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SPECIAL REPORT

The Role of the Envelope Glycoprotein in the Depletion of T Helper Cells in Human Immunodeficiency Virus Infection*

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Infection with the human immunodeficiency virus (HIV) causes gradual depletion of CD4+ T helper lymphocytes and destruction of the lymphoid tissue, which ultimately leads to a fatal defect of the cellular immune system. Paramount to the understanding of the pathogenesis of HIV infection is to elucidate the mechanism which underlies the loss of T helper cells. Various ideas have been proposed in order to explain this issue. Several hypotheses have focused on the role of the envelope glycoprotein in this process. This review summarizes the data obtained and concepts proposed regarding the involvement of the HIV glycoprotein in the pathology of CD4+ T cell depletion. (Pathology Oncology Research Vol 3, No 1, 62–67, 1997)

Key words: HIV, AIDS, envelope, T helper lymphocyte, pathogenesis, apoptosis

Introduction

Recent studies which demonstrate rapid turnover i. e. death and replacement of CD4+ T cells during human immunodeficieny virus (HIV) infection, support the concept that the gradual deterioration of the cellular immune functions in HIV infected individuals is the result of a physical loss of CD4+ T helper cells.[1-3] In addition; the observation that the proliferative response of mononuclear cells to mitogens and recall antigen is reduced in infected individuals suggests that functional defects of the T helper cell population develop during disease progression.[4,5] The decline of the CD4+ T lymphocyte number in the peripheral blood and the CD4+ T lymphocyte malfunction is accompanied by continued destruction of the lymphoid tissue.[6,7] Multiple hypotheses have been proposed in order to explain the mechanisms underlying the alterations in the CD4+ T helper lymphocyte population during the course of HIV-1 infection. Most of these concepts were established from experimental in vitro investigations or are based on empirical data obtained by studies using primary mononuclear cells from infected individuals. However, it has not been clarified which of the ideas are relevant to the in vivo situation. Several of the hypotheses focus on the envelope glycoprotein and suggest that this molecule plays a particular role in the depletion of CD4+ T lymphocytes during the course of infection with HIV. This review summarizes and evaluates the data that were generated regarding the function of the glycoprotein in this process.

The HIV-1 envelope glycoprotein

The HIV-1 glycoprotein gp160 is composed of the two glycoprotein molecules gp41 and gp120. Upon budding of the virus from the infected cell, it is incorporated into the viral lipid membrane envelope. The gp41 molecule is anchored in the lipid bilayer and contains a cytoplasmic region, a transmembrane portion and an extracellular part which is non-covalently linked to the gp120 molecule. The gp120 glycoprotein is located completely outside the membrane and easily shed from the viral surface.[8,9] This molecule contains several regions whose genetic sequence is relatively well preserved in different viral isolates, des-

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Abbreviations: HIV: Human Immunodeficiency Virus

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ignated C1-C4, and the regions V1-V5 which display substantial genetic variability. The cellular receptor for gp160 is the T cell receptor molecule CD4. Contact of gp120 with the CD4 molecule is mediated by a discontinous CD4-binding domain located in the C2, V4, C4 and C5 regions.[10,11] In addition, for entry of the virus into the cell, additional sequences possibly located in the V3 region have to interact with the coreceptor.[12,13] These coreceptors are members of the chemokine receptor family such as CCR-5 and CXCR-4 (fusin/LESTR) and possibly CCR-2b and -3.[14-20] Interaction of gp120 with CD4 and the coreceptor may result in conformational changes of the glycoprotein which exposes the fusogenic peptide of gp41 for interaction with the plasma membrane of the host cell.[21]

