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REVIEW

Cell Surface Effects of Human Immunodeficiency Virus

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Cell killing by human immunodeficiency virus (HIV) is thought to contribute to many of the defects of the acquired immunodeficiency syndrome (AIDS). Two types of cytopathology are observed in HIV-infected cultured cells: cell-cell fusion and killing of single cells. Both killing processes appear to involve cell surface effects of HIV. A model is proposed for the HIV-mediated cell surface processes which could result in cell-cell fusion and single cell killing. The purpose of this model is to define the potential roles of individual viral envelope and cell surface molecules in cell killing processes and to identify alternative routes to the establishment of persistently-infected cells. Elucidation of HIV-induced cell surface effects may provide the basis for a rational approach to the design of antiviral agents which are selective for HIV-infected cells.

KEY WORDS: human immunodeficiency virus; AIDS; cell fusion.

INTRODUCTION

Cell killing is an important pathogenic mechanism of many viruses. An example of the role of cell killing in viral pathogenesis of great contemporary significance is provided by human immunodeficiency virus (HIV), the etiological agent of the acquired immunodeficiency syndrome (AIDS) (1–3). HIV is capable of killing T-lymphocytes which express the CD4 cell surface marker (T4 lymphocytes) (1–7). During the course of HIV disease the number of circulating T4 lymphocytes undergoes a progressive decline which may be, at least in part, a consequence of the expression of the cytolytic capacity of HIV. Because T4 cells provide helper functions in several aspects of both cellular and humoral immunity, HIV-induced killing of T4 lymphocytes may explain, in part, why AIDS patients are severely immunosuppressed, at risk for infection by a number of opportunistic pathogens, and susceptible to developing certain malignancies. A limited number of other cell types, including monocytes and certain cells of the central nervous system, are also susceptible to HIV-induced cytopathology

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(8–10). Therefore, cell killing by HIV may contribute to various pathological features of AIDS.

CELL KILLING AND ESTABLISHMENT OF PERSISTENCE BY HUMAN IMMUNODEFICIENCY VIRUS

HIV Structure and Replication

On the basis of virion morphology and nucleic acid sequence similarities, HIV appears to be closely related to the subgroup of cytolytic and immunosuppressive retroviruses referred to as the lentiviruses (11). The HIV virion is composed of a complex nucleocapsid surrounded by a lipid-containing envelope. The nucleocapsid possesses a cylindrical core that contains the genomic RNA. The HIV genomic RNA is diploid, single-stranded, positive-polarity and contains coding sequences for at least eight sets of protein products. The *gag* (group antigen) gene encoded proteins comprise the major structural proteins of the nucleocapsid of the virus. The major *gag* protein of HIV is p24 (M_r in kilodaltons). Human antibodies directed against p24 of HIV-1, the predominant type of HIV which is epidemic in the United States and Central Africa, cross react with other primate retroviruses, including HIV-2 isolated in Western Africa, confirming the group antigenic designation. The products of the *env* gene are two glycoproteins designated gp41 and gp120. The transmembrane protein of HIV, gp41, serves as an anchor for the peripheral protein gp120 in the virion envelope and the infected cell plasma membrane. The envelope of HIV is acquired by budding from the cell surface. Associated with the HIV core is the product of the *pol* gene, a RNA directed DNA polymerase (reverse transcriptase). After the process of uncoating which occurs in the cytoplasm, the RNA genome is converted by the reverse transcriptase to linear double-stranded proviral DNA. Subsequently, the linear DNA is circularized and transported to the nucleus where it is integrated, apparently at random, into the host genome.

The HIV genome contains a number of genes which are not universally present among retroviruses (12–21). The transactivator gene, *tat*, encodes a protein which enhances translational and/or transcriptional expression of genes linked to the region of the integrated HIV provirus referred to as the long terminal repeat (LTR) (12–15). The product of the *trs/art* gene appears to be involved in regulating the steady state levels of genome-length progeny RNAs and subgenomic HIV mRNAs (16). The *sor* and *3′ orf* genes were originally identified as open reading frames in the HIV genome and subsequently have been shown to produce protein products (17–19). Studies with viral mutants generated by site directed mutagenesis indicate that the *sor* and *3′ orf* products are not required for production of infectious virions (17–19). The product of the *3′ orf* gene is myristylated, phosphorylated and possesses GTP-binding activity (19). The function of this protein, which is possibly membrane-associated, may be to regulate the expression of CD4, the HIV receptor (19). The function of the product of an eighth gene, *R,* is unknown presently (20). It has been proposed

that the products of *tat,* or other viral proteins which regulate viral gene expression, may also alter the expression of cellular genes, and thus, have a direct role in HIV pathogenesis (13, 21). However, HIV mutants carrying deletions in *tat, trs/art, sor* or *3' orf* appear to be as cytopathogenic as wild-type virus (15–20).

