Laboratory diagnosis of the first cases of HIV-2 infection in Canada

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Until recently the geographic distribution of infection due to human immunodeficiency virus type 2 (HIV-2) had excluded North America. We report the first two cases of such infection in Canada. Both people came from endemic areas of western Africa and were asymptomatic. The results of a commercial enzyme immunoassay specific for HIV-1 antibody were positive in both cases, but those of the Western blot technique were indeterminate. The Western blot technique specific for HIV-2 antibody and the indirect fluorescent antibody test were used to verify the presence of HIV-2 antibody.

Il y a encore peu de temps, la répartition connue de l'infection par le virus immunodéficitaire humain du type 2 (VIH-2) respectait l'Amérique du Nord. On rapporte ici les deux premiers cas canadiens de cette infection. Les sujets, en provenance de régions africaines où elle est endémique, sont asymptomatiques. Tous deux donnent un résultat positif à une immunoréaction enzymatique du commerce spécifique des anticorps anti-VIH-1 et un résultat incertain au Western blot. On confirme la présence d'anticorps anti-VIH-2 par un Western blot spécifique de ceux-ci et par la fluorescence indirecte.

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In 1986 a new retrovirus, subsequently called human immunodeficiency virus type 2 (HIV-2), was reported to have been isolated from patients with AIDS in western Africa.[1] Although this virus is distinct from HIV-1 its clinical consequences may be indistinguishable from AIDS due to HIV-1.[2] The pathogenic potential of HIV-2 may differ from that of HIV-1.[3] A single case of HIV-2 infection in an immigrant from an endemic area of western Africa has recently been reported in the United States;[4] this person had a 3-year history of weight loss and a recent onset of neurologic symptoms. Until then the global distribution of HIV-2 infection had excluded North America.[5]

The laboratory diagnosis of HIV-2 infection is somewhat problematic. In North America the enzyme-linked immunosorbent assay (ELISA) commonly used for testing is formulated from an HIV-1 viral lysate. The detection of HIV-2 with the use of HIV-1 screening tests has been successful in 28% to 93% of cases.[4]

We describe the methods used in the laboratory detection of the first two cases of HIV-2 infection in Canada.

Methods

The first case involved an asymptomatic pregnant woman 26 years of age who was originally

from Nigeria; she was screened for HIV antibody as a prerequisite for US immigration. The second case involved an asymptomatic 27-year-old man originally from Ghana who was tested to become a sperm donor for an in-vitro fertilization program.

Serum samples from the two patients, sent to the Central Laboratory, Laboratory Services Branch, Ontario Ministry of Health, Toronto, were found to be positive for HIV-1 with the use of a commercial ELISA kit. The results of the Western blot technique with the use of whole viral lysate as the antigen source were indeterminate, but the pattern evoked suspicion of HIV-2 infection. The samples were forwarded to the Bureau of Laboratories and Research Services, Federal Centre for AIDS, Ottawa, for comprehensive analysis.

Indirect fluorescent antibody (FA) test

Cultures of HUT 78 lymphocytes (American Type Culture Collection) were infected with either HIV-1 from a Canadian isolate or HIV-2 (ROD strain) obtained from Dr. Luc Montagnier, Pasteur Institute, France. When the cultures yielded giant cells, pathognomonic of HIV infection in vitro, the cells were washed once with phosphate-buffered saline and their concentration adjusted to yield 20 000 cells per 10 μl when spotted onto multiwell glass slides. Uninfected HUT 78 cells were also spotted onto the slides for comparison. The slides were air dried, acetone fixed and frozen (to −70°C) until ready for testing.

For the test 10 μl of a 1:10 dilution of serum in phosphate-buffered saline was added to the infected and uninfected wells and incubated in a humid chamber at 37°C for 1 hour. The slides were washed three times (10 minutes each time) in phosphate-buffered saline and then air dried. Subsequently 10 μl of goat antihuman (IgG, IgM and IgA) fluoroisothiocyanate conjugate (Organon Teknika Inc., Scarborough, Ont.) was added to each well. The slides were incubated at 37°C for 1 hour, washed as previously described, counterstained with Evans blue (1:10 000 dilution) for 10 minutes at room temperature, washed again, rinsed in distilled water, air dried, mounted and observed through an ultraviolet microscope.

