antibodies may delay the development of ARC and AIDS. In March 1988 we undertook an open uncontrolled trial at Saint Stephen's Hospital (London) to determine the long-term effects of passive immunization on 10 ARC and AIDS patients. These patients have received monthly transfusions of hyperimmune plasma, collected from healthy HIV-1-infected individuals with high titers of antiviral antibodies. In a previous paper, we reported the experimental foundations for the trial, its design, and the results up to the end of month 3 (10). We have studied only 10 patients, most of whom had long been ill and had many of the opportunistic diseases that affect AIDS patients; 5 also had Kaposi sarcomas, and 1 had developed a non-Hodgkin lymphoma. In advanced ARC and AIDS patients, disease progression is usually rapid. A 22-month follow-up of the present group of patients demonstrated that passive immunization is a safe procedure and may benefit patients with ARC; in patients with advanced AIDS, improvement lasted for an average of 10 months, but later 5 of 6 died. Using the polymerase chain reaction (PCR), we now provide conclusive evidence that neutralizing antibodies to HIV-1 clear the plasma from HIV-1 and maintain long-term neutralization of viremia.

METHODS

Trial Design, Patients, Plasma Collection, and Processing. These have been described in detail (10). ARC patients who had remained HIV p24 antigen-negative for 4 consecutive months had their monthly plasma infusion reduced by half (from 500 ml to 250 ml), while patient 3 received only 125 ml of plasma per month during months 11 and 12, no transfusion during months 13 and 14, and again only 125 ml per month from month 15 to 20.

PCR Amplification of HIV-1 DNA and RNA from Serum. Coded serum samples (250 μl each) from the trial five AIDS and three ARC patients were used in this study. Total nucleic acid was isolated from serum, and PCR amplification was performed with primers derived from the *gag* region of the HIV-1 genome (SK 38 and SK 39) (14). Under the conditions of our assay, as little as 5–10 copies of viral DNA could be detected with this primer pair. After amplification, the products were hybridized with an end-labeled oligonucleotide

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Abbreviations: PCR, polymerase chain reaction; ARC, AIDS-related complex; HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; AZT, 3′-azido-3′-deoxythymidine.

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Table 1. HIV-1 p24 antigen levels and detection of HIV-1-specific DNA and RNA in plasma by PCR

Patient no.	HIV-1 p24 antigen level in serum* and PCR detection (+/−) at monthly infusions																													
	Pre-1st			Post-1st		Pre-2nd			Pre-3rd			Pre-4th			Pre-5th			Pre-6th			Pre-7th			Pre-8th			Pre-9th			Pre-10th		
	DC	AB	PCR	DC	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR
1	10	190	+	0	+	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	E	−	0	60	−	0	110	−	0	40	−
2	30	380	+	0	+	0	40	+	0	E	−	0	0	−	0	0	−	0	350	+	0	E	−	0	0	−	0	120	+	0	0	−
3	50	520	+	0	+	0	0	+	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	30	−	0	0	−
4	210	2080	+	0	+	0	0	+	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	20	−	0	270	−	0	2740	−
5	65	200	+	0	+	0	320	+	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	30	0	−	125	0	+
6	80	310	+	0	+	0	0	+	0	0	−	14	170	−	18	560	−	0	0	−	0	0	−	0	0	−	0	E	−	0	120	+
7	100			0		†			†																						
8	100	360	+	0	+	0	0	+	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	E	−	0	0	−	0	0	−
9	120	380		0		0	0		0	0		0	0					‡														
10	340	2640	+	0	+	0	30	+	0	80	−	0	50	−	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−

DC, DuPont and/or Coulter (pg/ml); AB, Abbott (units/mmol); E, equivocal; †, loss of contact; ‡, death.
*In pg/ml for DC and in units/mmol for AB.

probe (SK 19) and run on 12% polyacrylamide gels followed by autoradiography. The presence of a 114-base-pair (bp) fragment was indicative of HIV-1 sequences in the sample. A pair of primers derived from the *HLA-DQα* chain locus was included in all samples as in internal control for verifying the capability of the sample to be amplified.

HIV p24 Antigen Assay. All samples were assayed according to the manufacturer's instructions by commercial HIV p24 ELISA kits (Abbott and DuPont or Coulter).