Role of CD4 as the Cell Surface Receptor for HIV

Several lines of evidence suggest that HIV employs the CD4 molecule as a cell surface receptor for virion attachment (1–4, 6, 7, 22–25). For example, some, but not all, monoclonal antibodies specific for CD4 are capable of blocking HIV attachment and HIV-induced cytopathic effects (CPE) (23, 24). Among cells of the lymphocyte lineage, CD4 is expressed predominantly on helper-inducer cells (T4-lymphocytes), providing an explanation for the T4 cell tropism of HIV. CD4 is also expressed on monocytes and certain cells of the central nervous system which may also serve as targets of HIV (25). HIV will productively infect a number of lymphocyte, monocyte, and other cell lines *in vitro.* These cell lines are a valuable resource to study the cytopathogenesis of HIV. It is important to note the possibility that HIV employs alternative cell surface receptors to CD4.

HIV-induced Cytopathic Effects

In general, two types of HIV-induced CPE are observed during the acute phase of infection in cultured cells (1–7, 26). The first type of CPE is characterized by formation of multinucleate (syncytial) giant cells, considered a hallmark of HIV infection of cultured cells. Because the giant cells are no longer capable of proliferation, cell-cell fusion may contribute to depletion of T4 lymphocytes in patients with HIV disease. The second type of HIV-induced CPE results in lysis of single cells. Single cell lysis is preceded by swelling of the cells (Fig. 1). In some instances, distinct regions of perturbance characterized by a change in the refractive properties of the membrane under phase optics can be discerned in single cells undergoing acute infection (R. F. Garry, unpublished observations). Certain cell lines and peripheral blood mononuclear cells from most normal donors fail to form large numbers of multinucleate cells, and in these cells HIV-induced cytopathology predominantly involves lysis of single cells (26). Therefore, the role of multinucleate cell formation in T4 cell depletion during HIV disease has been questioned (26). Significant numbers of cultured cells may survive the acute phase of HIV infection and form a persistently-infected culture. The mechanism(s) by which some cells survive the acute cytopathic effects of HIV are unknown. Cells persistently-infected with HIV produce 10^4 to 10^7 infectious units (IU) per ml depending on the cell type and virus strain, with a particle to IU ratio of approximately 10^2–10^3:1.

Substantial amounts of unintegrated linear proviral DNA are found during both acute and chronic phases of infection by HIV, and it has been suggested that this DNA might contribute to HIV-induced CPE (27). However, cells to which ultraviolet (UV)-irradiated HIV (approximately 100 particles/cell) has been