Western blot technique

All serum samples were tested by means of the Western blot technique with partially purified HIV-1 viral lysates (Organon Teknika, Charleston, South Carolina) as previously described.[6] Western blots with HIV-1 recombinant *env* glycoprotein (molecular weight 160 000) (MicroGeneSys, West Haven, Connecticut) were used to resolve further the HIV reactivity. Whole-cell lysates for blotting of HUT 78 lymphocytes infected with HIV-2 were prepared as described by Pan, Cheng-Mayer and Levy.[7] Uninfected HUT-78 cell lysates were also used to test the serum samples for reactivity against normal lymphocytes.

Results

HIV-1 serologic findings

When tested at the Central Public Health

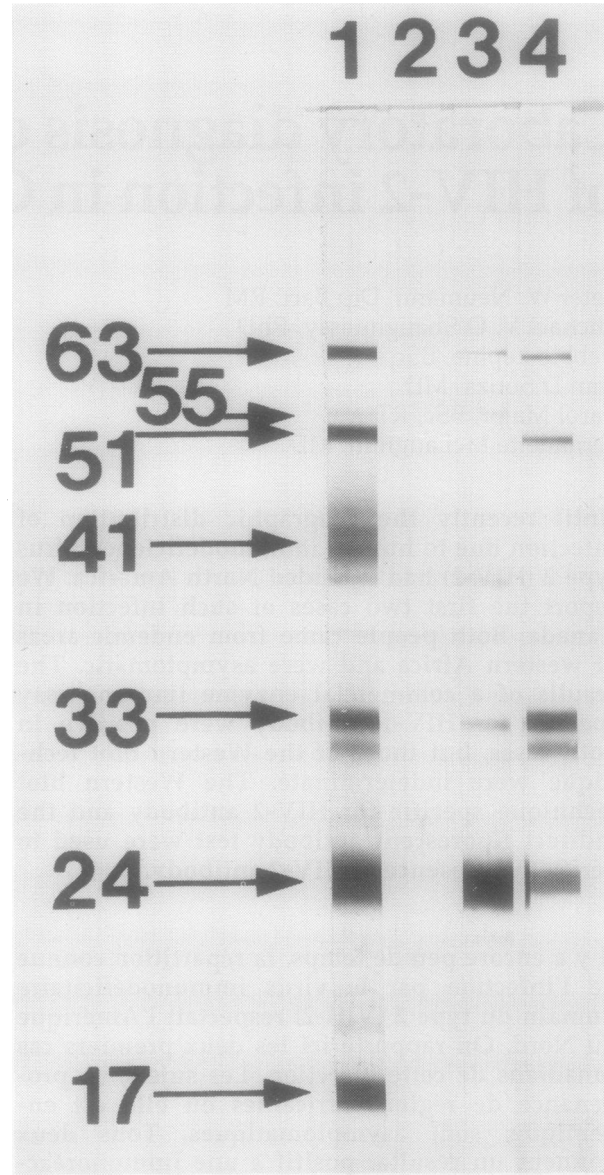

Fig. 1 — Results of Western blot technique using human immunodeficiency virus type 1 (HIV-1) viral lysate and alkaline phosphatase conjugate. Numbers on left represent molecular weights (× 1000) of specific viral proteins. Strips represent (1) control serum sample positive for HIV-1 antibody, (2) nonreactive control serum sample, (3) serum sample from first patient, with antibody reactive to viral proteins with molecular weights of 24 000, 33 000, 51 000, 55 000 and 63 000, and (4) serum sample from second patient, with antibody reactive to viral proteins with molecular weights of 24 000, 33 000, 51 000 and 63 000 (note no reaction to transmembrane glycoprotein with molecular weight of 41 000).

Laboratory, the Federal Centre for AIDS and the Centers for Disease Control, Atlanta, the serum samples from the two patients were found to be positive for HIV-1 antibody with the use of the DuPont HTLV-III ELISA (DuPont Co., Wilmington, Delaware) and the Genetic Systems LAV EIA (Genetic Systems Corp., Seattle). The reactivity exceeded the positive cutoff optical densities at least fourfold. The Western blot technique with the use of the HIV-1 lysate confirmed these findings. The samples appeared as indeterminate for HIV-1 antibody, because there was a conspicuous absence of antibody to the HIV-1 transmembrane glycoprotein with a molecular weight of 41 000 (Fig. 1), usually present in cases of HIV-1 infection,[7] even though cross-reacting antibodies to *gag* and *pol* proteins were present. There was no reaction to the *env* precursor glycoprotein with a molecular weight of 160 000.

