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Effects of passive immunization in patients with the acquired immunodeficiency syndrome-related complex and acquired immunodeficiency syndrome

(human immunodeficiency virus neutralization/immunotherapy)

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ABSTRACT **Infection with the human immunodeficiency virus type 1 (HIV-1) is usually followed by a vigorous immune response that temporarily protects against disease progression. After a variable asymptomatic period, acquired immunodeficiency syndrome (AIDS)-related complex (ARC) and AIDS develop in most infected individuals. We have demonstrated that healthy HIV-1-infected individuals have neutralizing antibodies and a high titer of antiviral antibodies. In contrast, AIDS patients have undetectable levels of neutralizing antibodies, low titers of antiviral antibodies, and, frequently, HIV p24 antigenemia. These observations prompted us to attempt passive immunization in ARC and AIDS patients. Ten consistently viral-antigen-positive patients (mean, >6 months) were treated, resulting in sustained clearance of p24 antigen. Patients either maintained or increased their antiviral antibody titers. The raised titers result from increased antibody synthesis by the recipients. Circulating CD4$^+$ cell counts were unchanged after 2 months. By the third month none of these patients remained in hospital. As this treatment was of minimal toxicity, it merits wider evaluation in ARC and AIDS patients.**

Acquired immunodeficiency syndrome (AIDS) and the AIDS-related complex (ARC) are caused by lentiviruses (1–3) designated human immunodeficiency type 1 and 2 (HIV-1 and HIV-2), the latter being common in West Africa but uncommon in Western Europe and the United States (for review, see ref. 4). Infection with these viruses is usually followed by a vigorous immune response that temporarily protects against, but ultimately fails to prevent, the depletion of CD4$^+$ T cells and the development of a severe immunodeficiency in most cases.

In 1985 we demonstrated the presence of neutralizing antibodies in healthy HIV-1-infected individuals and the absence of these antibodies in AIDS patients (5). Further studies reported here extended this initial finding of a correlation between the absence of neutralizing antibodies and disease progression. We also found a correlation between levels of antiviral antibodies and neutralizing antibodies in infected individuals.

Based on these results, we undertook an open uncontrolled trial to determine the effects of passive immunization of 10 patients (4 with ARC and 6 with AIDS) with hyperimmune plasma collected from healthy infected individuals with high titers of anti-HIV-1 antibodies. For ethical reasons we felt unable to treat patients with HIV-1 antibody-negative plasma as a control group.

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TRIAL DESIGN, PATIENTS, AND METHODS

Trial Design. This was an uncontrolled trial designed to assess the toxicity and possible short-term benefits of passive immunization with hyperimmune plasma in 10 patients. In an unpublished study in 1985, our results suggested clearance of viral p24 antigen in an antigen-positive patient after administration of 250 ml of hyperimmune plasma. We wished to determine whether this was a general phenomenon, and if so, whether it could be sustained. Five hundred milliliters of plasma was to be administered monthly for 3 months, in the first instance. The trial procedures were approved by the Ethical Committees of both Addenbrooke's Hospital, Cambridge, and St. Stephen's Hospital, London.

Recipients. Recipients were patients with ARC or AIDS with detectable HIV-1 p24 antigenemia on two or more occasions. All patients signed informed consent forms.

Donors. Donors were clinically healthy HIV-1-infected individuals with antiviral titers ≥1:320 (range, 320–5120), as determined by the cell test, who had volunteered to come to Cambridge to donate plasma. Five individuals donated twice; all donors were ABO typed.

Plasma Collection and Processing. One liter of plasma was collected at each donation on a Haemonetics (Braintree, MA) 30 cell separator by a standard procedure. Each unit was centrifuged to remove residual white and red blood cells, treated with 0.25% β-propriolactone with frequent stirring for 30 min at room temperature, aliquoted into three flasks, and then frozen at −20°C. This treatment renders the plasmas free of infectious viruses but also decreases their antiviral titers (data not shown). Before administration equal amounts of three-to-six ABO-compatible donations were pooled to make 500 ml. Each pooled unit was tested for bacterial sterility by adding 1 ml to standard blood culture bottles.

