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SPECIAL COMMUNICATION

THE XI AIDS CONFERENCE AND HIV-1 LYMPHOPATHY

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ABSTRACT

The random reverse transcription of HIV-1 RNA into the genes of large dividing lymphocytes and transport of integrated proviral DNA within and between persons via migratory small cytoplasm depleted lymphocytes derived therefrom causes deterioration of the entire lymphopoietic system. Secondary results are progressive failure in homeostasis, loss of sensitivity to potentially therapeutic drugs, and inability to produce preventive vaccines. The world-wide prevention of HIV-1 sickness and other lymphopathic retroviral diseases will depend on greater individual cooperation, especially with respect to minimizing the numbers of infected lymphocytes which migrate between persons through blood, semen, uterine endocervical secretions and maternal colostrum.

On July 7-12, 1996, 14,137 delegates convened in Vancouver, B.C. to share knowledge and experience at the XI International Convention on AIDS. The theme was "*One World. One Hope.*" The venue was subdivided into 4 tracks — A: Basic Science; B: Clinical Science; C: Epidemiology and Public Health; and D: Social Science: Research, Policy and Action. The outcome was mixed optimism and pessimism in all tracks, especially so with respect to drugs for treating human immunodeficiency virus (HIV) infections.

Human lymphotropic retroviruses (1), especially HIV-1, are proving to be the most debilitating and deadly infectious pathogens the civilized world has yet encountered. Therefore, it would seem timely for a lymphologist familiar with the *homeostatic* roles of lymph, lymphatics, lymph glands and lymphocytes to comment.

A-B Basic and Clinical Sciences — Messages from the Meeting

1. After the natural development of circulating antibodies to the gp120 component of HIV-1 virus capsules, the HIV-1 viral load measured in terms of polymerase chain reaction (PCR)-detectable viral RNA in circulating blood plasma correlates with rapid progression into clinical illness in the form of acquired immune deficiency syndrome (AIDS). The correlation appears more predictive of disease progression than measuring the reduction of small circulating lymphocytes identified as cluster differentiation (CD)-4 + cells in circulating blood.

2. In HIV-1 infected individuals with PCR-detected plasma HIV-1 viral RNA loads ranging from 10^3 to 10^5 copies per mL in circulating plasma, the therapeutic addition of one or more protease inhibitors to regimens consisting of one or more reverse transcriptase inhibitors reduces the plasma HIV-1 RNA load 10-100 fold or to undetectable levels during treatment for weeks or months,

hopefully with beneficial clinical effects. However, with cessation of treatment, the circulating plasma viral load usually returns, along with disease progression, at least in HIV-1+ adults.

3. An anti-viral factor (CAF) derived from circulating CD8 cells was postulated to play an important role in limiting HIV-1 production from infected CD4+ cells.

4. At least 3 chemokines, RANTES, MIP-l alpha and MIP-1 beta, were postulated to prevent entry of cell-free virus into receptive CD4 cells, at least in PHA-stimulated tissue culture.

5. A rapid rate of mutation of HIV-1 and the corresponding reverse transcriptase in the lymphocytes of infected humans has had three important consequences:

a. The identification of at least 10 different subtypes of HIV-1 of world-wide distribution, and variable classes, termed "clades," within subtypes whose pathogenicity and modes of transmission may differ.

b. The development of resistance to therapeutically administered reverse transcriptase inhibitors, as well as newly developed protease inhibitors.

c. Difficulties in producing a human anti-HIV-1 vaccine or vaccines which will prove effective.

C-D. Epidemiology, Public Health and Social Sciences—Messages:

The main themes concentrated on preventing the spread of cell-free HIV-1 from one person to another, especially via sexual intercourse in all nations; and via needles contaminated with blood in many well-developed nations. Much discussion centered on personal and governmental attitude development; condom distribution and use; the need for women to have additional choices for preventing coital transmission; the role of other sexually transmitted diseases in the global spread of AIDS; the need for supplying clean needles and syringes to persons addicted to "shooting" drugs; and the need for additional HIV-1 blood testing, especially for preventing sexual transmission and detecting mothers whose offspring are at risk for HIV infection. The summary session stressed personal and community, as opposed to governmental, approaches to prevention; the wide gaps which exist between nations with respect to personal income, facilities, health care and capacity to cope with AIDS, and the global need for mutual respect and cooperation to counter HIV infections. Encouraging were the numbers of volunteers in Vancouver who demonstrated such mutual respect and freely donated much time to help delegates and infected persons in attendance.

Comments on basics from a Lymphologic Point of View:

HIV-1 is unique in that it initially causes a self-limited transient "viremic" illness, often less symptomatic than a common cold or infectious mononucleosis. However, due to the random (2-4) manner wherein HIV-1 reverse transcriptase inserts viral RNA into the DNA of large lymphocyte precursors during mitosis when the chromosomes are most unstable (4), the outcome is lethal several years later as cell and gene failure develops in diverse portions of the lymphatic apparatus, as defined by Drinker and Yoffey (5). Debility and death usually ensue from impaired *homeostasis*, as described by Claude Bernard (6), and not just from "immunodeficiency" (4,7).

Normally in a 70 Kg individual, the body contains 100 trillion living cells, of which 25 trillion are circulating erythrocytes especially adapted to transport oxygen, concentrate carbon dioxide and buffer pH. Another 25 trillion lymphocytes grow in lymph glands (LG) and migrate via the blood circulation into and through all body tissues at random to perpetually extrude and supply essential nutrients, regulatory molecules and immunologic protection to remaining body cells (4,7). During the natural course of maturation from large precursor cells into small cytoplasm-depleted cells, the lymphocytes in lymph