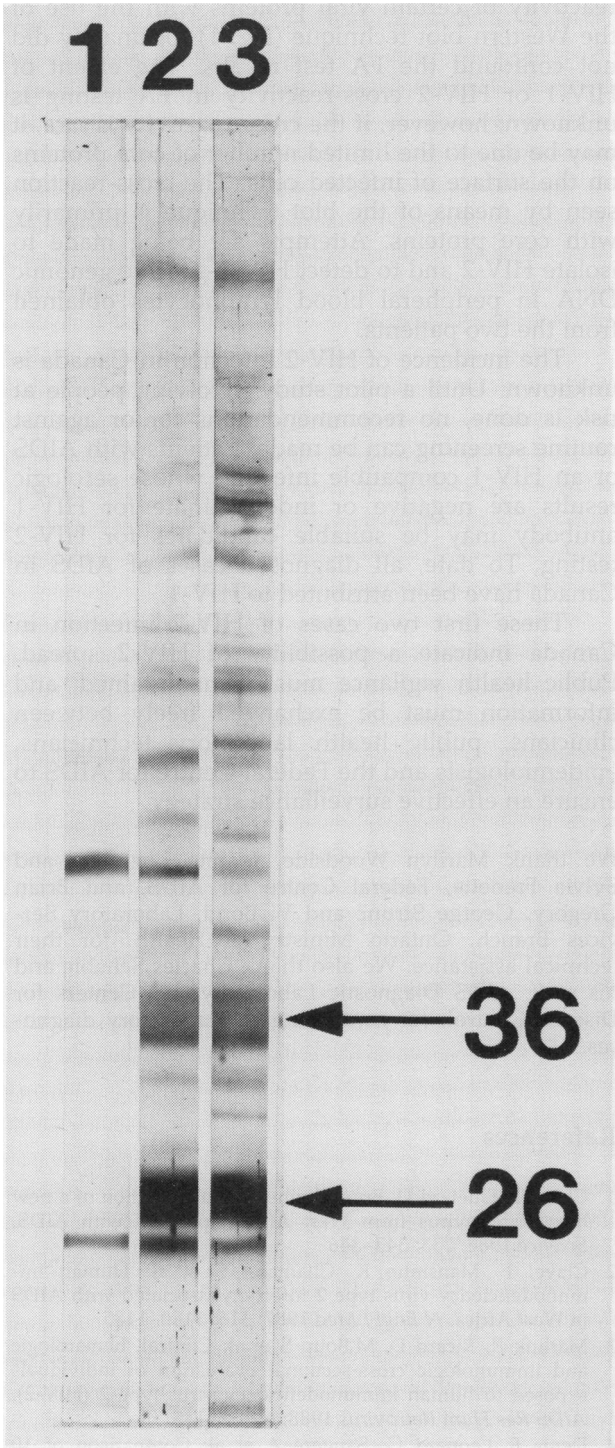

Fig. 2 — Results of Western blot technique using HIV-2-infected cell lysate. Numbers on right represent molecular weights (× 1000) of HIV-2-specific viral proteins. Strips represent (1) negative reaction for HIV-1 and HIV-2 antibodies but extensive reaction to lymphocyte component of lysate, (2) serum from first patient, with antibody reactive to HIV-2 *gag* protein with molecular weight of 26 000 and to HIV-2 *env* glycoprotein with molecular weight of 36 000, and (3) serum from second patient, with same antibody reactions as shown on strip 2.

