Minireview

HIV: from molecular recognition to tissue pathogenesis

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Abstract Dramatic progress has been made recently in identifying both viral and cellular molecules responsible for binding and fusion of HIV-1 to target cells. In vivo, HIV-1 infection is transmitted by viruses that recognize chemokine receptor CCR5, while viruses isolated at later stages of HIV disease often recognize another chemokine receptor, CXCR4. It is still not understood how this molecular tropism of HIV-1 is translated into the virus' ability to compromise normal cell functions, which results in impairment of lymphoid tissue and causes AIDS. Here, we discuss how the new molecular findings might relate to HIV pathogenesis in cells and tissues.

Key words: Human immunodeficiency virus type 1; Molecular tropism; Molecular recognition

1. Introduction

AIDS is the first pandemic to start in the era of molecular biology. As a result, we know much about the molecules encoded by the viral genome. In contrast, however, we know relatively little about the mechanisms of viral pathogenesis. AIDS is a complex disease, in part because HIV infects the cells that fight infection and disrupts multiple, little understood cell interactions in the lymphoid system. Moreover, the virus evolves rapidly and continuously over many years in the body under yet unidentified selective pressures, and its properties can be strikingly different early in infection compared with later, when severe immunodeficiency occurs [1,2].

Until recently, HIV-1 isolates were classified according to their ability to infect and induce syncytia in various cells lines or blood-derived cells in culture. Initial HIV-1 infection is transmitted by viruses that, in vitro, are able to infect both T lymphocytes and monocyte-derived macrophages, but that fail to induce syncytia in several test cell lines. In contrast, viruses isolated at later stages of HIV disease often have acquired the ability to induce syncytia in the same test cell lines but lose the ability to infect primary macrophages. The former are defined as M-tropic, or syncytia-inducing (SI), the latter are classified as T-tropic, or non-SI. Recently, it was discovered that various isolates recognize particular chemokine receptors on the target cells. As a result, a vague and controversial classification of HIV-1 based on a complex interaction of virus with particular cells has now been replaced by one built on the firmer basis of the viral molecular characteristics [3]. This new classification may have a profound impact on HIV research, similar to the impact of Mendeleev's Periodic Table on chemistry. The next task is to correlate the molecular characteristics of HIV and its counterparts on target cells with the mechanisms by which HIV compromises normal cell functions, using this knowledge to understand how HIV impairs the more complex lymphoid tissue and causes AIDS.

The aim of this minireview is to discuss how the new molecular findings relate to HIV pathogenesis in cells and tissues.

2. Molecular tropism: HIV co-receptors

HIV infection starts with the binding of the viral envelope to the target cell surface via the viral envelope glycoprotein (Env) gp120, eventually enabling the virus to fuse with the plasma membrane. The first cellular component of this interaction was identified several years ago as CD4, a member of the immunoglobulin superfamily. CD4 is necessary but not sufficient for fusion. The strongest indication that a co-factor is required is the inability of non-primate cells transfected with human CD4 to fuse with HIV-1- or with Env-expressing cells (reviewed in [4]).

It took, however, almost a decade more to identify a fusion co-factor [5]. The discovery of this molecule, originally named fusin, by Edward Berger and his colleagues [5] was a triumph of the purely molecular biological approach. They repeatedly subdivided a human cDNA library, made from a cell type permissive for HIV infection, transfected CD4-expressing non-human cells with this cDNA, and then tested the resultant cellular clones for their ability to fuse with Env-expressing cells. Three other HIV-1 co-receptors were reported only weeks after fusin by five independent groups [6–10], and a few others have been reported since [11,12].

All known HIV co-receptors belong to the chemokine receptor branch of the superfamily of seven-transmembrane G-protein coupled receptors [13]. Chemokine receptors bind a subfamily of cytokines that attract leukocytes to sites of inflammation [4,13]. According to a new nomenclature, fusin was renamed CXCR4. In vitro, HIV-1 utilizes various chemokine receptors, but evidence for in vivo usage has been published only for CCR5 and CXCR4 (see [4,14] and references therein).

Chemokine receptors on the cell surface are recognized by viral gp120, which forms a three-molecule complex with CD4 [15], but many details of this process remain unclear. Recently, a CD4-independent HIV-1 isolate, which (similar to HIV-2 [16]) uses only chemokine receptors for viral entry, was described [17]. Thus, the categorization of HIV-1-binding cell surface molecules into receptors and co-receptors reflects

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merely the history of their discovery rather than their relative importance.