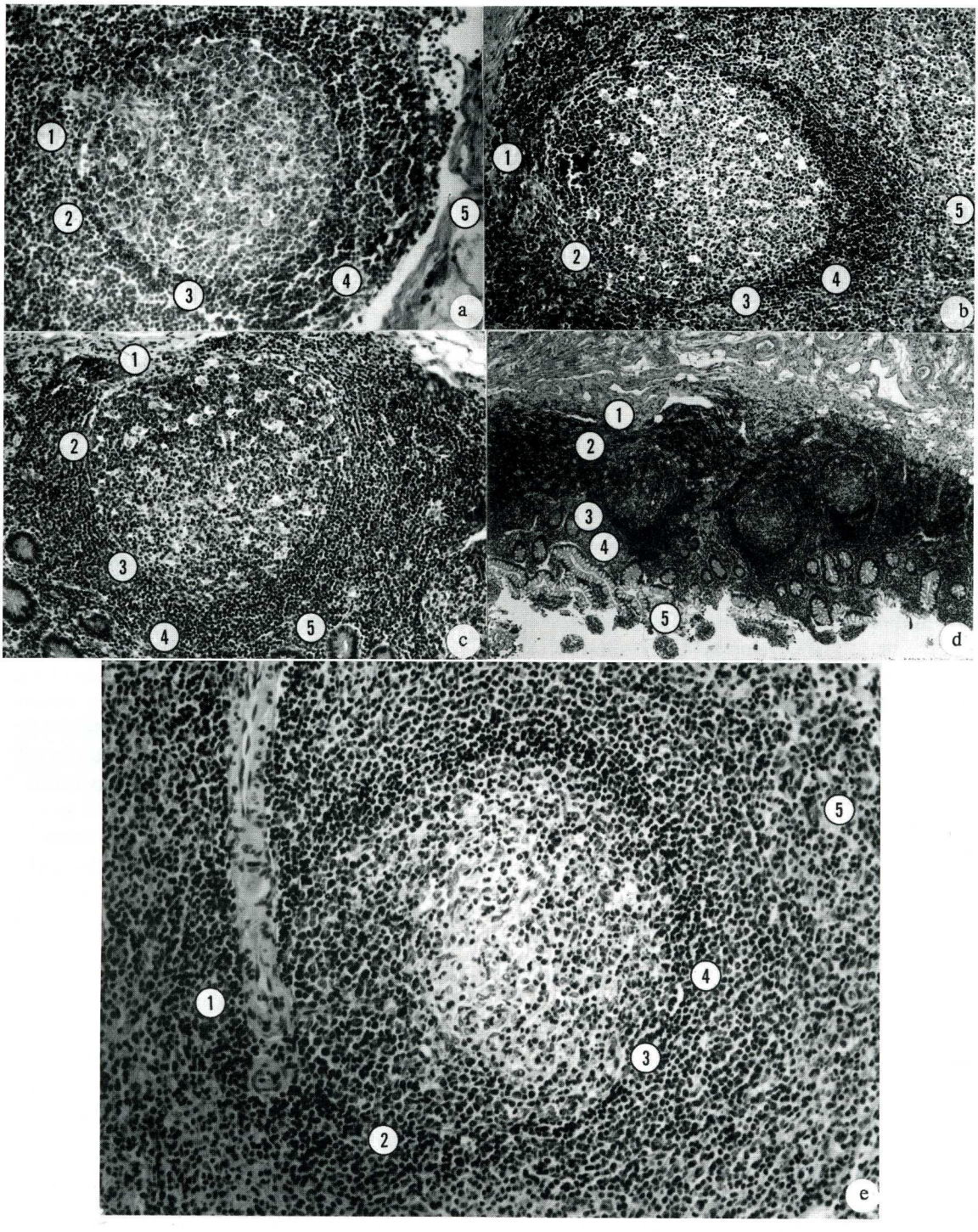

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Fig. 1. Photomicrographs of polarized germinal centers (GC) and secondary lymph follicles in normal ⓐ lymph node x100; ⓑ pharyngeal tonsil x100; ⓒ cecal appendix x100; ⓓ Peyer's patch of terminal ileum x40; and ⓔ spleen x200. Each GC normally develops to comprise:
① An efferent or central arteriole surrounded by a sheath of medium-sized lymphocytes (MSL).
② A juxta-arteriolar dark staining lymphocytopoietic pole containing closely packed large germinal center lymphocytes (LGCL), many of which exhibit mitosis. There are also many interspersed large germinal center macrophages (LGCM) containing apoptotic or pyknotic nuclear remnants of small cytoplasm-depleted lymphocytes (SCDL). The LGCM are supported in a fine reticular stroma formed by mesenchymal reticular cells (MRC) and follicular dendritic cells (FDC).
③ An ab-arteriolar pale staining reactive pole made up of macrophages. MSL and plasmacytes supported in a fine reticular stroma and pointing toward the local sources of exogenous, as well as endogenous antigens shown at ⑤. The pale pole usually is PAS-positive, indicating a relatively high concentration of mucopolysaccharides and RNA, whereas the dark pole is Feulgen-positive, indicating a high concentration of DNA.
④ A thick ab-arteriolar mantle zone or cap pointing toward ⑤ and a thin juxta-arteriolar mantle zone, both comprised of densely packed SCDL contained in fine reticular stroma.
⑤ The most likely source of exogenous or endogenous antigens toward which each GC is oriented, i.e., peripheral and penetrating lymph sinus in nodes (a) pinocytic crypt epithelium in tonsils (b), mucus producing epithelial glands in the appendix (c), epithelial pinocytes and crypt epithelium in Peyer's patches (d), and arteriovenous sinusoids in the red pulp of the spleen (e).

Although primary lymph follicles surrounding clusters of large dividing lymphocytes and macrophages develop during gestation in all vertebrates, such polarized GC in secondary lymph follicles do not normally develop in mammals until birth and the onset of oral feeding, breathing and exposure to the external environment. They do not normally develop in the thymus or bone marrow. Polarized GC normally develop from syncytially arranged periarteriolar mesenchymal reticular cells, first, in the terminal ileum, then in the spleen, tonsils and adenoids and finally in the lymph nodes. Development is rudimentary in germ-free mammals. Normally, the polarized GC develop proximally along the course of arterioles serving primary lymph follicles, but grow relatively rapidly to push aside the lymphocytes and other cells previously generated from primary follicles. Growth usually commences from periarteriolar clusters of macrophages and large lymphocytes forming the pale poles ③. However, owing to relatively rapid mitotic growth of the LGCL in the dark poles ②, the nearby mantle zones ④, consisting mostly of SCDL become greatly thinned near the afferent arterioles ① where MSL generated from the LGCL accumulate to form the periarteriolar lymphocyte sheath, as especially well shown in the spleen ⓔ.

Normally polarized GC achieve maximal development during childhood and until puberty. Thereafter, the polarized GC gradually involute in parallel with thymic age involution, but remain defined until old age and death. During regional infections or experimental inoculation of strong antigens, the regional polarized GC typically become hyperplastic ±4 days after serial responses of sinus macrophages and periarteriolar plasmacytes to an endogenous or exogenous antigen encountered for the first time. They usually involute with discharge of polyvalent soluble antibodies, followed by release of immunocompetent SCDL, within 10-14 days after first exposure to a foreign antigen, especially if the source of the antigen is eliminated. After subsequent exposures to the same antigen, the polarized GC usually evolute and involute more rapidly in concert with accelerated circulating antibody production, followed by reactive SCDL responses, both of which appear enhanced by reutilization of DNA from circulating "primed" or immunocompetent SCDL. However, during the course of HIV-1 infections, the polarized GC and surrounding lymphocytes in the spleen, gut, nodes, tonsils all become hyperplastic during latency, gradually lose their polarity, become dysplastic and, finally, disappear or change into malignant neoplasms during terminal phases clinically defined as AIDS.

glands progressively extrude cytoplasm (7-9) which dissolves to release a variety of soluble globulins, including immune globulins and lymphokines (4,7,10). The small cytoplasm-depleted lymphocytes (SCDL) generated in LG, may disintegrate *in situ*, especially under the influence of adrenal glucocorticoids (7,11), or migrate from LG via the vascular circulation to wander "inside round about" at random through the interstices and into most, if not all, body cells and body secretions (4,7,12-17). The SCDL undergo disintegration with release of high concentrations of phosphate-rich nucleotides which can be reutilized for energy, DNA repair or modulation of growth in recipient cells (7,18-23).

Although very large, large, medium and small-sized lymphocytes in a ratio of ± 1:10:100:1000 normally constitute 25 % of body cells (7), they normally constitute only ± 2% of body mass (5,12), owing to nuclear condensation and cytoplasmic depletion during maturation (7,8,12). As a result, the lymphatic organs (including the spleen,

thymus, nodes, adenoids, tonsils and diffuse lymphopoietic tissue of the gut) and SCDL normally contain concentrations of DNA-bound phosphate 5-20x than that found in remaining body organs and cells (7,18).