HIV-2 serologic findings

The results of the immunoblot tests for HIV-2 demonstrated the presence of antibody to the transmembrane protein (molecular weight 36 000)

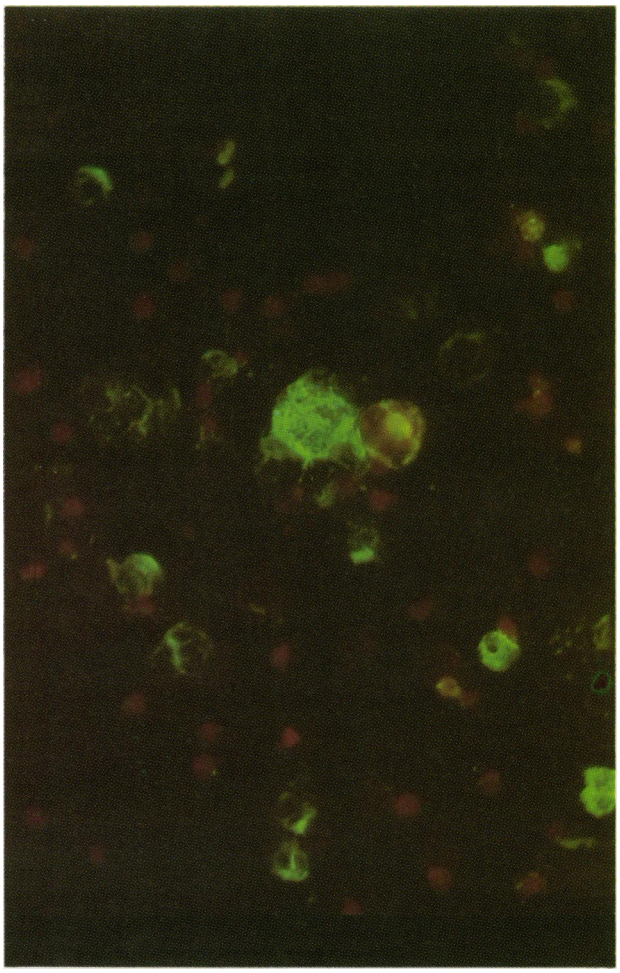

Fig. 3 — Results of indirect fluorescent antibody testing for HIV-2, showing strong cytoplasmic fluorescent reaction of serum from first patient to HIV-2-infected HUT 78 cells; serum sample from second patient displayed identical reaction (original magnification × 400, reduced by about 60%).

and *gag* viral protein (molecular weight 26 000) (Fig. 2). Such reactivity was not seen with the use of HUT 78 cell lysates.

Findings from indirect FA testing

The results of FA testing using HUT 78 cells infected with HIV-1 and HIV-2 revealed no specific HIV-1 antibody. The serum samples reacted strongly with the HIV-2-infected cells (Fig. 3) but not at all with the uninfected cells.

The wife of the second patient was tested for HIV-1 and HIV-2 antibody and found to be seronegative.

Discussion

HIV-2 infection is common in specific regions of western Africa.[5] Until this report the spread of such infection into North America appeared to be limited to a single case in New Jersey, which involved an immigrant from western Africa.[4] The identification of a seropositive person from an endemic area is not unexpected.

It is reassuring that the commercial kits detected HIV-1 antibody in the two cases presented here. A comprehensive evaluation of the ability of such kits to detect HIV-2 antibody is under way at the Centers for Disease Control (Richard George: personal communication, 1988). The degree of cross-reactivity of certain viral proteins with the use of the Western blot technique (Fig. 1) fortunately did not confound the FA test results. The extent of HIV-1 or HIV-2 cross-reactivity in FA testing is unknown; however, if the cross-reactivity is rare, it may be due to the limited number of core proteins on the surface of infected cells. The cross-reaction seen by means of the blot technique is primarily with core proteins. Attempts are being made to isolate HIV-2 and to detect HIV-2-specific genomic DNA in peripheral blood lymphocytes obtained from the two patients.

The incidence of HIV-2 infection in Canada is unknown. Until a pilot study involving people at risk is done, no recommendations for or against routine screening can be made. Patients with AIDS or an HIV-1 compatible infection whose serologic results are negative or indeterminate for HIV-1 antibody may be suitable candidates for HIV-2 testing. To date, all diagnosed cases of AIDS in Canada have been attributed to HIV-1.

These first two cases of HIV-2 infection in Canada indicate a possibility of HIV-2 spread. Public health vigilance must be maintained, and information must be exchanged freely between clinicians, public health laboratory technicians, epidemiologists and the Federal Centre for AIDS to ensure an effective surveillance strategy.

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