ISOLATION OF HUMAN IMMUNODEFICIENCY VIRUS FROM THE BLOOD OF LYMPHADENOPATHY PATIENT FROM BRATISLAVA

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Received June 15, 1988

Human immunodeficiency virus (HIV), the causative of the acquired immune deficiency syndrome (AIDS) (*1*) was recovered from patients suffering from AIDS, ARC, persisting generalized lymphhadenopathy, as well as from infected asymptomatic persons, using cultures of blood mononuclear cells (*2*).

The 24-year-old romiscuons homosexual man was treated in May 1986 for a severe respiratory tract infection. In April 1987 he developed lymphadenopathy and was found seropositive for HIV-1 by EIA and immunoblot assay. In August 1987 antibodies to the recombinant HIV envelope proteins in his serum titered 1 : 4096, but antibodies to recombinant core antigen were detectable at dilution 1 : 8. Virusneutralizing antibodies (*3*) titered 1 : 200 in the serum sample. HIV antigen (solid-phase EIA, Abbot) was found again in his serum sample in February 1988. At these interval the CD4/CD8 T cell ratios were 0.40 (766 CD4 cells per mm^3) and 0.33 (501 CD4 cells per mm^3), respectively.

For virus isolation the patient's lymphocytes were separated from fresh heparinized blood on the Ficoll gradient. Cultures of lymphocytes were established in RPMI-1640 medium containing 10 % foetal serum, 10 % T-cell growth factor (IL-2), polybren (2 μg per ml) and anti-alpha IFN serum. Two isolation systems were used: a) PHA-stimulated patient's lymphocytes were after three days incubation cocultivated with normal human lymphocytes (*4*); b) non-stimulated patient's lymphocytes were mixed with previously stimulated normal lymphocytes (*5*). To such culture, 10^6 normal lymphocytes were added at each passage. Every three of four days, the cultured cells were tested for the presence of the viral antigens by indirect immunofluorescence using a human serum with confirmed presence of HIV specific antibodies and the supernatants were tested for the presence of HIV antigens. Virus was recovered only from patient's lymphocytes which had not been stimulated from day 20 after establishment of the cultures. The isolate (BTSDN-1) subsequently infected cultures of normal lymphocytes, where the virus production started from the eleventh day after infection.

Our findings are consistent with the observation of Goudsmit *et al.* (*6*) concerning the more frequent HIV antigeneamia in persons showing lowered titres or absence of antibodies to core peoteins. These events may witness ongoing viral replication. Such findings may be useful for selection of HIV-seropositive subjects to start drug-therapy reduction of antigen load.

References

1. Barné-Sinoussi, F., Chermann, J. C., Rey, F., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W., Montagnier, L.: *Science* **220**, 868, 1983.
2. Levy, J. A., Shimabukuro, J.; *J. Inf. Dis.* **152**, 734, 1985.
3. Rey, F., Berré-Sinoussi, F., Schmidtmayerová, H., Chermann, J. C.: *J. virol. Meth.* **16**, 1987.
4. Barré-Sinoussi, F., Mathur-Wagh, V., Rey, F., Brun-Vezinet, F., Yancovitz, S. R., Rouzioux, C., Montagnier, L., Mildvan, D., Chermann, J. C.: *JAMA* **253**, 1737, 1985.
5. Michaelis, B., Walker, C. M., Legg, H., Whalen, M., Levy, J. A.: *Abstracts of III. International Conference on AIDS*, Washington, p. 112, 1987.
6. Goudsmit, J., Lange, J. M. A., Paul, D. A., Dawson, G. J.: *J. infect. Dis.* **155**, 558, 1987.