The LG renew their lymphocyte DNA at rates sufficient to replace the average lymphocyte in the body every ± 42 hours, as judged by mitotic indices (12), DNA-bound radio-phosphate turnover (24), and more recent calculations (25,26). Following birth in mammals, polarized germinal centers (GC), as shown in *Fig. 1*, develop around relatively large arterioles in all LG, except the thymus (27,28), to process endogenous and exogenous antigens from afferent lymph and circulating blood, to produce polyvalent but highly specific antibodies to a variety of antigens, and to produce relatively large numbers of SCDL, which recognize the source of the processed antigens (4,7). The average germinal center has a life cycle of 10-14 days during which the large germinal center lymphocytes (LGCL) undergo 6-10 mitoses with ± 6 hour intermitotic intervals to produce 64-1024 smaller lymphocytes (12,27,29) and untold quantities of soluble globulins derived from progressive extrusion of cytoplasm from LGCL (7,8). The bulk of antibodies to a new antigen usually leave the GC 6-10 days after exposure to a new antigen serially processed through sinus macrophages and plasmacytes (7,30). Smaller lymphocytes, on the other hand, break through the mantle to accumulate in periarterial sheaths (7) and leave the GC during the next 4 days (4,7,29). The SCDL derived from GC and other LG sites (8,12) migrate at random into and through body fluids, interstices and other cells at a rate of ± 0-40 µM per minute (31) as they fulfill their ultimate destiny, whether to modulate growth or to destroy other cells or microorganisms recognized as antigenic (7). Characteristically, the SCDL migrate at random through the host tissue until they encounter an object or cell which they encircle (peripolesis) or enter, wander within or pass through—hence, the term *emperipolesis*, "inside roundabout wandering" (13). Whereas *peripoletic* SCDL may lyse cells on contact; *emperipoletic* SCDL are prone to enter other cells, undergo intracellular nucleolysis, or exit without apparent effect (13-15). Humble (13) noted that *emperipoletic* SCDL are especially prone to enter other cells just before or during their mitosis (13). Sherwin (14) noted, however, that *peripoletic* SCDL seldom pass through thick layers of epithelial cells.

Apart from the LG where ± 98% of lymphocytes in the body of a healthy well-fed 70 Kg person normally reside (5,7,12), the numbers of migrating SCDL and small lymphocytes with residual immune globulins on their surfaces in body fluids are as follows: central lymph—$2\text{-}20 \times 10^6$; circulating blood—$2\text{-}5 \times 10^6$; semen—2.5×10^6; maternal colostrum—$1\text{-}3 \times 10^6$; uterine endocervical secretions—± 2×10^5; urine—$5\text{-}10 \times 10^3$; spinal fluid—$1\text{-}5 \times 10^3$; and sweat or tears $<10^3$ per mL during random sampling under normal conditions (32,33). The numbers in saliva vary greatly, depending on gingival cleanliness and blood contamination (33). The numbers of SCDL migrating within and between epithelial cells, based on differential counting in tissue biopsies freshly obtained in a series of 10 healthy individuals, are as follows: jejunal epithelium (75±6), bronchial epithelium (47±3), endometrium (35±8), basal layer of uterine cervix (87±43), and corneal basal layer epithelium (25± 10 per 1000) (7,16). Practically, no SCDL migrated in or through the *flattened* superficial layers of normal uterine cervical, vaginal or corneal epithelium (7,16). At least 50% of the intraepithelial SCDL in sampled tissues showed advanced stages of apoptosis, potocytosis or pyknosis (16). The numbers of intraepithelial SCDL found in a given sample appeared to correlate with the customary rate of cell turnover described by Leblond (34) for each cell class (16). In the germinal centers (GC) of LG, where LGCL mitoses are extraordinarily frequent, many GC macrophages contain the apoptotic remnants of 5-10 SCDL (7,12). This observation has led many to

conclude, after Trowell (22), that in addition to reutilization of DNA from SCDL in other tissues, large quantities of SCDL DNA are normally reused in GC during lymphopoiesis for sustaining immunologic memory, as well as to coordinate *homeostasis* within the lymphatic apparatus (7,18).

The basic research reported from Vancouver focused on the circulating HIV-1 RNA load and progressive depletion of CD4 lymphocytes as the harbinger and cause of AIDS, respectively. However, lymphologists should recognize that issues are more complex for the following reasons: 1. The random insertion of retroviral RNA into LGCL DNA during mitosis or during the S-phase of DNA synthesis and strand duplication lies at the crux of HIV-1 lymphopathy (4). 2. Because there may be ± 100,000 different genes in each LGCL (35), the consequences of random HIV-1 RNA nucleotide substitution into any given gene or number of genes coding for the expression of a phenotypic product is unpredictable, as well as cumulative during the prolonged course of HIV-1 infections (4). 3. Usually, 2-6 weeks after initial insertion of HIV-1 RNA into LGCL DNA, polarized GC (4,7,12, 28,29) cooperating with sinus macrophages and periarteriolar plasmacytes sequentially processing foreign and endogenous antigens (4), customarily produce circulating antibodies reactive with the gpl20 component of HIV-1 capsules (4,36). Such antibodies persist throughout the latent course of HIV-1 infections (36), even though integrated provirus, the corresponding viral RNA and reverse transcriptase may be mutated once or more during the course of each LGCL mitosis (37,38).

Following the demonstration of circulating anti-capsular antibodies in infected persons, encapsulated HIV-1 such as that seen in PHA-stimulated tissue culture and depicted in modern diagrams remains to be demonstrated microscopically outside GC, as well as in circulating blood or body secretions (4). Although occasional clusters of encapsulated HIV-1 have been found between LGCL and supporting follicular dendritic cells (FDC) (39-41), the outstanding features of HIV-1 lymphopathy during the long interim between the first development of circulating anti-gpl20 antibodies and the onset of AIDS with disappearance of most circulating CD4+ SCDL are as follows: 1. GC hypertrophy and hyperplasia throughout all lymphatic organs (39-41), except the thymus where GC do not normally develop (4,7,28). 2. PCR-detectable HIV-1 proviral DNA in ±1% of circulating SCDL and a frequency of infected cells 5-10 times higher in and around the GC within the spleen, nodes, Peyer's patches, tonsils and adenoids. (39-42). 3. Amorphous, electron-dense precipitates of HIV-1 RNA and antibodies to HIV-1 capsular proteins between hyperplastic LGCL and FDC in all these lymphatic organs (40,41). Such precipitates on the LGCL surfaces restrict the dissemination and circulation of encapsulated HIV-1, at least during latent phases of HIV lymphopathy (4, 40).

During later stages of HIV-1 infections, before GC atrophy and circulating lymphocytopenia supervene, loss of polar orientation and LGCL dysplasia, but not necessarily immunodeficiency are common. Circulating CD4 SCDL may not appear critically reduced below 200 to 400 per µL. The clinical sequelae include fatal autoimmune nephritis, autoimmune hemolytic anemia, platelet deficiency or granulocytopenia (4); or may include epidermal, oral, rectal or uterine cervical epithelial dysplasia, followed by corresponding neoplasms (4). Progressive angiodysplasia in the form of Kaposi sarcoma (43) is common, especially in association with focal trauma or anal transmitted herpes virus infections (44).

Ten-12 years after initial HIV-1 infection of the LGCL and, possibly as a result of ± 10,000 cumulative genetic mutations (38), the normal processes of DNA repair are impaired (7), the LGCL and GC disappear throughout the LG; most of the LG shrink to empty skeletons with lack of lymphocyte formation. The circulating SCDL mostly

disappear. Then, AIDS sets in as a multivariate *homeostatic* failure including combinations of profound body wasting, lack of resistance to a variety of opportunistic and common infections, dementia secondary to glial inflammatory or degenerative changes, and a high incidence of endothelial neoplasms, epithelial neoplasms, or tumors of cells like LGCL often containing detectable intranuclear EBV DNA (4).

