

## VIRUS DISSEMINATION VIA THE LYMPHOMYELOID COMPLEX

J.M. Yoffey

Department of Anatomy and Embryology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

### ABSTRACT

*In the previous AIDS symposium organized by the Society, Witte and Witte (1) made a number of predictions, one of which was that in AIDS patients, "Lymph from the thoracic duct should be strongly positive for HIV." Though direct evidence for this is lacking, some early experiments of ours with vaccinia virus (2) are fully in accord with this prediction, to which they lend indirect support.*

*In rabbits, nasally instilled vaccinia virus spreads via the lymphatic pathway (afferent peripheral lymph-deep cervical gland-efferent lymph-thoracic duct) in as short a time as nine hours. Virus is transported mainly in cells, for when the efferent lymph is centrifuged virus is found only in the cell sediment. It seems reasonable to assume that other viruses, including HIV, are similarly disseminated. Paradoxically, the lymphomyeloid complex both greatly facilitates the spread of virus, and at the same time, mounts the immunological defenses against the virus which it so effectively helps to disseminate.*

*Whatever the portal of entry of the virus, its transport by migrating cells ensures its dissemination throughout the lymphomyeloid complex, including the bone marrow. The bone marrow is an integral part of the complex, as the prime source of B lymphocytes, T lymphocyte precursors, and many of the antigen-presenting cells as well as the granulocytes. There is some evidence concerning possible*

*ways in which the bone marrow can contribute to the development of immune deficiency in AIDS patients. The bone marrow merits further study in this context.*

The lymphomyeloid complex is not only responsible for the immune response to viral infection. Paradoxically, it also greatly facilitates the dissemination of virus. This results from two basic factors, namely (1) the cytotropism of viruses, and (2) the extensive migration throughout the organism of virus-containing cells. As far as cell migration is concerned, the dissemination of virus is largely predictable on the basis of what is known about the Cellular Migration Streams which constitute so distinctive a feature of the lymphomyeloid complex (3).

### *Early work on virus dissemination*

Some of the factors involved in virus dissemination were originally noted in the case of vaccinia virus in rabbits (2). These experiments were a continuation of earlier studies in which, following nasal instillation, vital dyes or proteins appeared in cervical lymph, en route for the thoracic duct and blood stream, within 2-3 hours (4). Vaccinia virus, on the other hand, was not found in cervical lymph within the first nine hours after nasal instillation. But after 12 hours, and for seven days thereafter, virus could practically always be found in the cervical lymph. The virus does not pass through the nasal mucous

membrane immediately but appears first of all to settle in the mucosa, where it proliferates after its administration. From the primary focus of infection in the nose a continuous stream of virus passes through the cervical lymph gland into the blood, via the main cervical lymph duct draining into the terminal portion of the thoracic duct.

However, virus does not just pass through the node as free virus particles, for when the virus-containing efferent lymph was centrifuged, virus was not found in the supernatant fluid, but only in the cellular sediment, which consisted predominantly of lymphocytes together with smaller numbers of other cells (4,5).

In these early experiments with vaccinia, thoracic duct lymph was examined for its virus content for a period of only 7 days. But the virus may be presumed to have been present in thoracic duct lymph for at least as long as is needed for immunity to develop. In infections with other viruses, virus may be presumed to be present in thoracic duct lymph for as long a period as is required to reach the immune stage. In the case of HIV, this usually would mean indefinitely.

I have emphasized the presence of virus-containing cells in thoracic duct lymph in the case of vaccinia, since this fits in well with the prediction by Witte and Witte (5) that a similar situation would be found to exist in the case of those AIDS patients in whom infection was occurring via lymphatic channels. "In AIDS patients, central lymph from the thoracic duct should be strongly positive for HIV."

#### *Infection of lymph glands through the bloodstream*

It would appear in fact to be a fairly safe prediction that even in those patients where the original HIV infection does not occur via lymphatic channels, as for example where the virus is conveyed through blood transfusion, the lymphatic system is bound sooner or later to become involved, with ultimately the same sequence of events in many lymph glands,

culminating in the prolonged presence of virus in thoracic duct lymph. Once host lymphocytes become infected, they can find their way into any lymph gland either via peripheral lymph (4,5) or through the post-capillary veins (6,7). The gland in which the infected lymphocytes are found can then serve as a further center for the proliferation and dissemination of virus. Infected lymphocytes can leave the lymph gland and enter the blood stream through three routes, namely the post-capillary veins (7), the thin-walled veins (8), or the efferent lymph.