Assay for Neutralizing Antibodies. This assay was performed as described (5). Briefly, 10^4 infectious virus particles of strain C-LAV-1 (3, 5) in 0.1 ml of RPMI 1640 medium were incubated with 0.1 ml of serum/plasma for 1 hr at 37°C. After this incubation 10^4 Karpas T cells in 0.1 ml of medium were added, and the incubation was continued for another hour. Then the virus/serum/cell mixture was suspended in 6 ml of medium and seeded into three wells of 24-well plastic plates. Cells in the plates were observed for 10 days for the development of a characteristic cytopathic effect. This method is reproducible, but results are not readily converted to a quantifiable form, because assessment of the end point

Abbreviations: AIDS, acquired immunodeficiency syndrome; ARC, AIDS-related complex; HIV, human immunodeficiency virus; PGL, persistent glandular lymphadenopathy.

is partly subjective. This stricture also applies to proposed plaque assays.

Assay for Antiviral Antibodies. The principles and methodology of the cell test have been described (5, 6). To determine total antiviral antibody titers, Teflon-coated glass slides with 3 rows of 10 wells each were used to determine levels of antiviral antibodies in positive serum samples. A starting dilution of 1:10 was used with a 2-fold increase (1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, and 1:5120). Ten microliters of the corresponding dilution of serum were placed on each well. End point titrations were determined by microscopic examination.

HIV p24 Antigen Assay. All samples were assayed, according to the manufacturer's instructions, by a commercial HIV p24 ELISA (DuPont). Samples from before and after the first plasma infusions were also tested by the Abbott HIV-antigen ELISA. All samples were positive before the infusions and negative after the infusions.

RESULTS

We tested our previous finding (5) of a correlation between the presence of neutralizing antibodies and lack of disease progression in 38 serum samples. Twenty-three of these were tested in the absence of clinical information. All healthy infected individuals (20/20) and patients with persistent glandular lymphadenopathy (PGL) (3/3) and three of four ARC patients had neutralizing antibodies, whereas one ARC patient and all AIDS patients (11/11) lacked these. Six seronegative individuals had no detectable neutralizing activity.

A similar correlation between high levels of antiviral antibody titers and lack of disease progression was evident (Table 1) when these titers were compared with the clinical states of these patients, although there was some overlap between states.

When the sera were tested for HIV-1 p24 antigenemia, none (0/16) of the healthy infected individuals tested had detectable levels of antigen. In contrast, 8/11 AIDS patients and 2/3 ARC patients were antigen-positive with titers that ranged from 20 to >500 pg/ml.

From these results we undertook the passive immunization of 10 patients (Table 2) to determine toxicity, possible clinical benefits, and the effects on viral p24 antigen levels, antiviral antibody titers, and circulating $CD4^+$ cell counts. Donors were examined for disease progression and compared with controls.

Table 1. Relationship between clinical state, antibodies, and p24 antigen

Clinical state	Number tested	Neutralizing antibodies	Antiviral antibody titer*	Viral p24 antigen
AIDS	11	−	1:5–1:160	8/11
ARC	1	−	1:80	
	3	+	1:160–1:320	1/2
PGL	3	+	1:160–1:1280	0/3
Healthy	2	+	1:160	0/2
(HIV-1	6	+	1:320	0/6
seropositive)	6	+	1:640	0/4
	3	+	1:1280	0/2
	3	+	1:5120	0/2
Healthy (HIV-1 seronegative)	6	−	−	ND

ND, not done.
*Antiviral antibody titers were determined by the cell test and are reported as the end point dilution.