Cell-cell fusion and the formation of syncytia

After virus infection and upon replication of HIV in the cell, gp160 is inserted in the cellular membrane before viral particles bud from the surface. Interaction of cells expressing the HIV envelope glycoprotein on the surface with cells expressing the CD4 molecule and members of the chemokine receptor family causes fusion of the cells.[16] The fusion process requires the expression of the complete gp160 molecule in a fashion that allows intracellular cleavage to gp41 and gp120 and mutations in the cleavage site abolish the formation of syncytia.[22] Cell-cell fusion can be extensive and result in the formation of large syncytia, a phenomenon which is readily observed in HIV-infected CD4+ T lymphoblast cultures.[23-25] Because syncytia are not viable for an extended time period in vitro and the formation of syncytia coincides with the death of the infected cell culture, it was hypothesized that cell-cell fusion may underly T cell depletion in the infected individual.[23-25] However, an important argument raised against this hypothesis is the experience that syncytia are rarely detectable in vivo, and lymphoid tissue does not exhibit massive syncytium formation.[6,26-28]

Although this argument lead to the introduction of multiple additional hypotheses, several lines of evidence support the view that cell-cell fusion plays a role in vivo. For instance, cell-cell fusion products which contain dendritic cells possibly fused with T helper lymphocytes have been found in adenoidal lymphoid tissue.[29-31] Similarly, syncytia are readily detectable in the central nervous system where they are composed primarily of cells of the monocytic lineage including microglial cells and macrophages.[32,33] The view that syncytia may occur in lymphoid tissue in vivo is further supported by an in vitro model system in which blocks of human tonsils were kept in long-term histoculture and small syncytia could be generated by implantation of glycoprotein-expressing cells.[34] In addition, the experience that syncytia are not easily detected in lymphoid tissue may in part be due to the fact that the lymphoid organs are densely packed with lymphocytes, a fact which makes it difficult to identify small syncytia by histologic analysis.[35]

An additional point which has to be considered is that formation of large syncytia in cell culture is not observed with all viral isolates. HIV-1 strains have therefore been termed as either syncytium-inducing (SI) or non-syncytium-inducing (NSI) according to the ability to form syncytia and to cause cytopthology after infection of MT-2 cells.[36,37] In addition, virus isolates of the SI phenotype are typically T cell-tropic and NSI virus strains are macrophage-tropic. The phenotypic difference is mirrored by genotypic differences between these groups in the V1-V2 and V3 region[38,39] and the ability to form syncytia corresponds to the presence of a particular HIV coreceptor.[17,18,40] It was previously suggested that a shift in the dominance from NSI to SI isolates in the peripheral blood of infected individuals correlates with the progression of disease; a concept which favors the assumption that SI viruses are more virulent than NSI isolates and implies that syncytium formation plays a role in T helper cell depletion.[41,42] However, this may not always be the case, since the viral phenotype does not necessarily correlate with progression to AIDS.[43,44] Moreover, it was demonstrated that isolates which have been classified as NSI strains in MT-2 cells may still cause fusion of a few cells without progression to large syncytia[45] or even result in overt syncytium formation and cytopathology in primary T cells[46] and other cell culture systems.[47]

The notion that cytopathology associated with cell-cell fusion may constitute a relevant mechanism of T cell depletion in vivo is further supported by the observation that cell-cell fusion products may be more fragile than previously appreciated. For instance, contact of primary CD4+ T cells with envelope-expressing B lymphoblasts results in rapid lysis of a significant fraction of the cells in a few hours and the formation of relatively few and small syncytia. In contrast, contact of glycoprotein-expressing cells with transformed T cell lines like Jurkat and CEM induces the generation of large syncytia and no detectable cell lysis for more than 8 hours of incubation.[48] Additional data from our laboratory obtained by quantitative flow cytometric analysis demonstrate that coincubation of envelope glycoprotein-expressing cells with unstimulated primary PBMC causes selective disappearance of the majority of normal CD4+ T cells in a matter of hours by both syncytium formation and rapid cell death.[49]