Circulating antibodies vs viral capsular gpl20 do not protect infected individuals from such diverse sequelae. However, it is obscure why such antibodies persist in the face of almost infinite possibilities for random HIV-1 RNA insertion into genes of dividing lymphocytes. In this respect it should be recalled that the plasmalemma of the infected cell initially forms the HIV-1 capsule (45,47) and that the retroviral capsular glycoproteins remain remarkably stable, despite rapid rates of error-prone mutation in HIV-1 proviral DNA, retroviral RNA and reverse transcriptase inside infected cells (47). Therefore, it seems probable that the rate of HlV-1 provirus and secondary HIV-1 retroviral RNA mutation during successive lymphocyte mitoses perpetually exceeds the capacity of GC and remaining portions of LG to produce adequate humoral, as well as cellular defense against each successive mutant within the 10-14 days customarily observed for a given GC to react appropriately. Thus, despite qualitative changes in the HIV-1 RNA encapsulated by the cell plasmalemma, antibodies to the capsular gpl20 continue to be produced and circulate, along with ± 1% of circulating SCDL containing native or mutant provirus for as long as the GC remain functional.

Prevention in a Capsule

Using the foregoing data, one might argue that HIV-1 infections primarily alter the genes of dividing LGCL at random to produce diverse homeostatic failures through error-prone and cumulative interference with the normal production of soluble globulins and migratory SCDL (4). One might suggest further that *emperipoletic* HIV-1 provirus-infected SCDL, or soluble provirus derived therefrom, are common vectors of HIV-1 infection between, as well as within individuals (4,17). However, the principal speakers in Vancouver maintained that capsulated cell-free HIV-1, such as that first identified shedding from immunologically naive umbilical cord lymphocytes or foreign neoplasm cells in PHA-stimulated tissue culture (45), is the vector, as well as the cause of human AIDS. Data refuting this popular point are as follows:

1. Encapsulated cell-free HIV-1 (ECF-HIV) remain to be identified microscopically in body secretions or anywhere in the body of a seropositive person, except between LGCL and FDC on rare occasion (40,41) and, possibly, as particles shed from glial cells in the central nervous system (46) wherein the diffusion of circulating globulins through the "blood-brain barrier" is restricted, although *emperipoletic* SCDL normally pass through into cerebrospinal fluid (4,33).
2. As opposed to HIV-1 provirus, ECF-HIV does not fulfill Koch's postulates as the cause of AIDS or related conditions in seropositive individuals (4).
3. In tissue culture the capsule of ECF-HIV is formed by the plasmalemma of transfected malignant or transformed cells microscopically resembling LGCL (4,45,47) *(see Fig. 2).* Thus, the HIV-1 capsular gpl20 and, possibly other membrane-related parts such as transmembrane fusin (48), are really parts of the cell which genetically codes for production and extrusion of the virus. However, in the body of an infected person, many of whose LGCL usually contain PCR detectable HIV-1 proviral DNA, the plasmalemmal act of virus encapsulation remains to be demonstrated microscopically. Instead, the precipitation of HIV-1 RNA to form "immune complexes" with antibodies vs. gpl20 is almost uniformly found between the LGCL and FDC in the LG of the infected

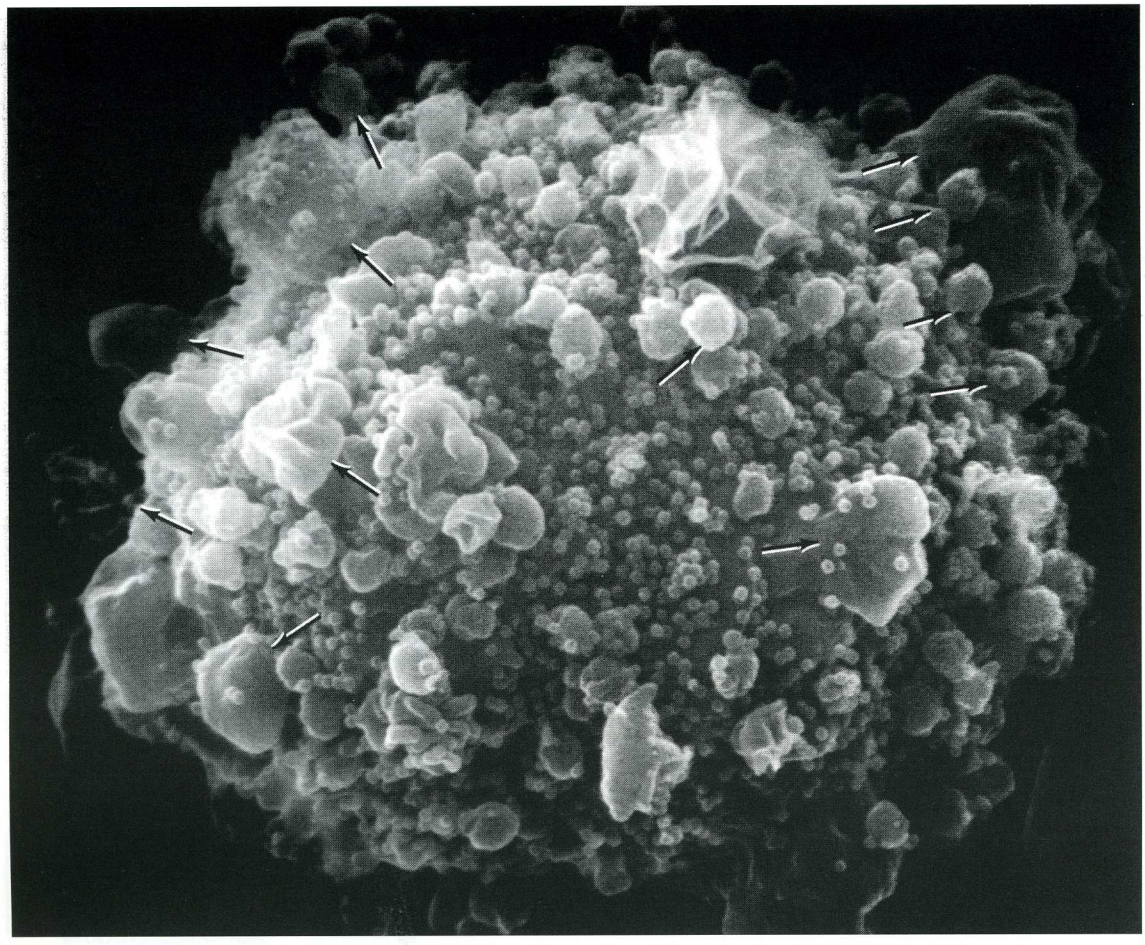

Fig. 2. Scanning electron photomicrograph (20,000x) [courtesy of Dr. Harvey Holmes at the National Institutes of Biological Standards & Control (NIBSC) in the UK] shows unusually prolific shedding of large cytoplasmic fragments (arrows) and tiny HIV-1 particles from a HIV-1-infected lymphoblastoid cell in phytohemagglutinin (PHA)-stimulated tissue culture with added interleukin-2 (IL-2). Note that:

1. The plasmalemma of the infected cell forms the envelope of the shedding retrovirus as well as that of the large extruding cytoplasmic fragments (compare with Barré-Sinoussi, 47).

2. In the body and in tissue culture, such shedding of cytoplasmic fragment is the means whereby large and medium-sized lymphocytes secrete a variety of soluble globulins, including antibodies and lymphokines, and reduce in size to become small cytoplasm-depleted lymphocytes (SCDL) (see 4,7-11,30,33,49,65, and especially Fig. 7 in ref. 4).

3. The shedding of encapsulated HIV-1 from lymphocytes has not been demonstrated in vivo, partly because antibodies to HIV-1 capsular components, especially gp120, precipitates amorphous antigen-antibody complexes consisting of HIV-1 RNA and anti-gp120 antibody between large germinal center lymphocytes and follicular dendritic cells (see text).