Table 2. HIV-1 p24 antigen levels in serum (pg/ml)

Patient no.	Pre-1st infusion	Post-1st infusion	Pre-2nd infusion	Pre-3rd infusion	Pre-4th infusion
ARC					
1	10	0	0	0	0
2	30	0	30	0	0
3	50	0	0	0	0
4	210	0	0	0	0
AIDS					
5	65	0	0	0	0
6	80	0	0	0	
7	100	0	ND	ND	
8	100	0	20	20	0
9	120	0	0	0	0
10	340	0	0	0	0

ND, not done.

The recipient group was composed of four ARC patients and six AIDS patients. Nine were homosexual or bisexual men, and one was a heterosexual man; all were treated in one center. The mean time from diagnosis to trial entry was 8 months, and all were consistently p24 antigen-positive before the trial period (mean, >6 months). Nine patients received three infusions of hyperimmune plasma (500 ml over 1–2 hr) at monthly intervals. A 10th patient (no. 7) received a single infusion before returning to Brazil; he was lost to follow-up and excluded from statistical analyses.

In general, the treatment was well tolerated. The only adverse effects were recorded in patient 3, of blood group A, who had received plasma from a group B donor. After 400 ml had been infused he developed headache, nausea, cold extremities with aching distal joints, and mild rigors. The infusion was stopped, and all symptoms resolved in 2 hr. A blood sample taken then showed mild hemolysis. Further administrations to this patient of correctly cross-matched plasma have been uneventful. Urea, electrolyte, and liver-function tests showed no significant change from base-line values in any recipient over the trial period (data not shown).

Viral p24 antigen levels showed striking changes after treatment (Table 2). Column 2 shows p24 levels immediately before the first infusion, whereas column 3 shows levels 2 hr after the infusion. In all cases, this antigen clearance was complete. Columns 4, 5, and 6 show antigen levels in serum before the second, third, and fourth infusions, respectively, and demonstrate that complete antigen clearance was sustained in most patients.

We measured antiviral antibody titers in the same serum samples (Table 3). All patients had low titers before treatment, consistent with our previous findings (column 2). Furthermore these titers were not doubled by the passive

Table 3. Antiviral antibody titers

Patient no.	Pre-1st infusion	Post-1st infusion	Pre-2nd infusion	Pre-3rd infusion	Pre-4th infusion
ARC					
1	1:320	1:320	1:640	1:320	1:320
2	1:160	1:160	1:160	1:320	1:320
3	1:160	1:160	1:1280	1:640	1:640
4	1:320	1:320	1:640	1:640	1:640
AIDS					
5	1:80	1:80	1:160	1:160	1:160
6	1:160	1:160	1:160	1:160	1:160
7	1:80	1:80	ND	ND	
8	1:40	1:40	1:40	1:80	1:160
9	1:160	1:160	1:320	1:320	1:640
10	1:320	1:320	1:320	1:320	1:320

Titers were determined by the cell test and are reported as the end point dilution. ND, not done.

administration of 500 ml of hyperimmune plasma (column 3). Nevertheless, seven of nine recipients increased their titers 2- to 4-fold over the following months.

Circulating $CD4^+$ cells were counted before each infusion. The mean $CD4^+$ cell count was 130 cells per mm^3 (range, 29–284); this mean increased marginally to 139 cells per mm^3 (range, 17–246) before the third infusion.

Patients were weighed before each infusion; the mean weight before the first infusion (66.5 kg) decreased (to 64.5 kg) before the third. However, >90% of this weight loss was attributable to one patient (no. 9), who was being treated for depression.

A clinical appraisal was carried out by the physicians (M.Y. and B.G.) responsible for the patients. Two patients were considered to have deteriorated (one progressed to AIDS by developing pneumocystis pneumonia, and one developed severe depression and lost 17 kg in weight), two patients have remained stable, whereas five patients were judged to have improved on the grounds of good responses to treatment of intercurrent illnesses or of improved well-being. All ten patients have since been discharged from hospital.

We compared disease progression in the donors with that in a group of 25 HIV-positive individuals from the same center. These were bi/homosexual men, who fulfilled the criteria for donation but were not asked to donate. One donor progressed to AIDS (9 months after donation); over an equivalent follow-up period two people in the control group progressed to AIDS and one to ARC. Thus, donation seems not to have enhanced disease progression.