The discovery of the molecular tropism of HIV-1 now allows molecular classification of these viruses according to their co-receptor usage (molecular tropism) as X4 (CXCR4-tropic), R5 (CCR5-tropic), or X4R5 (dual tropic) [3].

Shortly before HIV co-receptors were discovered, Gallo and his colleagues purified three anti-HIV substances, RANTES, MIP-1α and MIP-1β [18], which turned out to be CCR5-binding chemokines. Later a CXCR4-specific chemokine SDF-1 was identified [19]. Together with cell lines expressing CD4 and a single co-receptor, chemokines provide new research tools to determine which co-receptor is used by various isolates to infect different types of cell.

3. Cell infection: relation of molecular to cellular tropism of HIV

T lymphocytes, dendritic cells, monocytes/macrophages and brain glial cells are the main cells that co-express chemokine receptors and CD4+ [11], making them the natural targets for HIV-1 infection. In the laboratory, all HIV-1 isolates are universally propagated in peripheral blood mononuclear cells (PBMC) [20]. Cultured monocyte-derived macrophages, and various T cell lines are more selective, some isolates infecting only one type.

Designation of HIV-1 isolates as M-tropic or T tropic disguises the fact that in vitro both infect dendritic cells and activated T cells [3,4]. At molecular level the situation is less confusing, and also molecular tropism correlates with M- or T-tropism (see [4,14]): R5 HIV isolates are M-tropic while X4 isolates are T-tropic; dual-tropic R5X4 viruses have also been identified.

However, molecular tropism does not fully explain the cause of cellular tropism. For example, it is not clear why X4 HIV poorly infects macrophages even though these cells express both CD4 and CXCR4 (as well as CCR5) (see [12]). Whatever the explanation, the selective resistance of in vitro macrophages to X4 HIV-1 may be related to the fact that R5, rather than X4, HIV-1 transmits infection in vivo. Normally, mucosal macrophages and dendritic cells are the first cells to meet an antigen and to carry it to the regional lymph nodes to trigger an immune response [21]. This natural mechanism of defense is also used by HIV-1 for its transport to the lymph nodes [22]. If also in vivo macrophages are infectible only by R5 HIV-1 it would partially explain why only R5 viruses transmit infection. However, it is not clear how well cellular tropism in in vitro experiments reflects the spectrum of target cells in vivo. Moreover, it was found that the expression patterns of CCR5 and CXCR4 on the surface of macrophages [23] or dendritic cells [24] in culture differ from those present in vivo. Obviously, in vitro cells do not display some important physiological parameters that are critical for HIV infection in vivo.

Cell activation that modulates co-receptor expression [25,26] and thus HIV infection, may be one of these parameters. Moreover, the virus itself may send an activation signal through the chemokine receptors [27,28] fine-tuning of cells for viral replication. Such a signal is not required for in vitro PBMC infection [29–31] since such cells are already artificially activated by PHA and IL-2 to be able to propagate virus [20].

Cell activation may explain why, in vitro, CC-chemokines paradoxically enhance R5 HIV replication in macrophages instead of inhibiting it [32]. In vitro, in contrast to PBMC, macrophages do not need to be over-stimulated for HIV-1 infection, and stimulation by CC chemokines may enhance replication of those viral particles that enter cells through unoccupied CCR5 receptors. Such a phenomenon was not observed in over-stimulated PBMC or in constitutively stimulated T cell lines, in which CC chemokines inhibit R5 HIV-1 infection [6–8,18]. Recently, however, CC chemokine-enhanced HIV-1 replication was also described for T cells in lymphoid tissues ex vivo [33], in which lymphocytes do not require exogenous stimulation for productive HIV infection [34,35].

This is further evidence that one may not be able to extrapolate results of experiments relating to HIV co-receptor usage directly to cells in vivo. In conclusion, HIV-1 cellular tropism appears to be a function of the co-receptor tropism of HIV, albeit not a simple one.