4. Probably because PHA-transformed lymphocytes are prone to secrete antibodies toward antigens previously processed in lymph glands, it has not been, thus far, possible to demonstrate shedding of encapsulated HIV-1 from persons with circulating antibodies reactive toward gp120. Therefore, co-culture with immunologically naive or incompetent cells, such as umbilical cord lymphocytes, or neoplastic lymphoid cells lines obtained from HIV-negative persons, has been essential to isolation and electron microscopic demonstration of HIV-1 retrovirus shedding from infected cells and for harvesting/purification of the gp120 viral capsular antigen commonly used in ELISA tests for detecting HIV-1 infection in the body.

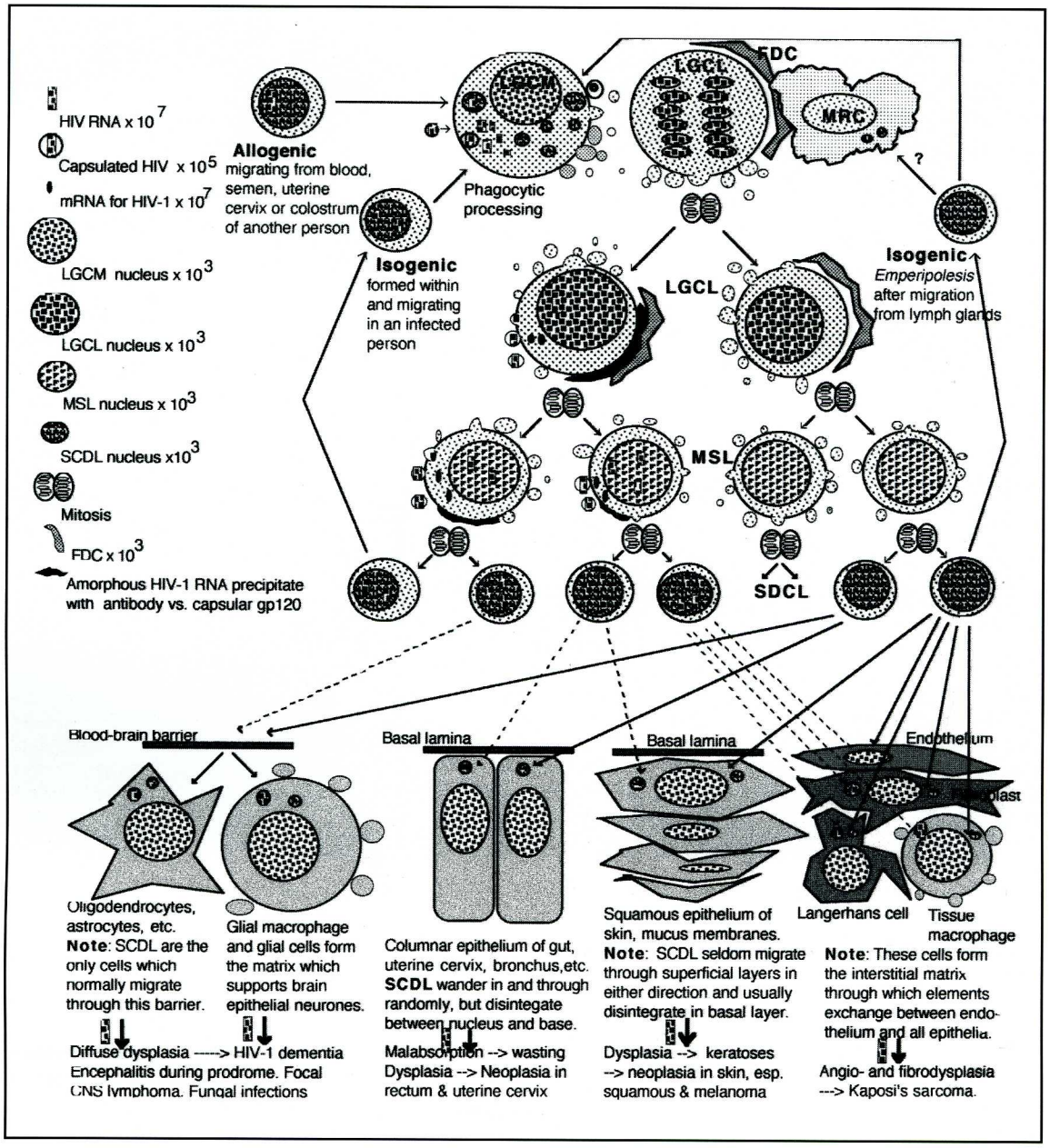

Fig. 3. Lymphotopoiesis and tropisms—normal and in HIV-1 infections. During normal lymphocyte formation in polarized germinal centers (GC), as depicted on the right, mesenchymal reticular cells (MRC) differentiate into large germinal center lymphocytes (LGCL), large germinal center macrophages (LGCM), endothelial cells (not shown), and follicular dendritic cells (FDC), which form a fine reticular stroma supporting all the former. Using influent substrates processed by the LGCM and FDC, the LGCL grow rapidly and divide by mitosis to generate a variety of soluble globulins, including nutritive globulins, antibodies, and lymphokines, along with many lymphocytes of smaller size, large, medium-sized lymphocytes (MSL), and small cytoplasm-depleted lymphocytes (SCDL) are generated from the larger cells by means of mitosis, followed by progressive nuclear chromatin condensation and progressive loss of cytoplasm through shedding in the form of plasmalemma-encased globules which de-polymerize and disintegrate to produce sols rich in dissolved globulins. These soluble globulins flow into

individual, at least until the GC and LGCL practically disappear throughout the body (40,41). Thus, despite the observation that the proviral DNA, viral RNA and reverse transcriptase appear to alter or mutate frequently, genetic stability apparently remains with respect to the formation and antigenic content of the HIV-1 capsule (47). See *Fig. 3.*

4. In order to explain the intrusion of HIV-1 into LGCL, SCDL, glial cells, Langerhans cells or other receptive cells, it is postulated that the CD4 cell surface receptors or fusins (48) facilitate endocytosis, such that intracellular enzymes may break down the capsule and other components. Nevertheless, as opposed to macrophages and other kinds of cells, lymphocytes, as a generic class, do not ingest insoluble foreign particles other than finely dispersed elemental carbon, and do not contain lysosomes or pinocytic vesicles (5). Conversely, GC macrophages, especially those in the juxta-arterial dark-staining lymphopoietic poles (7), appear indiscriminate with respect to matter ingested, are rich in lysosomes, and ingest and digest large numbers of SCDL (7,12) whose remains are shed by clasmatosis (49) to feed adjacent dividing LGCL (7,12).

5. Although it has been shown through radio-phosphate labeling of DNA-bound

lymphatics or into blood circulation, whereas the MSL and SCDL accumulate around afferent arterioles and then actively migrate into circulation. From circulation the SCDL continue emperipoletic migration throughout the body, into most organs, tissues and many dividing tissue cells, and into most body secretions to help regulate cell growth and sustain nutritive, as well as immunologic homeostasis.

In order to sustain homeostasis, each polarized GC responds to exogenous and endogenous antigens processed by LGCM and FDC, as well as other mononuclear cells located between the juxta-arterial dark lymphocytopoietic pole and the epithelia or lymph sinuses toward which each pale reactive pole customarily points. Each GC evolutes with hyperplasia and involutes with discharge of soluble products and SCDL every ±10 days. Each SCDL derived therefrom has an average life span of ±42 hours during which it migrates to play key roles in DNA repair, modulation of growth in other tissues, and elimination of cells or microorganisms whose genes are recognized as incompatible or foreign. Normally the LGCL reutilize significant quantities of DNA from SCDL previously formed in other lymph glands to sustain the integrity of isologous SCDL DNA and to perpetuate genetic information acquired after processing of antigens derived from heterologous cells or genetically incompatible microbes. Homeostasis fails progressively with HIV-1 infection, as provisionally outlined below.