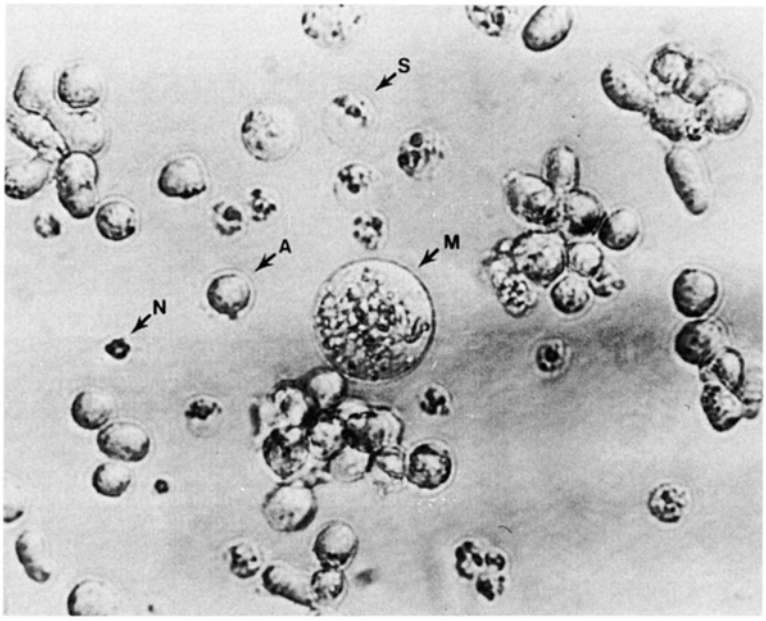

Fig. 1. HIV-induced cytopathology in H9 cells. HIV (strain HITI) was UV-irradiated (4800 ergs/mm^2) and added to H9 cells (10^6 cells/ml) at the equivalent of approximately 1 infectious unit/cell. Cultures were photographed 48 hr later. Cytopathology induced by irradiated HIV was indistinguishable from that induced by unirradiated HIV. Symbols: S, "swelling" single cell undergoing acute cytopathology; A, average sized "normal" cell, N, nucleus from lysed cell; M, multinucleated giant cell.

adsorbed display single cell cytopathology and syncytial cell formation which is identical to that observed during the first several days after exposure to unirradiated HIV (Fig. 1) (5). Cell killing occurs even with UV-irradiated HIV preparations which were incapable of synthesizing proviral DNA using the virion-associated genomic RNA as a template for reverse transcription. This observation suggests that accumulation of proviral DNA is not involved in the initial cell killing induced by HIV in cultured cells. Thus, HIV-induced cytopathic effects may develop when a virion component(s) is added to cells in sufficient quantities or when the component(s) accumulates to sufficient levels during active infection. Cytopathic retroviruses of other animal species, including lentiviruses, are also capable of cytopathology after UV-irradiation (28–30, Bose *et al.*, in preparation).

It is now generally accepted that envelope proteins are involved in the killing of cultured cells by HIV (31–35). Expression of molecularly cloned HIV *env* gene products (gp41 and gp120) results in cell-cell fusion of $CD4^+$ cells (31, 32). Furthermore, HIV contains a short sequence, designated the "fusion peptide" near the amino terminus of gp41, which is common to various strains and also similar to amino-terminal sequences of the fusion proteins of other viruses such as respiratory syncytial virus and influenza virus (33). The fact that HIV contains a

related "fusion peptide" sequence suggests that HIV may employ a cell-cell fusion mechanism similar to that utilized by other fusogenic viruses.

Further information regarding the roles of *env* proteins in HIV-induced cytopathology is provided by studies of HTLV-IV and STLV-III, primate retroviruses, which contain, in comparison to HIV, a carboxy-terminal truncation of gp41 (34). Interestingly, HTLV-IV appears capable of inducing cell-cell fusion, but is defective in the ability to kill single cells. Furthermore, HIV, produced from a cloned provirus in which a region at the 3′ end of the *env* gene encoding the 14 carboxy-terminal amino acids of gp41 was deleted, was shown to be as competent at replication as wild-type virus (35). This mutant was fusogenic, but attenuated in the ability to kill single $CD4^+$ cells. Perhaps, gp120 *per se* is not sufficient for killing of single cells, and different domains of gp41 are required for fusion and single cell killing.

A MODEL FOR THE ROLE OF CELL SURFACE EFFECTS OF *env* PROTEINS IN HIV-INDUCED CYTOPATHOLOGY

Cell surface effects of HIV envelope proteins appear to play a critical role in the process of cell killing. To approach the goal of defining the mechanism(s) by which HIV envelope proteins induce cell killing, a model is described which outlines the process of cell-cell fusion and hypothesizes a role for cell surface effects in single cell killing (Fig. 2). The purpose of this model is to define the potential roles of individual viral envelope and cell surface molecules in cell killing processes. This model borrows heavily from previous models of cell-cell fusion and cell surface-mediated killing by other fusogenic and/or lytic viruses (37–43), and is discussed in subsequent sections. Because HIV readily establishes persistent infections, an important feature of the proposed model, which distinguishes it from earlier models, is to identify alternative routes to the establishment of persistently-infected cells. Moreover, to the extent that information is available it shall be noted where effects of HIV on cell surfaces differ from the effects of other viruses.