4. Lymphoid tissue: HIV co-receptors and viral pathogenesis

Nowadays, it is generally accepted [2] that the critical events of HIV disease take place within lymphoid tissue, in which complex interactions between both infected and uninfected cells occur. 98% of CD4+ T lymphocytes, the potential HIV-1 targets, reside in lymphoid tissue. Even during the apparent clinical 'latency' stage of HIV disease, HIV continues to replicate in lymphoid tissue [36], which eventually deteriorates and fails to replenish the massive loss of lymphocytes [37].

Suspensions of peripheral blood lymphocytes in vitro or transfected cell lines, which have been used to obtain the vast majority of experimental data on HIV infection, lack the complex intercellular interactions and the rich cellular repertoire typical of lymphoid tissue. Thus, for experimental studies of AIDS, HIV pathogenesis may be more closely approximated using integral lymphoid tissue.

Two experimental systems have been developed to study HIV pathogenesis in whole lymphoid tissue: HIV-1-infected SCID-*hu* mice with transplanted human neonatal thymus [38], explants of human adult lymph nodes or tonsils [34,35] or of neonatal human thymus [39,40]. All these systems support replication of HIV-1. In ex vivo lymphoid tissues, X4 HIV-1 infection is associated with severe depletion of CD4+ T lymphocytes, while R5 viruses deplete CD4+ T lymphocytes only mildly [34,35,41]. This correlates with different stages of HIV disease in vivo: the early stages are dominated by R5 viruses, while the late stages, associated with CD4+ T lymphocyte depletion and immunodeficiency, are often dominated by X4 or dual tropic HIV-1 [42,43].

Why do X4 isolates deplete CD4+ T cells in human lymphoid tissue ex vivo, while R5 do not? Perhaps the life and death of an individual T cell depend on which co-receptor the virus has used to infect the T cell. Alternatively, R5 and X4 HIV may infect different CD4+ T cell subsets, only those infected by X4 dying. These questions remain to be answered.

The ex vivo studies [35,44,45] mimic clinical observations [36,46] on the apparent discrepancy between the small number of productively infected cells and massive cell killing by X4 viruses. Apparently, besides the infected cells, uninfected (by-

stander) cells may be killed as well [47,48]. Many mechanisms have been proposed to explain this, including an excess of cytotoxic viral proteins (reviewed in [49]), oxidative stress [50,51], secreted cellular factors, or defective cell-cell interactions [2,47,49], although none of these hypotheses has yet been proved. At least for ex vivo tissue, productively infected cells may die quickly [37]; even though a relatively small number of them die at any time, the integrated number dying over weeks of infection may account for all cell death without invoking the bystander death hypothesis. Ultimately, this controversy should be resolved by the development of methods to monitor individual HIV-infected cells in ex vivo experiments with whole tissues.

Many other aspects of HIV pathogenesis also require tissue integrity. One of them is immunodeficiency, the hallmark of HIV disease, which can now be studied in human lymphoid tissue ex vivo [52]. Ex vivo antigen challenge of these tissues leads to production of specific antibodies. This response is dramatically inhibited by X4, but not by R5 HIV-1 isolates [52]. This provides experimental evidence that a switch in HIV co-receptor tropism may be a cause of immunodeficiency in vivo rather than merely an associated phenomenon.

What is the driving force for such a switch? Endogenous CC chemokines may play an important role [2] in this process. Their upregulation in X4-infected but not in R5-infected human lymphoid tissues, which has been observed ex vivo [33], may inhibit R5 viruses, giving advantage to X4 infection. Whatever the factors regulating tissue infection, they are either secreted locally or transmitted in the course of cell-cell contacts. In both cases, the relative positioning of the cells producing and accepting these factors should be extremely important.

The critical role of tissue cytoarchitecture in HIV pathogenesis was fully appreciated only relatively recently [36], and experimental approaches to address this problem have still to be developed. As a result, this field poses many more questions than it has answers.

5. Conclusions

Significant progress has been made in identifying both viral and cellular molecules responsible for virus binding and fusion to target cells. Primary cellular targets for HIV-1 that harbor virus and transfer it into lymphoid organs have been identified, and their relevant molecular characteristics have been extensively studied. The next challenge is to understand how these newly discovered molecular and cellular mechanisms of HIV infection fit into the integral picture of HIV pathogenesis in vivo. More generally, a multidisciplinary approach to HIV pathogenesis offers a better opportunity to determine how a natural multi-cellular system operates and how a relatively simple pathogen, whose molecular characteristics are known, diverts this operation to its own benefit.

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