DISCUSSION

Infection with HIV, as with animal lentiviruses, elicits an efficient immune response, which inhibits replication of, but fails to eliminate, the virus. This response is dependent on $CD4^+$ cells, which HIV destroys, inducing a severe immune deficiency, often many years after infection.

The role of antibodies in this infection is undetermined. Antibodies appear unable to prevent infection in chimpanzees when administered[¶] or induced (7) before challenge with large doses of infectious virus. Yet in monkeys[‖] and in man, high titers indicate a relatively good prognosis. These titers fall as disease progresses. Whether this fall is the result of loss of helper T-cell function or the cause of disease progression is unknown but of obvious importance for understanding the pathogenesis of this infection and for vaccine strategies.

Our previous work suggested that neutralizing antibodies might have a protective function, as they could be detected in healthy infected individuals but not in those with AIDS. These antibodies were clearly incapable of eliminating the infection; nor, it seemed, were they capable of providing lasting protection against depletion of $CD4^+$ cells. A plausible but undemonstrated explanation proposes that the virus, by virtue of its high mutation rate, produces antigenic variation in its envelope, thus evading the immune response of the host.

Other groups, using different assays, were unable to produce a clear relationship between clinical state and the presence or absence of neutralizing antibodies (8). Therefore, we examined additional sera, some coded, to check our initial finding. We found a clear relationship between the presence of neutralizing antibodies, high titers of antiviral antibodies in the cell test, absence of viral p24 antigen, and clinical well-being.

We examined, therefore, by passive administration whether antiviral antibodies were responsible for clearance of p24 antigen and simultaneously carried out a subjective appraisal of the recipients' clinical state. We pooled three to six plasma donations to minimize the effects of antigenic variation between strains. Administration of this hyperimmune plasma produced sustained clearance of viral antigen in all recipients, who had been consistently antigen positive before the trial. In primary HIV infection HIV antigenemia is commonly associated with the ability to isolate virus from plasma (9), and the presence of antigenemia was strongly associated with disease progression in the San Francisco Hospital cohort (10).

Some clinical improvement was apparent in five of nine recipients; two recipients deteriorated, while two recipients remained stable. Some of the clinical benefit might be attributed to the wide spectrum of antibody activity in the administered plasma, which is diminished in patients with ARC and AIDS. The treatment appears less toxic and more effective in reducing measurable viral p24 antigen than currently available treatments (11, 12).

Unexpectedly most patients increased their antiviral titers to levels beyond those expected from the amounts of antibodies administered. Several explanations of this phenomenon are possible, ranging from stimulation of immune function by factors in the administered plasma to the synthesis of anti-antiidiotypes. These explanations remain speculative.

No further depletion of $CD4^+$ cells occurred during the trial period. The mean circulating $CD4^+$ cell count increased to 139 cells per mm^3 before the third infusion from a pretrial mean of 130 cells per mm^3, which we consider of no clinical significance. This suggests that the treatment does not stimulate antibody-dependent enhancement of viral infectivity at the concentrations of antibody achieved.

Some caution is indicated before concluding that antiviral antibodies are responsible for clearing circulating viral p24 antigen. We have not attempted to demonstrate that nonimmune plasma fails to clear antigen nor that immunoglobulin fractions from the donors are responsible for the reduction. Further, one possible explanation of the decreased levels is that the administered antibody masks p24 antigen in the assay (13). Nevertheless, our results are consistent with *in vivo* neutralization of circulating viruses.

A limitation of this uncontrolled trial is its inability to determine whether hyperimmune plasma protects $CD4^+$ cells against further depletion or improves the prognosis of recipients. A controlled trial is required to determine whether the improvement in prognostic indices demonstrated here and the apparent lack of acceleration in disease progression in donors reflects a real improvement in the recipients' prognosis that can be achieved without detrimental effects on the donors.

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