HIV-induced Cell-Cell Fusion

Two distinct mechanisms for virally-induced cell-cell fusion have been described, termed fusion from without (FFWO) and fusion from within (FFWI) (38). In FFWO, the virus envelope may fuse simultaneously with two cells thereby forming a "bridge" between them. Some studies have suggested a mechanism of HIV entry into the cell cytoplasm which would preclude FFWO (25). These studies have suggested that the mechanism of HIV penetration might be similar to receptor-mediated endocytosis in which the input virus particles are taken up in clathrin-coated vesicles. Subsequently, fusion of the endocytic vesicles with lysosomal vesicles could lower the intravesicular pH a process which may permit fusion of the viral envelope with the vesicular membrane and release of the viral nucleocapsid into the cytoplasm. Results from several laboratories suggest that uptake of several enveloped viruses, including alphaviruses and

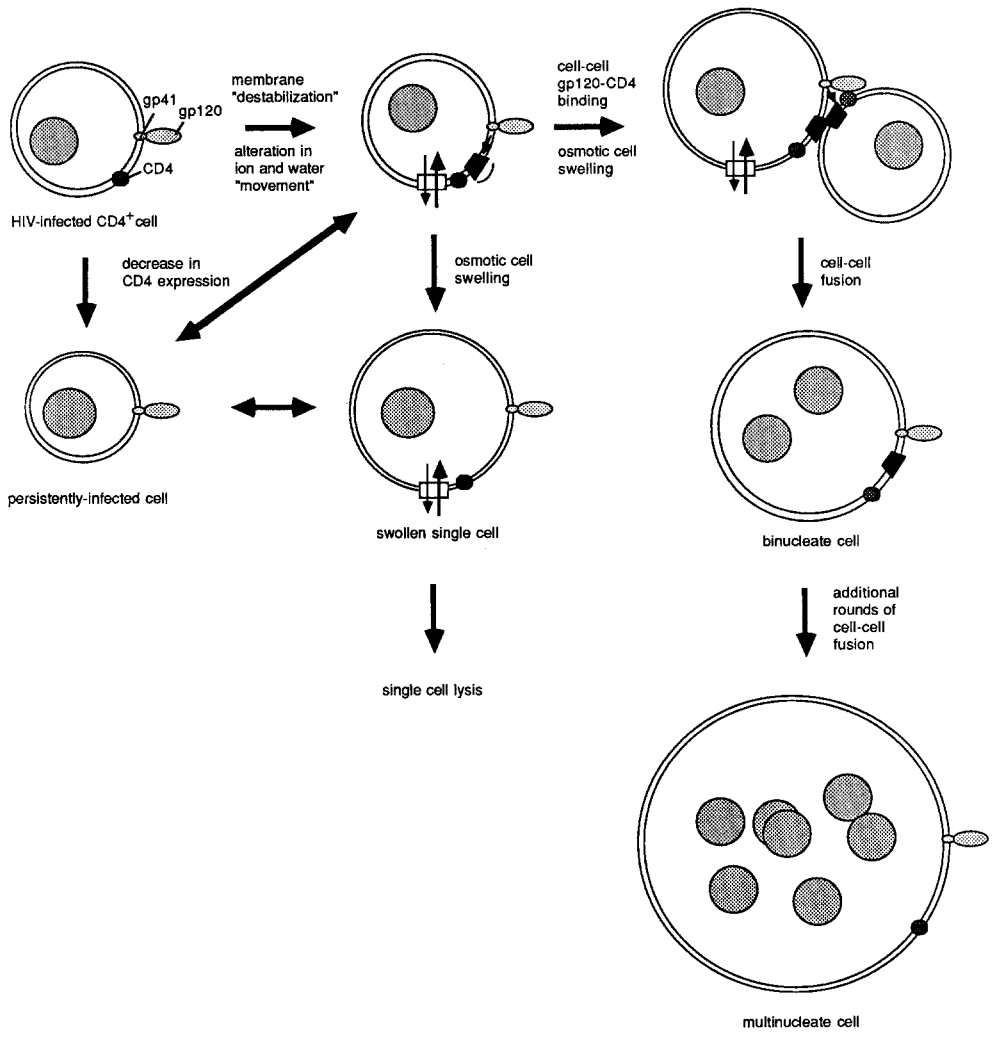

Fig. 2. Cell surface effects in HIV-induced cytopathology. HIV envelope proteins designated gp41 and gp120 may be inserted into the plasma membrane of infected $CD4^+$ cells during viral adsorption and penetration or as a result of *de novo* synthesis. One or both envelope proteins, possibly as a result of interaction with the CD4 molecule, may initiate a process which results in an alteration of ion and water movement and membrane destabilization. Subsequently, cell-cell fusion may occur if an area of destabilized membrane is juxtaposed to another cell. Binding of cell surface gp120 with CD4 on another cell may drive contact between the two cells. Osmotic swelling which occurs as a result of net influx of ions and water may initiate or drive these fusion events. Additional rounds of cell-cell fusion result in formation of multinucleated giant cells. Killing of single cells may occur as a result of osmotic swelling. Persistently-infected cells arise if membrane destabilization and/or alteration of intracellular ion and water content does not occur or, alternatively, if these processes are reversed. Compared to uninfected cells, cells which are persistently-infected with HIV are smaller and express lower levels of CD4.

influenza A, is by an endocytic route, although this remains a matter of some controversy (reviewed in 45). Recent studies have indicated strongly, however, that the HIV entry process is pH-independent and, as in the case of paramyxoviruses, involves fusion of the virion envelope to the cell plasma membrane. This mechanism of HIV penetration would potentially permit FFWO (36) and would also deposit input viral envelope proteins directly into the plasma membrane.

