

Immunodeficiency and other clinical immunology

Immune complexes from HIV-1+ patients contain infectious virus able to infect normal lymphocytes

Rosa García, BSc, PhD,^a Esperanza Feijoó, BSc, PhD,^a
Manuel-Fernández Guerrero, MD, PhD,^c Miguel de Górgolas, MD, PhD,^c
Maria Angeles Muñoz-Fernández, BSc, PhD,^b
Eduardo Fernández-Cruz, MD, PhD,^b and Fernando Ortiz, MD, PhD^a
Madrid, Spain

It has been suggested that circulating immune complexes containing HIV-1 could be involved in enhancement of the infection through Fc receptors. To test this hypothesis, immune complexes precipitated from the sera of 25 HIV-1-seropositive individuals, at different stages of the disease, were assayed for the presence of infectious virus. When added to phytohemagglutinin-activated peripheral blood lymphocytes, seven of 25 complexes were able to sustain a productive infection. This was demonstrated by release of HIV-p24 antigens in the supernatant of activated but not resting peripheral blood lymphocytes after several days of culture. Moreover, peripheral blood lymphocytes cultured with complexes from HIV-1+ patients but not from control subject, contained integrated HIV-1 provirus in their DNA. These results demonstrated the presence of infectious virus in immune complexes from HIV-1+ patients. (J Allergy Clin Immunol 1996;98:827-30.)

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Elevated levels of circulating immune complexes (CICs) have been noted by various authors in patients infected with HIV, using different detection techniques.¹⁻⁴ These immune complexes contain HIV-1 virions more frequently in later stages of the disease (C1-C3),⁵ and their presence correlates with immune defects such as depressed T helper cell counts. The inverse relationship among high levels of CICs and low CD4 cell count can be explained by the anti-CD4 activity found in HIV-immune complexes.⁶ On the other hand, as indi-

Abbreviations used

CICs: Circulating immune complexes
PBMCs: Peripheral blood mononuclear cells
PCR: Polymerase chain reaction
PHA: Phytohemagglutinin

cated by some authors, CICs from HIV-1+ patients may be involved in the enhancement, through the Fc receptor, of HIV infection in monocytes,⁷ normal mononuclear cells,² and cell lines.⁸ Here, we present clear evidence that naturally occurring CICs, isolated by precipitation from the serum of HIV-1+ patients, in different stages of the disease, could infect normal lymphocytes by integration into the DNA of normal activated cells.

METHODS

Patients

Twenty-five patients positive for HIV-1 antibody in at least two serum samples, taken at different time

From ^aDepartment of Immunology, Fundación Jiménez Díaz;

^bDivision of Immunology, General Hospital Gregorio Marañón, Complutense University of Madrid; and ^cDepartment of Infectious Diseases, Fundación Jiménez Díaz.

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Reprint requests: Rosa García, Department of Immunology, Fundación Jiménez Díaz, Reyes Católicos 2, 28040 Madrid, Spain.

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points, were studied. Clinically, four individuals were classified in group A (free of symptoms), six in group B, and 15 in group C according to the classification for AIDS of the Centers for Disease Control.⁹ Two HIV-seronegative patients with more than 200 $\mu\text{g/ml}$ of CICs (with chronic hepatitis B) were used as control subjects.

Isolation of circulating immune complexes

CICs were isolated from patients' sera with 1.04 mol/L ammonium sulfate as described by Feijó et al.⁶ Briefly, 4 ml of diluted serum (0.5 ml of serum in 3.5 ml of phosphate-buffered saline) was mixed with 1.4 ml of 4 mol/L ammonium sulfate and incubated for 1 hour at 37° C. After an additional 30 minutes at room temperature, the resulting precipitate was spun down at 3000 g for 20 minutes, washed once with 1.04 mol/L ammonium sulfate, and finally redissolved in 500 μl of phosphate-buffered saline. The protein concentration was calculated from the optical density of the redissolved precipitate at a wavelength of 280 nm, taking 1.4 as the extinction coefficient (the approximate mean extinction coefficient of human immunoglobulins). The assay of control sera from 228 healthy persons gave a mean value of $36.05 \pm 22.8 \mu\text{g/ml}$ with a cutoff level (mean $\pm$ 2 SD) of 81.65 $\mu\text{g/ml}$. A solution of 1.04 mol/L ammonium sulfate was demonstrated to not precipitate free p24 antigen.