Starting upper left, a migrating allogenic provirus-infected SCDL is engulfed by a LGCM and partially digested therein to release soluble proviral DNA by shedding cytoplasm (clasmatosis). The adjacent LGCL in mitosis, when the chromosomes and genes are most unstable, incorporates the partially digested, "processed" proviral DNA into one or more chromosomes. Depending on which out of 80 to 100 thousand genes in 24 chromosome pairs are being expressed at the time, the proviral DNA may or may not be expressed to code for the production of retroviral RNA. However, during the same mitosis or subsequent mitotic divisions, the left daughter cell (below) inherits the provirus and passes it on to progeny generated with subsequent mitoses. When the provirus-containing nucleotide sequences in the genes of any of the former or their progeny are stimulated by endogenous or foreign signals to produce a phenotypic product, the genes may code for the expression of an altered or defective product, as well as the expression of HIV-1 whose reverse transcriptase (RT) is error-prone and the smooth portion of whose capsule is initially formed by the plasmalemma of the provirus-infected cell. As a result, each time a provirus-altered nucleotide sequence in a given gene is "turned on" to express a phenotypic product, the error-prone RT of HIV-1 expressed inserts HIV-1 RNA at random into the genes in the same or in other chromosomes, as indicted by placing the symbol for HIV-1 RNA in differing nuclear sites. However, the circulation of encapsulated HIV-1 appears limited because: 1. LGCL or MSL plasmalemma initially forms the retroviral capsule; 2. anticapsular antibodies are usually formed by LGCL within 10-12 days after initial infection; 3. the retroviral capsular antigens are stable and not prone to mutation; and 4. anticapsular antibodies precipitate the HIV-1 RNA on the surfaces of infected cells for as long as the GC remain functional. When the GC, LGCL, MSL and SCDL are destroyed by genetic failure and/or autoimmune reactions, AIDS ensues as a global homeostatic failure. Although it might appear that this failure is simply due to circulating SCDL depletion, it is likely that many peculiar features of AIDS are attributable to the transfection of HIV-1 provirus via infected SCDL to other kinds of cells with rapid rates of DNA turnover, such as glial cells, gut, bronchial and uterine cervical epithelium, and cells which form the dynamic interstitial matrix between endothelium and all epithelia, as depicted on the bottom of the illustration.

phosphorous that LGCL reuse significant quantities of DNA from circulating SCDL during lymphopoiesis, it is uncertain whether reutilization is via macrophages or via syncytially arranged mesenchymal reticulum cells from which the LGCL and macrophages are commonly derived (4,7,8).

6. When allogenic SCDL having distinctive markers, such as X or Y chromosome, are transfused for the first time into a healthy person, all marked SCDL disappear from the blood circulation in 30-60 minutes, reappear at 48 hours, attain circulating numbers 6-10 times that inoculated at 6-10 days; and disappear before 14 days without apparent reaction (12). These findings, along with data using other stable, but tolerable chromosome aberrations as markers combined with experience gained from studying GC reactions during allografting of solid tissues, suggest that the DNA of allogenic SCDL is reutilized and replicated temporarily until polarized GC have undergone ± 10 day cycling in first response to an intolerable exogenous antigen (12). With solid tissue allografting, the duration of tolerance is shortened with subsequent allografting unless the growth of GC and circulation of sensitized SCDL is limited by medication, which inhibits LGCL DNA synthesis and adrenal steroids which lyse SCDL (12). Alternatively, isogenic LGCL and SCDL can be eliminated by massive doses if X-irradiation and/or radiomimetic drugs followed by bone marrow transplantation to achieve tolerance to allogenic cells. Either way, the cost of tolerance is reduced resistance to a wide variety of opportunistic and common infections, namely, high incidence of neoplasms, especially lymphoma or Kaposi sarcoma; and graft vs. host reaction, especially when the genes of allogenic SCDL and isogenic SCDL are significantly mismatched. These considerations, when applied to HIV-1 infection, may be significant in that widespread GC reutilization of provirus-infected SCDL DNA during the first ± 10 days before circulating antibodies to gpl20 appear, is probably responsible for the wide dissemination of infection throughout the lymph glands, as well as the burst of "viremia" associated with large numbers of circulating infected SCDL during prodrome. After circulating antibodies become demonstrable, it appears that the error prone (47) or random insertion of HIV-1 RNA into the DNA nucleotide sequences of multiple differing genes is what ultimately makes this retroviral sickness intolerable.

From these considerations, it appears integrated provirus particles in migratory SCDL, or soluble DNA fragments derived therefrom (4), are more likely sources for transfection of rapidly dividing LGCL than encapsulated cell-free HIV-1 or soluble HIV-I RNA.

Lymphologists may suggest further that ECF-HIV and plasma HIV-1 RNA possess no obvious means of locomotion or enzymes to facilitate cell entry. Lymphocytes are the most motile diploid cells, as well as the most common eukarocytes in the body (7,12). The inherent capacity of lymphocytes to migrate from LG into and through blood, endothelium, interstices and other cells, especially just before mitosis (13), is well known (4,7,12-18). In terms of numbers, during latency in HIV-1 infections, ± 1% out of $2\text{-}3x10^6$ SCDL per mL in circulating blood and in some secretions more or less constantly contain proviral HIV-1 DNA (36,42). Compared with undetectable numbers to 10^3 PCR-demonstrable HIV-1 RNA particles per mL of plasma during latency in seropositive persons, PCR-demonstrable HIV-1 DNA particles in SCDL per mL of whole blood usually number 100-10,000 times as many. Finally, when the GC disappear, the LG become lymphocyte-depleted and circulating CD4 cells fall below 400 µL and the plasma HIV-1 RNA particle level may rise to 10^6 per mL (37) — a number almost equal to the number of SCDL that normally circulate. However, the percentage of remaining CD4+ SCDL containing HIV-1 proviral DNA increases sharply and thereby likely increases the capacity of a given inoculum to transmit HIV sickness (37).

Therefore, in terms of quantity, as well as quality, migratory SCDL seem relatively important as vectors of HIV-1 infection.

Prevention of person-to-person spread throughout the world hinges on minimizing the numbers of HIV-1 provirus-infected SCDL which migrate between persons, especially through blood, semen, uterine endocervical secretions and colostrum, each of which normally contain 2-3x$10^{5\text{-}6}$ SCDL per mL. Considering these routes apart from racial, socio-economic and behavioral issues involved, the following recommendations are offered:

1. Hematogenous transmission is most efficient, in part because ± 2 x10^4 HIV-1 provirus-infected SCDL/mL of whole blood from a seropositive person that gain access directly to the bloodstream of another, are mixed efficiently during passage through the lungs, and uniformly are widely distributed within 1-2 minutes to wander via arterial capillaries into the GC of LG throughout the body (4,7). Therefore, preventive measures must target on avoiding sharing blood with seropositive persons and on hollow bore needles used to inject medications directly into blood vessels or to withdraw blood (50-53). Because hematogenous spread now accounts for ± 40% of > 700,000 cases of AIDS cumulatively reported from Western Europe and the U.S.A., *it is imperative to discourage intravenous drug abuse and to supply well-shielded, as well as sterile needles for use within and apart from health care settings*. Parenthetically, it should be noted that processed blood products, such as packed red blood cells, concentrated platelets, hemophilia and immune globulins, given via needles into veins, usually contain few intact SCDL/mL. On the other hand, pooled plasma globulins from multiple donors and lymphocytolyis during steps of processing without pasteurization, cannot entirely eliminate soluble HIV-1 RNA or HIV-1 proviral DNA from the plasma of seropositive donors contributing to the pool (52).