Accumulation of glycoprotein-CD4 complexes

Several viral proteins including the envelope glycoprotein have been implicated in mediating direct cytopathicity (reviewed in: 50). For instance, expression of gp160 (but not gp120) in CD4+ T lymphoblasts induces the death

of single cells by apoptosis.[51-53] Since the expression of gp160 causes retention of the CD4 molecule in the endoplasmatic reticulum,[54] it was hypothesized that the cytopathicity observed is due to the accumulation of gp160-CD4 complexes at nuclear pores which may affect the transport of biomolecules to and from the nucleus.[55]

Binding of gp120 to the CD4 molecule: induction of anergy and mediation of antibody-dependent cellular cytotoxicity

There is some evidence that gp120 shed from the virion surface and from infected cells is present in sera of HIV-infected individuals[56] and may bind to the CD4 molecule on T lymphocytes.[57] It was proposed that this interaction may interfere with normal antigen-specific activation of T cells simply by masking the CD4 antigen for interaction with MHC class II molecules.[58] This interaction may contribute to the functional defects of the cellular immune response which precedes the decline of CD4+ T-cells in HIV-infected individuals.[59] Moreover, it was demonstrated in vitro that glycoprotein-specific antibodies can crosslink CD4 molecules when they are coated with soluble gp120. This process reduces interleukin-2 production,[60,61] inhibits proliferation of the T helper lymphcytes[62] and renders the cells anergic,[63] possibly by initiating phosphorylation and activation of the tyrosin protein kinase $p56^{lck}$.[64] Upon subsequent activation of the cells through the T cell receptor these cells will be hyporesponsive[64] and undergo programmed cell death.[65-67] Finally, by adhesion of the HIV glycoprotein to the CD4 molecule, uninfected cells will be recognized and lysed by natural and lymphokine-activated killer cells which present anti-gp120 antibodies on their surface bound to Fc receptors. This antibody-dependent cellular cytotoxicity has been shown by several groups to be effective in vitro.[68-71]

Autoimmunity

In addition to the mechanisms described above several other characteristics have been attributed to the HIV envelope glycoprotein. These include the induction of autoimmunity based on structural similarities detected between the HIV-1 envelope glycoprotein and immunologically important molecules such as MHC class II antigens, the Fas/Apo-1 protein and functional domains of immunoglobulins[72,73] and superantigen-like activation of particular V T cell subsets in vitro.[74] However, these observations have not been uniformly confirmed and are still a matter of dispute.[75,76]

Apoptosis

Cells may die either by necrosis or by apoptosis. Necrotic cell death may be regarded as non-physiologic because it is usually the result of physical or chemical alterations in the environment causing damage to the cellular metabolism or structure. This type of cell death causes in vivo secondary damage to neighbouring cells by enzymes and toxic products released by the dying cell and induces an inflammatory response in the affected tissue. In contrast, apoptotic cell death is the outcome of a process intrinsic to a particular cell during which the cell actively starts and executes its own death program upon signaling from outside or infection of the cell. In the course of apoptosis, the cell disintegrates into membrane-enveloped subcellular particles, so called apoptotic bodies, which contain cytoplasm, morphologically intact organelles and parts of the nucleus. Apoptotic bodies are subsequently taken up and digested by neighbouring cells including macrophages and epithelial cells. The apoptotic cell death does not cause an inflammatory reaction (reviewed in: 77,78). Similar to the induction of necrosis, multiple stimuli and events can give rise to apoptotic cell death. Therefore, the presence of apoptotic cells in a particular tissue does not provide any indication as to which mechanism underlies the cell death observed.

Several studies have demonstrated an increased incidence of apoptotic cell death in HIV infection.[66,79] However, in HIV-infected individuals an increased incidence of apoptosis after in vitro stimulation has not only been observed with CD4+ T cells but also with CD8+ T cells when compared with cells from unifected controls.[80,81] A possible explanation for this observation is that in HIV infection both CD4+ and CD8+ T cells are activated. Stimulation of lymphocytes in the wake of a viral infection may cause increased levels of apoptotic lymphocyte death.[82] Alternatively, apoptosis of CD4+ and CD8+ T lymphocytes are due to different processes.