HIV may also induce FFWI, a mechanism which is temporally displaced from the processes of virus adsorption and penetration. Insertion of viral envelope proteins following *de novo* synthesis may induce stable or quasi-stable alteration(s) in the plasma membrane of cells (Fig. 2). These regions of perturbance may contain HIV proteins, such as gp41, the HIV fusion protein, or they may develop as a result of the effects of HIV proteins on other cell surface components such as CD4. These regions of perturbance, if subsequently juxtaposed with another cell may permit fusion between the plasma membranes of the cells. Conceivably, such regions of perturbance, which permit cell-cell fusion, may also develop as a delayed (post-penetration) consequence of the insertion of viral envelope proteins into the plasma membrane during direct fusion of the envelope of input virions with the plasma membrane. This mechanism may explain the ability of UV-inactivated HIV to induce cell-cell fusion at times up to 24–48 hrs after the adsorption and penetration steps by processes which do not involve formation of cell-virus-cell bridges (5).

Cell-cell fusion obviously requires contact between the two fusing cells. Contact between two cell membranes may be driven by an interaction between gp120 on an infected cell and a virus receptor (i.e. CD4) on another cell and by the hydrophobic portion of gp41 (Fig. 2). Interestingly, it has been observed that cell-cell fusion is most effective between an HIV-infected cell and an uninfected $CD4^+$ cell (46). CD4 may also have other important roles in HIV-induced cytopathology. Evidence for a possible role of CD4 in HIV cytopathology beyond its role as virion receptor is provided by two important observations: (1) transfection of a full length DNA clone of HIV into $CD4^-$ cells results in HIV production but not CPE (47), and (2) cell surface expression of CD4 is substantially lower in cells which are persistently-infected with HIV than in uninfected cells, a possible function of the 3′ *orf* product (19, 48).

Role of Ion Fluxes in HIV-induced Cytopathology

Fusogenic Sendai virus has been reported to induce changes in the plasma membrane which result in alterations in Na^+ and K^+ flux, the net effect of which is an entry of water and osmotic cell swelling (39–44). The alterations in ion fluxes have been grouped with other "toxin-like" effects of viruses, because they are reminiscent of the action of certain toxins such as the bee venom protein melittin (43). Moreover, it has been proposed that osmotic swelling is the force which initiates or drives cell-cell fusion events (42, but see also 43, 44). As proposed in the model, it is possible that osmotic cell swelling induced by HIV may be involved in cell-cell fusion events (Fig. 2). Certain fusogenic viruses, such

as early-harvest Sendai virus do not cause alterations in intracellular ion levels or cell swelling and therefore do not induce the formation of stable binucleate cells (41). The changes in intracellular ion levels induced by fusogenic viruses such as late harvest Sendai virus are usually transient in nucleated cells and may be reversed by cellular homeostatic mechanisms (43). Unlike the case with Sendai virus, however, it appears that cell swelling induced by HIV is irreversible in the majority of infected cells.