2. Seminal transmission of HIV, though more common, is less efficient than hematogenous transmission, probably because seminal ejaculates seldom exceed 4 mL. (35), contain numbers of SCDL ranging from 10^5 to 10^7 per mL, along with sperm numbering 60-200x10^6 per mL sampled from males without vasectomy (32,60), and depends on migration of infected SCDL through a single layer of columnar epithelial cells lining the uterine endocervical canal or rectum of a consenting sexual partner (17). Whereas seminal SCDL depend on random migration at a velocity of 0-40 µM per minute through columnar endocervical epithelium, basal lamina, interstices and peripheral lymphatics to gain access to the GC of regional LG (4,17), sperm normally migrate at a velocity of 1-2 cm per minute toward an alkaline environment inside the uterine cervix, uterus and Fallopian tubes to find a receptive ovum. Relatively large numbers of columnar cells lining a lumen of wide circumference may make SCDL transmission of HIV-1 provirus through the rectum ±10 times more efficient than transmission through the endocervical canal (54). Therefore, during ano-rectal intercourse, irrespective of the gender of the receptive partner, condom use is universally stressed (55). Because during vaginal intercourse, *emperipoletic* SCDL do not usually migrate through the flattened superficial layer of squamous epithelial cells lining the vagina and external portions of the uterine cervix (16,17), the use of a female condom, uterine cervical cap or vaginal diaphragm is an additional option, which is nearly as efficient as the condom in preventing pregnancy (56,57). Moreover, the use of a cervical cap or vaginal diaphragm, in conjunction with low concentrations of soluble Nonoxynol-9 (N-9), better prevents pregnancy than a uterine cervical barrier alone, and is also more efficient than a condom in preventing transmission of other cell-borne sexual transmitted diseases (STD) in women having many male sexual partners (56,58). Because SCDL and other mononuclear cells in semen are 10-100 times more

sensitive than sperm to the detergent, migration-stopping and surface lipolytic effects of low concentrations of N-9 (59), *it seems desirable to upgrade use of such female contraceptive devices to prevent the coital spread of HIV-1 infections, and other cell-borne STDs.*

3. Transmission of HIV via uterine endocervical secretions appears less efficient than via semen or blood, but it still is the most common form of spread from women to men, especially in nations where female prostitution is common, or males have many heterosexual partners (61). Male risk for transmission of HIV from a seropositive female is reduced ± 10 times by circumcision, probably because a thinned epithelium under redundant foreskin permits passage of provirus-infected SCDL (54,62-64). *Accordingly, circumcision, use of male condoms or female-choice barriers, N-9 and special attention to genital cleanliness may help protect men from AIDS as well as other STDs* (53,63).

4. Colostral transmission of HIV appears relatively inefficient compared with direct hematogenous, transplacental (4) and some forms of perinatal transmission, perhaps because the globulins and SCDL in maternal milk must gain access to the infantile intestine before exerting detrimental or beneficial effects (7,28). Whereas *emperipoletic* SCDL can migrate in either direction through single layer columnar cells lining the jejunum (7), other cogent factors include the rate at which gastric cells produce acid and pepsins, the rate at which the submucosal gut lymphopoietic system develops with oral feeding (65), rate of thymic involution (7,12), rate at which polarized GC first develop in the distal ileum (27,28) and the rate at which the SCDL and immunoglobulins fall with rising fat content in maternal milk (33). Abstaining from breastfeeding and substituting formulated milk has been highly effective in lowering the vertical transmission of HTLV-1 infections by infected SCDL in Japan. It also seems to be beneficial with respect to HIV-1 transmission in nations where nurseries are clean and milk formulas are readily available at affordable cost (61).

5. Although the receptive partner is disproportionately at higher risk during vaginal or anal intercourse, the high frequency of unprotected heterosexual intercourse both for pleasure and procreation, has made conventional coitus the most common route of HIV-1 transmission worldwide (61), especially in poorer nations where ready access to medical care, needles, syringes, narcotics, condoms and female contraceptive options is severely limited. In addition, the frequency of other cell-borne sexually transmitted diseases (STDs), especially Chlamydia infections, gonorrhea and genital herpes, increases chances of interpersonal HIV transmission (61), partly related to ulceration or thinning of genital surfaces in close contact during coitus and by increasing the numbers of *emperipoletic* SCDL in shared genital secretions (53). An additional possible co-factor is that use of oral or implanted progesterone for birth control increases female risk of acquiring HIV by effacing the uterine cervix such that more columnar epithelial cells are exposed to migrating seminal lymphocytes (53).

6. Unprotected homosexual male anal intercourse was the most common route of HIV-1 transmissions during the early stages of the AIDS pandemic in North America, Europe and Oceana. However, hematogenous spread by means of syringes and needles used to "shoot" illicit intravenous drugs has become after sexual intercourse, the most common alternative route of spread (53). With the lack of an effective way to control intravenous drug abuse, and the need to supply 13 billion needles and syringes each year throughout the world to withdraw blood or give prescribed medications, it becomes incumbent on caring people to mandate that *intravenous drug abusers apart from medical care settings be taught how to use, allowed to purchase or be supplied with sterile syringes/ needles which can only be used once and,*

then, be safely discarded. It is further incumbent on health care settings to mandate that syringes/needles and related paraphernalia used to withdraw blood from intravenous drug abusers, from other persons possibly HIV or hepatitis virus infected and needles used to give medications into veins or under the skin be sterile, adequately shielded, used only once, and discarded safely (52,53).

7. The combination of spread through vaginal or anal sexual intercourse, along with hematogenous transmission from persons "shooting" intravenous drugs, accounts for most infants infected during pregnancy, delivery, or nursing.

8. Many of factors and co-factors probably contribute to the transmission, course and manifestations of HIV-1 lymphopathy. Nonetheless, from a lymphologic point of view, it needs to be reemphasized that lymph transports migrating lymphocytes, the antigenic products of all peripheral cells and invading microorganisms to lymph glands, and carries lymphocytes and soluble products from lymph glands to the bloodstream (4). Accordingly, the symptoms and signs of HIV-1 infections derive not only from lymphocyte deficiency, but also likely from spurious DNA nucleotide sequences carried by lymphocytes to peripheral tissues, especially the gut, uterus, lung epithelium and glial cells where cell DNA turnover is rapid (4,12)

9. Levy has long contended (66) that infected CD4+ T-helper cells are both the critical victims and the primary vectors of HIV-1 infection which progresses to AIDS. He has suggested that CD8+ cytotoxic lymphocytes secrete a cell anti-viral factor (CAF) which limits the production of HIV-1 from CD4 lymphocytes. Possibly, this CAF is a polyvalent soluble globulin which complexes with CD4 receptors in cell plasmalemma, and with HIV-1 capsular glycoproteins. However, Livingstone et al (67) found that CD8+ lymphocytes become infected with HIV-1 provirus more or less proportional to CD4+ lymphocyte depletion, and that circulating dendritic cells and monocytes become infected to a lesser, but appreciable extent. They emphasized further that glial cells are frequently transfected with HIV-1 provirus (67).

