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Research Letter

HIV-1 group N among HIV-1-seropositive individuals in Cameroon

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A remarkable genetic diversity of HIV is observed in the west-central African country of Cameroon. At least three distinct groups of HIV-1 (M, O and N) have been identified. Most of the known group M subtypes have been detected, although HIV-1/M subtype A is predominant [1]. HIV-1 group O, reported mainly in native Cameroonians, is endemic in Cameroon, with an estimated prevalence of 3.3% among HIV-1-infected individuals [2]. Recently, a new group of HIV-1 (HIV-1/N) was identified in two Cameroonian patients [3]. HIV1-N is very closely related in the env gene to SIV from chimpanzees (*P.t. troglodytes*), suggesting zoonotic transmission [4,5]. However, no information is available to date about the prevalence and distribution pattern of HIV-1 group N in Cameroon.

In order to estimate the rate of HIV-1/N infection among HIV-1-seropositive individuals in Cameroon, serological studies were performed using a strategy that allows the serological discrimination of HIV-1/M, O, and N [2,3,6]. Blood samples originating from the north, the centre, the littoral and the west of Cameroon were referred to the Centre Pasteur du Cameroun for diagnosis (or confirmation) of HIV infection. Sera were first screened for HIV by means of an enzyme-linked immunosorbent assay (ELISA indirect HIV-1 and HIV-2, GenElavia Mixt; Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). Positive samples were confirmed using an ELISA based on competition with a group M-specific antigen (Wellcozyme rec HIV-1; Murex, Dartford, Kent, UK). Positive HIV samples were analysed using a group-specific enzyme immunoassay (GSEIA). This assay used different HIV V3 synthetic peptides specific for HIV-1 group M, group O, or group N or for SIVcpzGab [3,6]. Briefly, the peptides used as antigens were individually allowed to adhere to microplate

wells, and a classic enzyme immunoassay using an anti-human immunoglobulin-G labelled with peroxydase was then performed to detect the presence of anti-V3 antibodies in HIV-positive sera. The reactivities on wells coated with the different group-specific peptides were compared to discriminate among sera reactive with HIV-1 group M, O or N infection.

Between 1 July 1997 and 30 November 1999, 6446 HIV-1-positive sera were analysed using GSEIA. Ninety seven and 2% of these sera, respectively, were reactive with group M and O peptides. Four (0.06%) showed strong reactivities with the V3 peptide of the HIV-1/N prototype strain (YBF30) and the V3-SIVcpzGab reference peptide (Table 1). The remaining sera (0.94%) could not be serotyped as group M, O or N viruses. For most of them, this could be explained by a loss of specific antibodies that occurred during the clinical phase of HIV infection. With respect to the remarkable genetic diversity of HIV in Cameroon, we can also not exclude that some viruses are too divergent from the group M, O and N V3 consensus sequences to be detected by GSEIA.

Table 1

The samples positive with HIV-1 group N/SIVcpz peptides were from two patients with AIDS (YBF106 and YBF116) and from two other individuals for whom no clinical information was available. Patient YBF106 was a 51-year-old man from the centre part of Cameroon. He was found to be HIV seropositive in October 1997, and died of AIDS in December 1998. Attempts to isolate the virus from the peripheral blood mononuclear cells (PBMC) were unsuccessful. Patient YBF116, was a 7-year-old boy from the same region in Cameroon. He was diagnosed as HIV positive in June, 1999 and died of AIDS in October 1999. Mother-to-child infection was suspected but could not be confirmed because both his mother and father died from unknown clinical signs, in July and November 1997, respectively. A viral isolate, HIV-1/YBF116, was obtained from patient YBF116's phytohaemaglutinin-activated PBMC. No cells were available from patients YBF115 and YBF117.

No epidemiological link could be evidenced between these four patients nor between these four individuals and the two HIV-1/N previously identified [3].

Genetic analyses were performed in order to confirm that each of the four patients with sera reactive with the group N and SIVcpzGab V3-specific peptides was carrying HIV-1/N. For HIV-1 /YBF106, YBF115 and YBF116, direct sequencing of pol and gp41 polymerase chain reaction products derived from plasma or PBMC DNA demonstrated that the viruses were indeed members of the HIV-1/N group [7]. No viral sequences could be amplified from patient YBF117 who showed the highest cross-reactivity with the HIV-1/N V3 peptide. In five of the six cases tested, the presence of HIV-1/N sequences was confirmed genetically and in one case it is highly suspected. Moreover, the genetic distances were significantly high enough to exclude an epidemiological link between these infections. No viral sequences could be amplified from the serum from patient YBF117.

Together with the two first cases previously published [3], only six HIV-1-positive Cameroonian patients have thus been serologically found to be infected by HIV-1/N. There is, apparently, a very low prevalence of infection by this newly identified HIV-1 group N in the seropositive Cameroonian population. The serological assays commercially available,

including the rapid tests routinely used in Cameroon, allow the diagnosis of HIV-1 infection even with these highly variant strains.

Whether HIV-1/N are an emerging or declining HIV is unknown, implying that a serotyping survey has to be undertaken. There is no clear evidence for a specific location of HIV-1/N infection in a given area of Cameroon. The home villages of at least three of the six known HIV-1/N-infected patients have been clearly identified. Ongoing studies are currently performed in order to define more precisely the distribution pattern of HIV-1 group N and SIVcpz in these different areas of Cameroon. Because group N probably derived from a chimpanzee SIV, characterization of additional HIV-1/N and SIVcpz might bring new insights about the origin and evolution of primate lentiviruses.

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