Signs of apoptotic cell death were detected in various in vitro studies, most of which have been linked to the envelope glycoprotein. As outlined above, antigenic activation of helper cells, which were previously rendered anergic by crosslinking of CD4 molecules, results in lymphocyte apoptosis.[65] In addition, accumulation of gp160-CD4 complexes in infected cells may cause single cell apoptosis.[53] Alternatively, it was demonstrated that contact of HIV glycoprotein-expressing cells with CD4+ T cells causes the CD4+ T cells to rapidly die by apoptosis in cell culture.[83,84] In this system, apoptotic cell death occurs upon contact of gp160 and the CD4 molecule[85-89] and depends on the presentation of a cleavable gp160 molecule on the cellular surface.[86,90] In addition to the CD4-binding region, the V3 loop seems to be critical because point mutations in this area and monoclonal Ab directed at this region which inhibit cell-cell fusion but not binding to the CD4 molecule abolish induction of apoptosis[91] and rapid cell lysis.[48] Apoptotic cell death upon interaction of infected with uninfected cells can be detected in single cells and in cells fused in syncytia,[86,88] both in primary CD4+ T lymphocytes[85,89] and in T lymphoblast cell lines.[83,84,88,89,92]

Conclusions

Although the hypotheses presented are not mutually exclusive, they cannot be unified to a single concept. However, several of the hypotheses are linked by a common prerequisite for induction of apoptosis or may describe related processes. For instance, gp120 shed by infected cells constitutes the basis of antibody-dependent cellular cytotoxicity, CD4 masking, crosslinking and induction of anergy with subsequent apoptosis. The exertion of such mechanisms requires some kind of immune response, either cellular or humoral. Alternatively, syncytium formation, rapid lysis and induction of apoptosis were described as a result of the interaction of gp160 on the cell surface of infected cells with uninfected CD4+ lymphocytes. Crucial to the latter concept is that HIV replicates in vivo. In these circumstances, the cytopathicity observed should correlate with the proportion of HIV-infected and virus-replicating cells. The fact that primary CD4+ T cells die in a matter of days after infection with HIV in cell culture indicates that virus infection in vitro is highly cytotoxic in the absence of any immune response.

Common to the two groups of hypotheses is that they deliver an explanation for the death of uninfected CD4+ T cells. This is in cotrast to the idea that accumulation of gp 160-CD4 complexes causes cytotoxicity, a process which would account only for the death of HIV-infected cells. This concept has to compete with other mechanisms involved in killing of HIV-infected cells; like lysis by cytotoxic T lymphocytes.[93,94]

However, several lines of evidence suggest that not only infected but also uninfected cells are destroyed during HIV infection. For instance, histopathologic studies point to the fact that loss of CD4+ T cells is not restricted to infected cells but uninfected CD4+ T cells are similarly affected in vivo.[81]

In addition, the number of HIV-infected cells is markedly lower than the number of CD4+ T cells dying and being replaced day by day.[95] Finally, recent measurements in HIV-infected individuals undergoing antiviral combination therapy and mathematical modelling suggest that the number of virions produced each day exceeds the number of CD4+ T cells lost by a factor of approximately 10.[1,2,3,96] Since up to 99.99% of the virus particles produced are infection-incompetent[97] the ratio of virions produced and cells destroyed each day may be too low to account for lysis of only infected cells in the course of HIV disease.

In conclusion, although the cause of CD4+ T cell depletion in HIV-1 infection might be multifactorial and is still elusive, several lines of evidence indicate that the envelope glycoprotein may contribute to this process. However, the ideas ra.ised regarding the role of the glycoprotein are diverse and can only partially be harmonized into common concepts for the understanding of the role of this molecule in the depletion of T helper cells.

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