10. The proposition (4) that rapidly dividing LGCL, commonly designated as germinal center B-cells, are precursors of many T-cells, as well as sources of many soluble antibodies and lymphokines, and common victims of HIV-1 infection remains to be accepted generally. Yet, 15 years after recognition of HIV sickness as fatal; and 13 years after definitive description (45) of the causative retrovirus, few agree on the pathogenesis. Preventive vaccines and medicinal cures remain unavailable, partly owing to the error-prone manner (47) HIV-1 RNA reverse transcribes into the genes of large dividing GC lymphocytes sooner or later responsible for the cellular expression of HIV-1 RNA.

COMMENTS

Too many persons are ignorant, complacent or bored concerning AIDS. Yet, HIV-1 and other lymphopathic retroviruses which alter lymphocyte nuclear genomes and lymph glands and related tissues to generate a variety of fatal illnesses remain a clear and present danger. As emphasized by Merson (68), the venue at the Vancouver conference had "upsides" in encouraging therapeutic "breakthroughs" and "downsides" concerning whether treatment delays effective prevention. Even if effective vaccines for preventing and medicines for curing AIDS and other retroviral sickness become available, cooperative prevention could still minimize morbidity and save innumerable lives. Whereas, it is not possible to define the value of a human life in terms of dollars or the productive earning power of an infected individual in a given nation, it is abundantly clear that no individual or nation can afford the cost of an escalating AIDS epidemic. Combined administration of reverse transcriptase of HIV-1 polymerase inhibitors for treatment and PCR tests for monitoring plasma HIV-1 RNA now cost $15,000-20,000

per patient annually (69). These regimens can also be expected to increase the incidence of incurable HIV-related neoplasms. The potential global and personal costs are infinite (69,70), especially if current scientific focus fails to emphasize prevention (68).

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Addendum:

Cogent literature since the XI Vancouver AIDS Conference is cited as follows:

A. Basic Science

1. Grouard et al (70a) showed that macrophages containing the apoptotic remnants of lymphocytes are found mainly in the dark juxta-arteriolar poles of germinal centers (GC).

2. Haase et al (71) showed in the tonsils, nodes and spleen of HIV-1+ persons with ±200-800 CD+ cells/μL of blood, that the concentration of polymerase chain reactive (PCR)-detectable HIV-1 RNA associated with GC mononuclear cells is approximately 10x that in circulating plasma, and that the concentration of [insoluble] HIV-1 RNA associated with follicular dendritic cells (FDC) is ±100 to 10,000x that in plasma. Therefore, they suggested that FDC store virus particles emanating from GC mononuclear cells or, at least, limit their circulation during latent phases of HIV-1 infection. Comparing serial tonsilar biopsies, they noted that patient treatment with reverse transcriptase inhibitors made no significant difference.

3. Han et al (72) showed in murine splenic and Peyer's patch GC that antigen-driven V(D)J mutations coding for cell surface antigen receptors in T-cells, as well as B-cells, are identical and of local, not bone marrow, origin.

Such observations, after those of Pataleo (4) and Embretson (41), indicate that germinal centers are critical during HIV-1 infections.

B. Clinical Science

As major "breakthroughs" in '96, *Newsweek* (2 Dec.), *Nature* (12 Dec), *Science* (20 Dec.), and *Time* (30 Dec.) signaled: (a) newly developed protease inhibitors for treating HIV infections; and (b) transmembrane co-receptors, along with CD4 or apart from CD4, providing means for HIV-1 entry into cells. With respect to (a), reports from the 4th Conference on Retroviruses and Opportunistic Infections, 22-26 January 1997, Washington, D.C., were cloaked "in shades of gray" (73), partly because:

1. PCR-detectable circulating HIV-1 RNA, although an unfavorable prognostic index, does not, necessarily correlate with clinical illness (73).

2. According to Cavert, reduction in circulating HIV-1 RNA load does not, necessarily, correlate with reduction of HIV-1 RNA load in the lymph nodes "wherein 99% of the HIV in the body resides" (73).

3. According to Cavert and Haase, even after optimized treatment with reverse transcriptase and protease inhibitors with apparent elimination of HIV-1 RNA load in sampled tonsils, PCR-detectable HIV-1 DNA is still present (73).

4. According to Perelson and Ho, "it will take 2.3 to 3.1 years of viral suppression to rid the bod of HIV" (73), [even if started early].

5. Several reports at the conference "indicated that in the real world, many patients don't follow drug regimens strictly and it may only take a few days of treatment for resistant strains to appear" (73).

With respect to (b): transmembrane cell surface co-receptors for HIV-1 are assessed by experiments in PHA and IL-2 stimulated cultures of potentially receptive allogenic cells. Dittmar et al (74) from the U.K. found that the cellular tropisms of HIV-1 *in vitro* and immune escape from neutralizing antibodies *in vivo* depend on the variable domains V1/V2 and V3 of the outer-envelope glycoprotein (gp120) of HIV-1, and not on 7-transmembrane cell-surface receptors. In the body, immune complexity ensues, partly because LGCL shed polyvalent antibodies and form the

capsules of HIV-1 simultaneously. See *Fig. 2.*

Items omitted in the clinical advances cited, but emphasized in *Basic Science* and in this text are: (a) GC and their polarity; (b) the normal proclivity of LGCL to shed soluble cytoplasmic products and reduce in size, as well form the capsular envelopes of HIV-1 RNA; (c) the precipitation of amorphous, insoluble, electron dense complexes of antibodies and HIV-1 RNA between large GC lymphocytes and follicular dendritic cells; and (d) the capacity of SCDL derived from GC to infect other GC via the lymphatic system and blood, as well as other persons during the normal course of *emperipoletic* migrations through semen, uterine cervical secretions, and colostrum.

Thus, it will likely take longer than most appreciate to eliminate AIDS by current approaches using protease inhibitors.

C-D. Epidemiology, Public Health and Social Sciences

Most agree, after Merson (67) and Piot (70), that prevention is shamefully underfunded and needs real "breakthroughs." Part of the problem lies in the fact that many still look upon AIDS as a syndrome affecting definable subpopulations, instead of as a deadly infectious retroviral sickness which takes ±12 years to evolve, and which is indiscriminate with respect to race, religion, color, and occupation. Therefore, it is prudent to:

1. Dispel ignorance and stress universal education, as emphasized in Vancouver.
2. Care for others as you would like to be cared for yourself (53).
3. Always use a condom during anal intercourse (55).
4. During vaginal intercourse *unless conception is intended*, all persons conceivably at risk for AIDS, or other cell-borne STDs, should uniformly use condoms and/or female-choice uterine cervical barriers, preferably containing tolerable concentrations of N-9 (54,56,57).
5. Use hollow bore steel needles, syringes and multiple dose vials only once; shield them properly before and after use, and dispose of them promptly and safely (52).

Notes added in proof:

Based on references 39-42, we stated that, during the course of HIV sickness after prodrome, ± 1% of circulating SCDL contain integrated HIV-1 provirus. However, using advanced PCR technology, Chun and his cohorts (75) report that during the apparently latent stage of HIV-1 infection, the numbers of provirus-infected CD4+ lymphocytes in nodes and in circulating blood are usually 0.5% of the total. Moreover, 0.5% contain HIV-1 proviral DNA in linear integrated RNA transcriptionally silent form, 0.05% in transcriptionally silent circular form, and 0.005% in replication-competent linear form. They conclude that the latter, irrespective of serum or nodal HIV-1 RNA burden, CD4+ cell counts and treatment with reverse transcriptase inhibitors, might continually serve as reservoirs for HIV-1 infection. Whereas 0.005 seems a small percent, the absolute numbers can be large when one considers that a 1-2% mass of lymphocytes in a healthy adult normally comprises 10-25 trillions growing and dividing in organized lymphopoietic tissues, along with *emperipoletic* billions migrating through lymph, tissues, blood, semen, uterine secretions or colostrum.

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