#### *Bone marrow*

It seems to be generally accepted that the CD4<sup>+</sup> (helper-inducer) T cell is the major target of HIV (9) though the virus has also been found in other cells, including phagocytic cells such as monocytes or macrophages. Wong-Staal and Gallo (10) emphasize that HIV can be found in many different cell types, while Gallo et al (11) have suggested that the macrophage could well be the primary early target of HIV, and that the CD4 cells are infected secondarily after interaction with macrophages.

The presence of HIV in macrophages and monocytes raises the possibility that the reticuloendothelial system has a part to play. The problem arises not so much in the case of virus-containing cells as in the case of virus particles set free in the blood stream, through the disintegration of virus-containing cells. The possibility that the reticuloendothelial system may be involved calls for special consideration in the case of the bone marrow which constitutes a major component of the RES. The clearance of any HIV particles from the blood stream could conceivably take place through those parts of the reticuloendothelial system with which they come into contact, including inter alia the endothelium of the marrow sinusoids. The initial uptake could be through rapid vesicle transport across the sinusoidal endothelium into the marrow parenchyma, as in the case of inanimate particles (12-14). Once the virus particles are in

the parenchyma, their effect will depend upon the cell types which they can infect and in which they can proliferate.

The bone marrow is the primary source of T cells, through the continuous production of thymocyte precursors migrating to the thymus. The involvement of other cells is presumably responsible for the anemia, thrombocytopenia, and leukopenia frequently observed in AIDS patients. Geller et al (15), in a study of 30 marrow biopsies in cases of AIDS, observed a "typical AIDS bone marrow, with decreased numbers of segmented granulocytes and erythrocyte precursors, and increased numbers of immature granulocytes and large lymphoid cells." Stella et al (16) noted defective *in vitro* growth of several colony-forming cells, namely CFU-GEM, CFU-GM, and BFU-E and CFU-Mk. Their findings suggest that HIV may have a special tropism for the more primitive marrow cells.

If the marrow does in fact become infected with HIV through the uptake of free virus particles, this need not exclude infection by the uptake of virus-containing T cells. T cells are continually entering and leaving the marrow (3). With appropriate immunohistochemical techniques, it is possibly to demonstrate the widespread distribution of T cells in human marrow (17).

Whatever the route by which the marrow becomes infected with HIV, whether through the uptake of free virus particles, virus-containing cells, or both, there appears to be no doubt that it does become infected in cases of AIDS, and it seems likely that this infection may be an important contributory factor in the development of the characteristic immune deficiency and blood changes (18).

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Dr. J.M. Yoffey  
 Department of Anatomy &  
 Embryology  
 Hebrew University-Hadassah  
 Medical School  
 P.O. Box 1172  
 Jerusalem 91010, ISRAEL

## BIOLOGIC ACTIVITY OF HUMAN RETROVIRUSES WITH SPECIAL REFERENCE TO IMMUNOSUPPRESSIVE POTENTIAL

**F. Barré-Sinoussi**

Head of Laboratory Biology of Retroviruses Unit, Pasteur Institut, Paris, France

### ABSTRACT

*Possible mechanisms involved in the lymphopenia observed in HIV infected individuals include the cytopathic effect for CD4 positive cells, cell fusion phenomena, autoimmune mechanisms and release of suppressive factors. Infection of T cell precursors might also explain the hematologic abnormalities seen in AIDS patients. Studies showing that immature bone marrow cells are susceptible to HIV infection give support to this hypothesis. Moreover, an abnormal differentiation of infected bone marrow progenitors, such as the appearance of CD<sub>1</sub><sup>+</sup> cells, is observed in vitro and might be relevant to virus integration into the cellular genome. Recently, by in situ hybridization and immunohistochemistry experiments, we also evaluated the*

*susceptibility of immature thymocyte sub-populations to HIV-1 infection. Preliminary data on the nature of immature thymic cells permissive to HIV infection and the possible consequences on the pathogenesis of AIDS will be discussed.*

Dr. Françoise Barré-Sinoussi  
 Head of Laboratory Biology of  
 Retroviruses Unit  
 Pasteur Institute  
 28, rue de Dr. Roux  
 75724 Paris Cedex 15, FRANCE

[Dr. Barré-Sinoussi was unable to participate but her questions are included in the Questions Posed by Panelists in the Summary.]