Infectivity assay of normal peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by means of Lymphoprep (Nyegaard, Oslo, Norway) at 400 g for 20 minutes. PBMCs were collected, washed, and adjusted to 2×10^6 cells/ml in RPMI medium (Whitaker, Md.) supplemented with 10% fetal calf serum, penicillin G (200 U/ml), streptomycin (200 U/ml), and L-glutamine (0.3 mg/ml). A total of 2×10^6 cells were stimulated with 20 μl of phytohemagglutinin (PHA) (1 mg/ml; Gibco, Grand Island, N.Y.) in 24-well plates (Costar, Cambridge, Mass.) for 72 hours at 37° C in 5% CO₂ atmosphere. After 72 hours, supernatants were replaced by 1 ml of whole RPMI medium containing 100 U/ml of rIL-2 (Amersham Corp., Arlington Heights, Ill.) and 200 $\mu\text{g/ml}$ of isolated CICs from HIV-infected patients or control subjects. Culture supernatants were collected on days 2, 4, 6, 8, and 10 after infection and monitored for HIV-p24 production. P24 antigen was quantified by using an antigen-capture enzyme immunoassay (Abbott recombinant HIV Ag EIA; Abbott, Germany). Cell viability was measured by Trypan blue exclusion. Lymphocytes were kept frozen for polymerase chain reaction (PCR) analysis.

DNA extraction and PCR analyses

PBMCs were resuspended in PCR lysis buffer (10 mmol/L Tris-HCl, pH 8.3; 1 mmol/L ethylenediamine-

tetraacetic acid; 0.5% NP40; 0.5% Tween-20; and 300 $\mu\text{g/ml}$ proteinase K) at a concentration of 10^6 cells/100 μl buffer and digested with proteinase K overnight at 37° C. The proteinase K was then inactivated for 15 minutes at 94° C. Samples from the patients produced positive signals with primers PC02-PC04 specific for the human β -globin gene¹⁰ and were therefore suitable for amplification.

All samples were analyzed for the presence of HIV-1 DNA by PCR with two different sets of nested primers specific for regions of the pol and env genes (JA17 to JA20 and JA13 to JA16, respectively) as previously described.¹¹ Briefly, the samples were first amplified for 24 cycles with the outer primers, then one tenth (5 μl) of the product of the first PCR was amplified for 30 cycles with the inner primers. Negative controls were included in each run and consisted of lysis buffer alone and samples from healthy blood donors. Positive controls consisted of samples from peripheral blood lymphocyte cultures infected with HIV-1. The PCR product was analyzed by electrophoresis on a 1.5% agarose gel stained by ethidium bromide. Samples were considered positive if the two different primer sets repeatedly gave a specific amplification product.

RESULTS

In vitro infectivity of CICs from HIV-1+ patients

Levels of circulating immune complexes in 25 HIV-1+ patients studied ranged from 240 to 5100 $\mu\text{g/ml}$, well above the cutoff level of healthy control subjects. All the 25 isolated CICs demonstrated anti-HIV activity, and IgG was the predominant immunoglobulin. CICs isolated from seven of 25 infected patients were able to infect normal activated lymphocytes because we were able to detect the synthesis of viral p24 antigen in PHA-stimulated normal mononuclear cell cultures (Fig. 1). Five of these seven patients belonged to group C, according to the Centers for Disease Control classification, and three demonstrated less than 200 CD4⁺ cells/ μl (Table I). The maximum levels of p24 were observed between 4 and 6 days of culture and declined thereafter; the decline was probably due to a loss of cell viability. In a similar assay, Kobayashi et al.⁸ infected U937 cells with unfractionated p24-positive serum and did not detect p24 synthesis until 7 or 12 days of culture. The CICs from two HIV-seronegative patients with levels of CICs above 200 $\mu\text{g/ml}$ were not able to induce p24 synthesis in the same conditions.

To further confirm that the p24 antigen detected in PHA supernatants was due to the presence of an infectious virus and not the result of time-dependent dissociation of p24 antigen carried over in the isolated CICs, the presence of proviral DNA inte-