Alterations in intracellular concentrations of Na^+ and/or K^+ are also observed during cytolytic infections by picornaviruses (49, 50), alphaviruses (51–53), coronaviruses (56), rhabdoviruses (57), adenoviruses (58), herpesviruses (Koch *et al.*, in preparation) and during retrovirus transformation (59, 60). The mechanism for inducing changes in ion flux during lytic infections by certain of these lytic viruses involves inhibition of ion transport systems (51), and thus appears to be distinct from the "toxin-like" effects of fusogenic viruses such as paramyxoviruses (43). It has been suggested that alterations in the intracellular concentration of monovalent cations may be involved in the shutoff of host protein synthesis by certain lytic viruses (37, 49, 51). However, contradictory results regarding the role of monovalent cations in inhibition of host protein synthesis have been obtained (51–56). Nevertheless, numerous vital functions other than protein synthesis are regulated by monovalent cations and thus, alterations in Na^+ or K^+ may be involved in the development of the cytopathic effect induced by certain lytic virus (37, 49, 51).

Rasheed, Gottlieb, and Garry (5), Gallo (6), and Ho, Pomerantz, and Kaplan (7) have suggested that killing of single cells by HIV, like cell-cell fusion, may involve a membrane modification(s) by HIV envelope proteins that destabilizes or alters the "permeability" or ion transport properties of cellular membranes. It has also been suggested that killing of single cells by HIV may involve a type of "autofusion" event between regions of plasma membrane on the same cell (31). "Autofusion" need not necessarily be deleterious to the cell, however, events which are associated with cell-cell fusion, i.e. ion driven osmotic swelling, could result in cell death. Thus, osmotic swelling induced by HIV may result in single cell cytolysis (Fig. 2). A possible explanation for the irreversible nature of the HIV-induced cell swelling is that the activity of an ion transport system involved in maintaining ion gradients may be altered by HIV.

Effects of HIV on Ion Transport Systems

A critical test of the proposed model for HIV-induced CPE is whether HIV induces alterations in intracellular ion levels. The effect of HIV on the intracellular levels of $^{86}Rb^+$, a K^+ tracer, has been investigated. Equilibrium levels of $^{86}Rb^+$ were quantitated 24 hr after exposure to HIV, a time at which approximately 75% of these cells excluded trypan blue. The results of this experiment, which are representative of multiple experiments, indicated that a low multiplicity of intact HIV was capable of inducing a significant ($P < 0.01$)

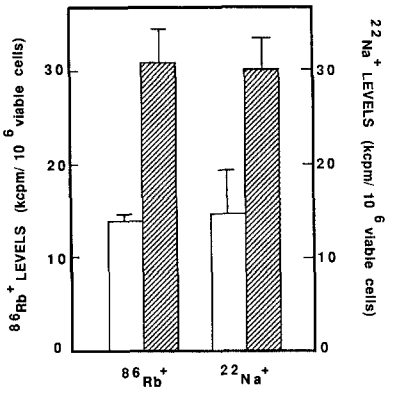

Fig. 3. Intracellular $^{86}Rb^+$ and $^{22}Na^+$ levels during the acute phase of HIV-induced cytopathology. H9 cells were exposed to HIV (the UV-inactivated equivalent of 1 infectious unit/cell; striated bars), and after 24 hr, a time at which approximately 75% of these cells excluded trypan blue, equilibrium levels of $^{86}Rb^+$ or $^{22}Na^+$ (0.1 μCi/ml, a labeling period of 30 min) were quantitated. Cells were washed by centrifugation through silicon oil (cell recovery was greater than 95%) and $^{86}Rb^+$ was quantitated by counting Cerenkov radiation on a scintillation counter. Mock-treated cells were exposed to concentrated supernatants from uninfected H9 cells (open bars). Standard error of the mean of triplicate samples is represented by the error bars.

increase in the intracellular levels of $^{86}Rb^+$ prior to cell lysis (Fig. 3). Studies in which cells were lysed with a Dounce homogenizer indicate that nuclei of H9 cells contribute less than 15% to the total level of cell-associated Rb^+ (data not shown). Other studies suggest that intracellular concentrations of Na^+ were also increased significantly ($P < 0.05$) at an early time after treatment of H9 cells with HIV (Fig. 3). In this experiment more than 80% of the cells exposed to HIV failed to exclude trypan blue by 48 hr pi, suggesting that the early alterations in ion content could contribute to cell death. Because net influx of water molecules generally follows the net influx of monovalent ions, the net increases in intracellular Na^+ and K^+ induced by HIV could drive the swelling which is potentially involved both in cell-cell fusion and in the lysis of single cells (Fig. 2).