Assay for Antiviral Antibodies. The principles and procedures of the Karpas cell test (2) and the methodology to determine total antiviral titers (10) have been described. Sharp increases or decreases in antibody titers were confirmed by comparing the earlier serum samples in parallel on the same slide.

Immunoblots (Western Blots). Serum samples from each of the patients were obtained before and immediately after every transfusion and were assayed for the profile of antibodies to the various HIV-1 structural proteins. Initially DuPont Western blots were used, but Bio-Rad strips were used later for reasons of economy. The assays were performed according to the particular manufacturer's instructions.

RESULTS

Clinical Development. The detailed condition and illnesses of each patient prior to and during the 22 months of passive immunization will be reported (15). The six AIDS patients all had advanced disease with opportunistic infections, and five also had Kaposi sarcoma. The ARC patients were not on any antiviral drugs nor did they receive prophylactic treatment against pneumocystis carinii pneumonia.

PCR Amplification of HIV-1 DNA and RNA from Serum. HIV-1 sequences were detected in all patients before the administration of hyperimmune plasma and at the end of month 1 (Table 1). Both viral DNA and RNA became undetectable by the end of the second month after infusion and by and large remained so for the length of the study. Thus, the PCR results suggest that <5–10 copies of the viral genome were detectable in the plasma of patients who received monthly transfusions of hyperimmune plasma. Amplification of HLA sequences was observed in all samples, indicating that DNA was present.

Viral p24 Antigen. In contrast to the PCR, which gave a positive reaction for viral nucleic acids after the first transfusion and before and after the second transfusion, the ELISA antigen tests recorded immediate antigen clearance (Table 1). From month 2 onward there was a good correlation between the PCR and the DuPont and Coulter tests. The Abbott test gave far higher readings than either of these tests with occasional positive readings, which were inconsistent with the other ELISA test or the PCR.

Levels of Antiviral Antibodies. In most patients the titer of antiviral antibodies was maintained (Table 2). By contrast, the titer of antibodies of three AIDS patients who had previously been monitored kept dropping (unpublished observation). The most remarkable observation was the 8-fold increase (from 1:160 to 1:1280) in the titers of antibodies of ARC patient 3. He has maintained a high level of antibodies in the past 22 months in spite of receiving only 250 ml from the fifth through the 10th monthly transfusion and only 125 ml per month during months 11 and 12. Infusions were discontinued for 2 months, but after HIV p24 antigen reappeared with the Abbott test, the monthly transfusions of 125 ml were continued. Patient 1 had a moderate increase in the level of antibodies.

Helper T Cell ($CD4^+$). Circulating $CD4^+$ cells were counted before each transfusion. The already low helper T-cell count in the AIDS patients continued to fall, but in the three ARC

Table 2. Antiviral antibody titers as monitored by the Karpas cell test

Patient no.	Anti-HIV-1 antibody titers at monthly infusions										
	Pre-1st	Post-1st	Pre-2nd	Pre-3rd	Pre-4th	Pre-5th	Pre-6th	Pre-7th	Pre-8th	Pre-9th	Pre-10th
1	1:320	1:320	1:640	1:320	1:320	1:320	1:640	1:320	1:1280	1:640	1:640
2	1:160	1:160	1:160	1:320	1:320	1:320	1:320	1:320	1:320	1:160	1:160
3	1:160	1:160	1:1280	1:640	1:640	1:1280	1:640	1:640	1:2560	1:2560	1:5120
4	1:320	1:320	1:640	1:640	1:640	1:320	1:320	1:320	1:320	1:640	1:320
5	1:80	1:80	1:160	1:160	1:160	1:160	1:160	1:80	1:160	1:80	1:40
6	1:160	1:160	1:160	1:160	1:160	1:160	1:320	1:160	1:160	1:320	1:160
7	1:80	1:80	*	*							
8	1:40	1:40	1:40	1:80	1:80	1:80	1:40	1:40	1:40	1:40	1:80
9	1:160	1:160	1:320	1:160	1:160		†				
10	1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:160	1:320

*, Loss of contact; †, death; ND, not done.