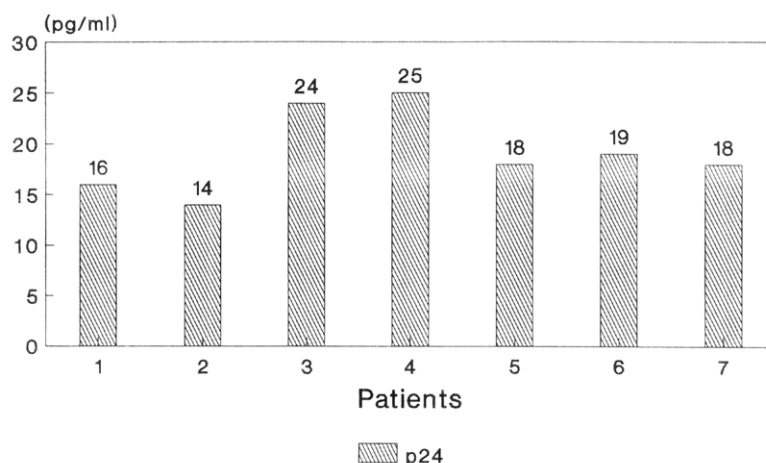


FIG. 1. In vitro production of p24 antigen by PHA-activated normal peripheral blood lymphocytes infected with isolated CICs from seven HIV+ patients (days of culture, 4 to 6). Clinical grades of these patients were: 1(C3), 2(A1), 3(C2), 4(C3), 5(B1), 6(C2), and 7(C3).

TABLE I. Centers for Disease Control classification, CD cells per microliter, and levels of p24 antigen in serum and culture supernatants of seven patients with CICs able to infect normal lymphocytes

Patient No.	CDC	CD4 (cells/ul)	p24 (pg/ml serum)	p24 (pg/ml supernatant)
1	C3	150	17	16
2	A1	768	Neg	14
3	C2	300	840	24
4	C3	110	Neg	25
5	B1	500	Neg	18
6	C1	450	Neg	19
7	C3	196	70	18

CDC, Centers for Disease Control.

grated in lymphocyte DNA was tested by PCR. Env-specific sequences were amplified in two samples of DNA isolated from activated normal lymphocytes incubated with CICs (Fig. 2, lanes 3 and 4). In contrast, PCR was negative in lymphocytes incubated with two CICs isolated from two HIV-1+ patients in which no p24 synthesis could be achieved (Fig. 2, lanes 5 and 6), further confirming that not all HIV+ CICs were able to infect activated normal lymphocytes. The same results were obtained with pol-specific sequences.

DISCUSSION

These results demonstrate the ability of circulating immune complexes from some HIV-1+ pa-

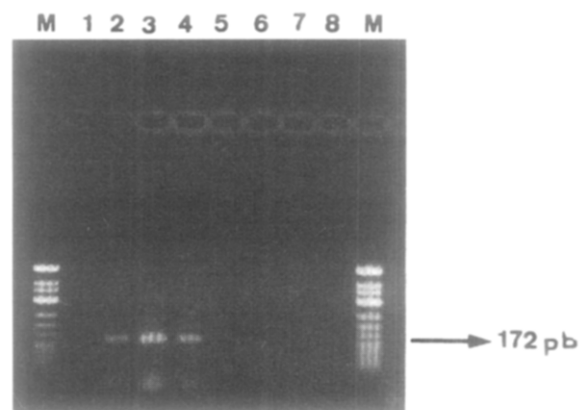


FIG. 2. Agarose gel electrophoresis of DNA amplified with *env* nested primers. Lane 1, Negative control; lane 2, positive control DNA from an HIV+ positive serum sample; lanes 3 and 4, DNA from PHA-activated peripheral blood lymphocytes incubated with "infectious" CICs from HIV+ patients (patients 6 and 7 from Fig. 1); lanes 5 and 6, DNA from PHA-activated peripheral blood lymphocytes incubated with "noninfectious" CICs from HIV+ patient; lanes 7 and 8, lysis buffer; M, DNA molecular weight markers (OX174/*Hinfl*).

tients, mainly those in advanced stages of the disease, to infect normal activated peripheral blood cells by integrated proviral DNA in the lymphocyte genome. The precise mechanism of the infection of normal human lymphocytes by isolated CICs from HIV-1+ patients is not well defined. Various lines of evidence now point to the versatility of the entrance of HIV-1 into the cells. Bolognesi et al.¹⁰ reported that immunoglobulins

facilitated the uptake of a virus-antibody immune complex through the Fc receptor. Also, Kobayashi et al.⁸ demonstrated that human aggregated IgG inhibited the infection of a monocyte cell line with positive p24-containing sera. It is therefore likely that CICs containing HIV virions may be taken up by macrophages and replicated inside them; and later, this newly produced virus could enter activated lymphocytes, propagating the infection. Alternatively, CICs may directly infect activated lymphocytes.

Only seven serum samples obtained from 25 patients contained CICs able to infect normal lymphocytes; the reason for this is not known at present. However, it could be related to AIDS pathogenesis; therefore, it might be important to distinguish between CIC enhancers and nonenhancers of the progression of HIV infection.

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