Intracellular levels of monovalent ions are regulated by a variety of membrane associated transport systems (61–63). Quantitatively, the most important of these are the Na^+/K^+ ATPase and the $Na^+/K^+/2Cl^-$ cotransport system. Certain cells, in particular lymphocytes, may also contain selective or "nonselective" ion channels. Preliminary results suggest that HIV inhibits the Na^+/K^+ ATPase and increases the activity of the $Na^+/K^+/2Cl^-$ cotransport system which could contribute to the increase in intracellular Na^+ levels. Reducing the activity of the Na^+/K^+ ATPase does not preclude a net increase in intracellular K^+, if other K^+ uptake mechanisms such as the $Na^+/K^+/2Cl^-$ cotransport system are activated or if K^+ efflux is inhibited. An additional possibility to account for the increase in intracellular K^+ is that an ion channel for K^+ efflux was blocked by HIV. Several preliminary experiments suggest that HIV treatment of H9 cells alters the activity of a tetraethylammonium chloride (TEAC)-sensitive K^+ channel which in turn results in an increased net level of K^+ (data not shown). These results do not exclude "toxin-like" effects of HIV components or the possibility that insertion of a "leaky" HIV envelope into the host cell surface could contribute to increases in ion flux. Obviously, further studies are required to quantitate the extent to which various directional components of $^{86}Rb^+$ and Na^+ flux are altered in HIV-treated H9 cells.

Role of CD4 in HIV-induced Effects on Membrane Transport Systems

As proposed, CD4 may have an additional role(s) in HIV-induced cytopathology beyond its role as a cell surface receptor for the virus. The potential role of CD4 in mediating increased levels of monovalent ions has been examined. Leu3a, a monoclonal antibody (MAb) to CD4, was added to H9 cells to block attachment of HIV and possibly prevent the HIV-induced increase in intracellular $^{86}Rb^+$. However, an increase in intracellular $^{86}Rb^+$ levels occurred in HIV-treated cells pretreated with Leu3a (Fig. 4A). Furthermore, an increase in $^{86}Rb^+$ levels was observed in Leu3a treated control cells not exposed to HIV. Leu3a antibodies were able to increase net $^{86}Rb^+$ levels, in various experiments, in a concentration-dependent manner (Fig. 4B). Monoclonal antibodies which are specific for CD4 can be divided according to those which block and those which fail to block HIV attachment (24). OKT4 MAb, which binds to CD4, but fails to block HIV attachment (Fig. 4A), and a MAb to HLA_{dr} (data not shown), another lymphocyte cell surface protein, both failed to increase $^{86}Rb^+$ levels in uninfected cells. These results suggested that increased intracellular $^{86}Rb^+$ is not an universal consequence of an interaction of a MAb with CD4 or lymphocyte cell surfaces. Unlike HIV, Leu3a failed to increase $^{22}Na^+$ levels or to inhibit the Na^+/K^+ ATPase (data not shown). Thus, volume regulation is intact in H9 cells exposed to Leu3a. In addition, it was observed that TEAC fails to alter intracellular Rb^+ levels in H9 cells exposed to Leu3a (data not shown). Collectively, these results suggest that the mechanisms by which Leu3a and HIV increase $^{86}Rb^+$ levels both involve the CD4 molecule. Further studies will be required to determine if Leu3a and HIV increase intracellular K^+ levels by