HIV-1 p24 antigen level in serum* and PCR detection (+/−) at monthly infusions																																
Pre-11th			Pre-12th			Pre-13th			Pre-14th			Pre-15th			Pre-16th			Pre-17th			Pre-18th			Pre-19th			Pre-20th			Pre-21st		
DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR	DC	AB	PCR
0	0	−	‡																													
0	0	−	0	260	−	‡																										
0	E	−	0	0	−	0	0	−	0	270	−	13	120	−	0	100	−	17	140	−	0			0	0	−	0	0	−	0	0	−
0	410	−	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	0	−	0	0		0	0	−	0	0	−	0	0	−
0	0	−	0	0	−	0	0	−	0	0	−	0	0								‡											
0	0	−	0	0	−	0	0	−	0	0																						
															‡																	
0	0	−	0	340	−	0	0	−	0	0	−	0	0		0	0		0	0		0	0		0	0	−	0	0	−	0	0	−
0	0	−	0	0	−	0	0	−	0	0	+	‡																				

patients (nos. 1, 3, and 4), the number of $CD4^+$ cells remained by and large stable (Table 3).

Western Blots. Western blots of each patient's serum before the first transfusion revealed a low level or depletion of antibodies to viral components in all patients. These ranged from a single weak positive band of antibodies to the gp160 in the serum of patient 8 to the presence of antibodies to most structural proteins in patient 3. However, the serum of even this patient lacked detectable levels of antibodies to p55 and p31, while the level of antibodies to the other structural proteins was lower than that of healthy donors. Fig. 1 shows that after the transfusion of 500 ml of plasma, which represents <10% of the total blood volume, all of the missing bands reappeared in the AIDS patients (no. 8) and in the other eight patients (data not shown). Since all of the missing bands reappeared immediately after the transfusion, they must represent the transfused antibodies. By the end of each month, many of the bands in the AIDS patients became weaker or undetectable, and several bands in the ARC patients' sera were reduced in intensity. After every transfusion, the bands either reappeared or their intensity was restored.

Donors. To date, 90 HIV-1-positive people have donated plasma, several of them two or three times. One who donated five times between September 1988 and August 1989 was also monitored for his $CD4^+$ cell count. Table 4 shows that the number of his $CD4^+$ cells did not drop during the year in which he donated five liters of plasma. Two others who have donated three times since 1985 remain well. So far only one of the donors has progressed to AIDS (9 months after donation). In a control group of 25 potential donors who did not donate, two progressed to AIDS and one to ARC (10).

DISCUSSION

Our earlier work suggested that neutralizing antibodies might have protective function as they could be detected in healthy HIV-1-infected individuals but not in those with AIDS. Neutralizing antibodies are incapable of eliminating the infection and of providing lasting protection against the development of ARC and AIDS in the majority of infected individuals. Nevertheless, we have found a clear relationship between the presence of neutralizing antibodies, a high titer of antiviral antibodies in the cell test, the absence of viral p24 antigen, and clinical well being. This led us to initiate passive immunization (10). The recipients fell into two groups. The first comprised advanced AIDS patients who had had a number of opportunistic infections, including one patient (no. 9) who received his first infusion during his third episode of pneumocystis carinii pneumonia. Four AIDS patients (nos. 5, 6, 8, and 10) had been taken off AZT (3′-azido-3′-deoxythymidine) before passive immunization started because of its cytotoxic side effects; none received AZT after treatment started. The second group was composed of ARC patients who had also been persistently HIV p24 antigen-positive for at least 6 months and were PCR-positive when tested before the trial started. They had declined treatment with AZT. One of these (no. 2), who had a $CD4^+$ cell count of $40/cm^3$, was in fact an AIDS patient, since he was found to have also a non-Hodgkin lymphoma. Hence, we encountered a wide range of clinical manifestations even among this small group of ARC patients. Immediately after the first transfusion, the hyperimmune plasma eliminated the HIV-1 p24 antigen detectable by antigen ELISA tests in all 10 recipients. Jackson *et al.* (16) have reported a similar observation. On the other hand, the PCR test detected HIV-1 nucleic acid both before and even after the second transfusion. This suggests that immunocomplexes had been circulating for at least a month after the first transfusion and were still doing so immediately after the second transfusion. However, the negative PCR of the patients' plasma after the second transfusion confirmed that the free virus was neu-