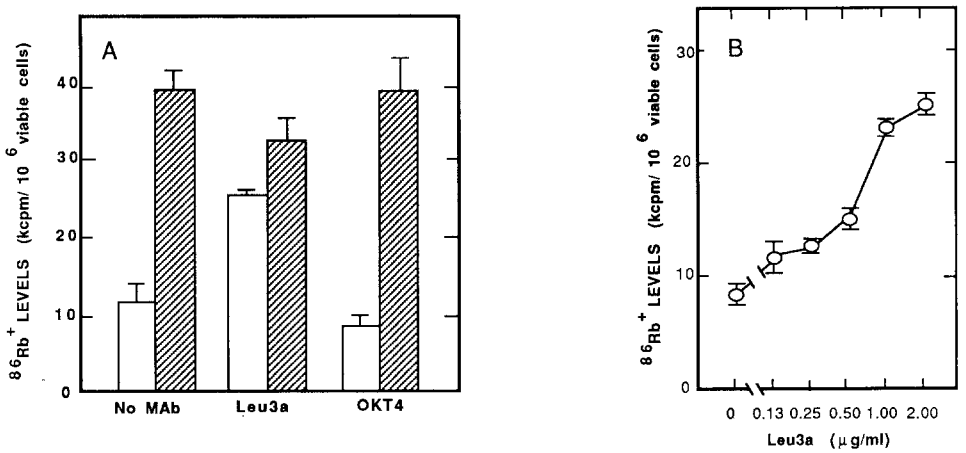

Fig. 4. $^{86}Rb^+$ levels in H9 cells during the acute phase of HIV-induced cytopathology in the presence and absence of Leu3a monoclonal antibody to the CD4 lymphocyte surface protein. Panel A: Leu3a or OKT4 monoclonal antibody (2 μg/ml) was added to H9 cells for 30 min at 37°, then cells were either treated with HIV (the UV-inactivated equivalent of 1 infectious unit/cell; striated bars) or mock-treated (open bars). After 24 hr, equilibrium levels of $^{86}Rb^+$ were quantitated as described in the legend to Fig. 3. Sera for cell growth were heated to destroy complement activity. Panel B: Leu3a in the amounts shown was added to H9 cells for 30 min at 37°, then intracellular levels of $^{86}Rb^+$ were quantitated. Standard error of the mean of triplicate samples is represented by the error bars.

related mechanisms. It is possible that a specific interaction with the portion of CD4 recognized by Leu3a and perhaps a HIV protein may be required to increase intracellular K^+ levels, possibly via blockage of a K^+ channel (Fig. 2).

Establishment of HIV Persistently-infected Cells

In a portion of HIV-infected cells the osmotic swelling may not occur or it may be reversible, as in the case of Sendai virus-infected cells (40, 43) (Fig. 2). This may allow for the survival of some HIV-infected cells and for formation of persistently-infected cells. Reversibility of osmotic swelling may occur by adjustments in the levels or activities of cell surface ion transport systems. In this regard it is interesting that H9 cells which are persistently-infected by HIV are significantly smaller than uninfected H9 cells (5). Ion transport systems, such as the $Na^+/K^+/2Cl^-$ cotransport system, have established roles in cell volume regulation (62), and the smaller volume of cells persistently-infected with HIV could be due to modulation of ion transport systems. Cell viability in cultures of H9 cells persistently-infected with HIV (about 85% trypan blue excluding cells) is considerably lower than in uninfected cultures (99% trypan blue excluding cells). Furthermore, HIV persistently-infected cells are susceptible to cell-cell fusion, but less so than uninfected cells. Thus, it is possible that the cell surface process(es) which allows survival of acute HIV infection fails occasionally after persistence is established (Fig. 2).

FUTURE DIRECTIONS

An important rationale for investigating the mechanism of cell killing by HIV is to establish how certain cells survive to form persistently-infected cells. In an infected individual, such persistently-infected cells provide a reservoir for HIV which can continuously release virus. Release of HIV from persistently-infected cells in which proviral DNA has been synthesized and integrated is not susceptible to the action of reverse transcriptase inhibitors such as azidothymidine (AZT) (64). HIV released from persistently-infected cells may not, in the presence of AZT, initiate further productive infections. However, AZT does not prevent the binding to cellular receptors of virus or viral products released from persistently-infected cells or insertion of viral proteins into the plasma membrane of susceptible cells. As discussed, such events could lead to cytopathology. Furthermore, viral proteins released from persistently-infected cells may be immunosuppressive by mechanisms other than direct $CD4^+$ cell killing (65–67). Insight into the features of HIV persistently-infected cells which distinguish them from uninfected cells or cells which undergo acute cytolytic infection may lead to strategies to reduce, eliminate or prevent expansion of the reservoir of persistently-infected cells from HIV-infected persons.

Elucidation of the cell surface effects of HIV during acute infection and in persistently-infected cells may also be useful in the rational design of a selective antiviral drug. Lytic viruses may either increase or decrease the rate of uptake of

various compounds (68–73, but see also 55). Furthermore, many compounds utilize transport systems which are monovalent ion dependent, and as demonstrated, HIV alters intracellular monovalent ion levels. Obviously, compounds which are not effectively transported intracellularly as a result of HIV infection may have reduced utility. In contrast, toxic analogs of compounds which are selectively transported by cells acutely or persistently-infected by HIV may be useful as antiAIDS drugs.

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