Anti-HIV-1 antibody titers at monthly infusions												
Pre-11th	Pre-12th	Pre-13th	Pre-14th	Pre-15th	Pre-16th	Pre-17th	Pre-18th	Pre-19th	Pre-20th	Pre-21st	Pre-22nd	Pre-23rd
1:640	†											
1:160	1:160	†										
1:2560	1:5120	ND	ND	1:1280	1:640	1:1280	1:1280	1:1280	1:640	1:1280	ND	1:640
1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:320	
1:80	1:80	1:80	1:40	1:40	1:40		†					
1:320	1:160	1:160	1:160		†							
1:80	1:40	1:40	1:40	1:40	1:40	1:40	1:40	1:40	1:40	1:40	1:40	1:40
1:320	1:320	1:320		†								

Table 3. $CD4^+$ (helper T cell) count at monthly intervals

Patient no.	Base count	Monthly count																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	274	144	243	290	ND	317	596	255	262	238	273										
2	40	53	25	52	22	17	37		23	3		4									
3	241	236	245	211		203	364	197	349	257	274	271	270	266	290	329	341	198		140	150
4	281	ND	241	238	227	275		241	309	238	295	274		210	262	175	187	180	153	281	188
5	20	17	ND	ND	26	3	12	6	5	4	5	6	4	7	4						
6	193	189	237	185	170	51	8	24	15	24	10	16	ND	14	7						
8	40	33	36	13	38	17	30	ND	33	22	16	9	17	18	33	15	10	10	8	8	2
9	81	31		35																	
10	117	108	213	64	89		66	37	62	51	27	34	31								

ND, not done.

tralized and that its clearance was sustained for a full month until the third infusion was administered. Table 1 shows that, by and large, the patients have remained HIV p24- and PCR-negative ever since the monthly transfusions were started in March 1988. Unfortunately we did not keep samples of white blood cells for PCR studies. However, since PCR is not yet a quantitative assay, and since anti-HIV-1 antibodies reduce but may not eliminate cellular infection, it is unlikely that we would have obtained negative PCR readings. In fact recent studies have documented infectious HIV-1 even in healthy HIV-1-infected individuals (17, 18).

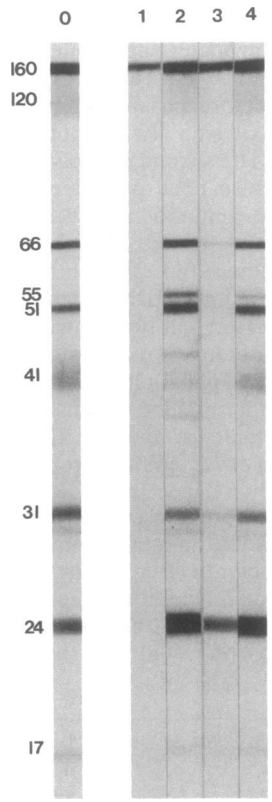

FIG. 1. Western blots of serum samples from one AIDS patient (no. 8) before and after infusion with hyperimmune plasma. Strips: 0, molecular weight $\times 10^{-3}$ of HIV structural proteins with control-positive serum; 1, profile of antibodies prior to first transfusion showing a weak band of antibodies to the glycoprotein gp160 only; 2, profile of antibodies immediately after the first transfusion showing the reappearance of antibodies to all of the structural proteins of HIV; 3, profile of antibodies 1 month after the transfusion showing weak bands of antibodies that reflect the low level of residual donor antibodies prior to the next transfusion; 4, profile of antibodies showing the reappearance of antibodies to all of the viral structural proteins immediately after the transfusion.

The second measurable effect was the level of antibodies. Most patients maintained their antiviral titers from the beginning of their treatment (Table 2). Usually the level of antibodies gradually decreases in ARC and AIDS patients. However, the most remarkable observation was the 8-fold increase (from 1:160 to 1:1280) in the titers of antibodies of the ARC patient (no. 3). He has been well for the past 22 months, during which period he received a total of just 6 liters of plasma. By and large the three ARC patients maintained their helper T-cell count (Table 3). In all of the AIDS patients, the helper T-cell count continued to decline, suggesting that in advanced AIDS the progenitor $CD4^+$ cells may be infected by the virus. By contrast Jackson *et al.* (16) found an increase in helper T-cell count after a single transfusion. Our findings are consistent with the observation that highly purified $CD34^+$ cells, containing precursors of all hemopoietic lineages, can be infected *in vitro* with HIV-1 (19).

Most patients on continuing monthly treatment improved during the first 6 months but several AIDS patients deteriorated during the second 6 months of the year, in particular patient 2, who developed non-Hodgkin lymphoma. This is the most aggressive form of malignancy in AIDS patients which kills most of those affected within a few months (20). Five of the six who died during the 17 months (nos. 2, 5, 6, 9, and 10) died of HIV-associated disease. In contrast to the AIDS patients, the three ARC patients (nos. 1, 3, and 4), who were consistently HIV p24 antigen- and PCR-positive before the passive immunization and who suffered from a range of illnesses, have been well following the first transfusion. ARC patient 1, who was reasonably well for 11 months, died of cardiac infarct (both his father and brother had died at a similar age from cardiac failure). The clinical remission of ARC patients 3 and 4 has been sustained during the past 22 months, while AIDS patient 8 had only a few infections during the same period.

The results of this trial cannot be compared directly to the results of AZT, since four of our AIDS patients could no longer tolerate its toxic effect and four had declined it. To judge by the patients' clinical histories and the disappearance of their helper T cells, the deaths of five of our six AIDS patients were not unexpected. All the same, our results suggest a marked improvement in well-being and prolonga-

Table 4. $CD4^+$ (helper T cell) count in plasma from a donor (JC) who gave 5 liters during 1 year

Date of plasma donation	$CD4^+$ cell count	Date of count
9/15/88	620	7/25/88
3/16/89	572	1/1/89
4/25/89	637	1/30/89
6/29/89	545	4/24/89
8/23/89	558	7/17/89

tion of life of the AIDS patients in the initial months of therapy, followed by a decline. We were extremely impressed by these patients' clinical improvement at the 3-month follow-up (10). This appeared to be maintained for about 6 months. All the opportunistic infections and most of the deterioration occurred after this period. The clinical features and the lack of recovery of the $CD4^+$ cell count indicate that there is such a severe impairment of immune function in these patients that long-term immunotherapy is unlikely to prolong survival markedly without an effective nontoxic drug. On the other hand, the sustained well-being of the ARC patients in the past 22 months suggests that passive immunization may benefit patients soon after the onset of HIV-associated disease. The rapid recovery in the ability to synthesize antibodies by patient 3 suggests that an earlier administration of passive immunization might induce a higher rate of immunological recovery. Ideally this should be used together with a nontoxic drug such as *N*-butyldeoxynojirimycin (Butyl-DNJ) (21) and some of the peptide-based HIV proteinase inhibitors (22). The administration of AZT has had to be discontinued in a significant number of the patients because its toxicity gave rise to hematological side effects (23, 24). Besides, an AZT-resistant HIV-1 mutant is liable to emerge in patients who receive the drug (25).

On the other hand, passive immunization appears to be remarkably free of side effects. Donation of 5 liters of plasma over the period of 1 year did not affect the donor's $CD4^+$ cell count. This observation indicates that plasma donations by healthy HIV-infected individuals do not accelerate disease progression. If meaningful results are to be obtained in a controlled study in patients with HIV constitutional (early) disease, careful attention would have to be paid to parameters other than mortality. A sustained improvement in the $CD4^+$ cell count would have obvious prognostic value, while the development of opportunistic infections could be used as a marker of treatment failure. Other aspects of the immune system, in particular cell-mediated immune function, need also to be studied. Possibly the administration of neutralizing antibody may also improve the function of both macrophages and lymphocytes. Our study does not establish passive immune therapy as a proven treatment, but it does indicate the need for further carefully controlled studies to determine whether the immunodeficiency of HIV-infected patients can be reversed by the administration of neutralizing antibodies in the disease, before the immune function has been seriously impaired.

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