The Role of Human Immunodeficiency Virus Type 1 Envelope Glycoproteins in Virus Infection*

Eric O. Freed and Malcolm A. Martin†

From the Laboratory of Molecular Microbiology, NIAID, National Institutes of Health, Bethesda, Maryland 20892-0460

Enveloped viruses enter cells by a two-step process. The first step involves the binding of a viral surface protein to receptors on the plasma membrane of the host cell. After receptor binding, a membrane fusion reaction takes place between the lipid bilayer of the viral envelope and host cell membranes. This fusion reaction can occur either at the plasma membrane or in acidic endosomes following receptor-mediated endocytosis. In either case, the membrane fusion reaction delivers the viral nucleocapsid into the host cytoplasm, allowing the infection to proceed. Viral proteins embedded in the lipid bilayer of the viral envelope (known variously as surface, spike, or envelope proteins) catalyze receptor binding and membrane fusion reactions. The critical involvement of these viral envelope proteins in receptor binding and membrane fusion has stimulated intensive investigation aimed at understanding the mechanisms by which these proteins function. In this article, we provide a brief overview of the roles envelope (Env) glycoproteins play in the human immunodeficiency virus type 1 (HIV-1) life cycle.

Env Precursor Biosynthesis and Processing

The Env glycoprotein of HIV-1, like those of other retroviruses, is synthesized as a polypeptide precursor molecule which is proteolytically processed by a host protease to generate the surface (SU) and transmembrane (TM) subunits of the mature Env glycoprotein complex. The unprocessed Env precursor has been designated, based on its apparent molecular mass, gp160. The mature SU and TM Env glycoprotein subunits are designated gp120 and gp41, respectively. Sequence comparison of a number of HIV-1 isolates indicated that (i) gp120 is highly variable between virus isolates and (ii) this variability is nonuniform, leading to the designation of conserved (C) and hypervariable (V) domains within gp120 (Fig. 1; 1-3). A series of highly conserved Cys residues, which are involved in intramolecular disulfide bonding crucial for achieving and maintaining Env tertiary structure (4), are found throughout gp120 and gp41.

As with other glycoproteins destined for the plasma membrane, gp160 is synthesized on the rough endoplasmic reticulum (ER) and is cotranslational glycosylated and inserted into the lumen of the ER. A single stop-transfer, membrane-spanning sequence is located in the central portion of the gp41 domain (Fig. 1; 5, 6). Shortly after synthesis, gp160 monomers oligomerize (7-10), a process which is thought to be required for transport from the ER to the Golgi complex (11). Once in the Golgi, some of the high mannosyl-ER-acquired N-linked oligosaccharide side chains are modified to more complex forms, and gp160 is proteolytically cleaved to gp120 and gp41 (10, 12). The HIV-1 Env glycoprotein is extensively glycosylated; approximately half the molecular mass of gp120 is composed of oligosaccharides (13). All 24 potential N-linked glycosylation sites in gp120 from the HTLV-III, HIV-1 isolate and at least three of the five sites in the ectodomain of gp41 appear to be utilized (Fig. 1; 4, 14). It has been suggested that HIV-1 Env also contains O-linked carbohydrates (15).

Proteolytic cleavage of gp160 in the Golgi is inefficient (12) and is catalyzed by a host protease at a Lys/Arg-X-Lys/Arg motif (where X is any amino acid) that is highly conserved among viral Env glycoprotein precursors (16-19). Several studies have suggested that the host enzyme responsible for cleaving gp160 (and other viral Env precursors) is furin or a furin-like protease (20, 21). Other enzymes may also be capable of mediating gp160 precursor processing, since cleavage can occur in a furin-deficient cell line (22), and a basic pair of amino acids at the cleavage site is not absolutely required for gp160 processing (18). Following gp160 cleavage, the oligomeric, noncovalently associated gp120-gp41 complexes are transported to the cell surface, where they are incorporated into budding virions.

Env Incorporation into Virus Particles

Because of the role HIV-1 Env plays in receptor binding and membrane fusion (see below), the virion incorporation of Env is essential for the formation of infectious virus particles. In certain virus systems (e.g., the alphaviruses), an interaction between the Env protein intracytoplasmic tail and the viral capsid has been demonstrated directly, and this interaction is required for virus release (23-25). In the case of retroviruses, which do not require Env expression for virus assembly and release (for review, see Ref. 26), the picture is less clear. Evidence derived from Env and Gag mutagenesis and pseudotyping studies has accumulated over the past decade both for and against the existence of a specific interaction between the TM cytoplasmic tail and the matrix protein (MA), which forms the membrane-proximal component of the retroviral core (27-35). In a recent study, it was demonstrated that mutations in HIV-1 MA that blocked the virion incorporation of full-length HIV-1 Env did not affect the incorporation of heterologous retroviral Env glycoproteins with short cytoplasmic tails or HIV-1 Env mutants containing large truncations in the gp41 cytoplasmic tail (36). This latter finding implies that the incorporation of Env glycoproteins with long cytoplasmic tails (i.e., lentiviral Env glycoproteins) depends upon a specific interaction between sequences in the cytoplasmic tail of the TM glycoprotein and the HIV-1 MA, whereas the incorporation of Env glycoproteins with short cytoplasmic tails into HIV-1 virions does not (36).

CD4 Binding

The initial step in HIV-1 infection involves the binding of virion-associated gp120 to the cell surface molecule CD4, which serves as the major receptor for HIV-1 and the related HIV-2 and simian immunodeficiency viruses (SIVs) (37-39). The Env determinants of CD4 binding map to gp120, in particular C3 and C4 (40-42). CD4 binding to gp120 induces conformational changes in both gp120 and gp41 that result in the exposure of Env domains (see below) that are thought to be involved directly in the membrane fusion reaction (43-46).

Following the identification of CD4 as the primary receptor for HIV, it was determined that soluble CD4 (sCD4) could neutralize virus infectivity (47-51). This neutralization was demonstrated to be primarily a result of an enhanced shedding of gp120 from virions following treatment with sCD4 (52-54). Initially, it was suggested that the ability of sCD4 to neutralize HIV-1 might be exploited therapeutically. Unfortunately, however, primary, non-laboratory-adapted HIV-1 isolates are neutralized poorly by sCD4 (55, 56), in part as a result of the relative resistance of primary isolates to sCD4-induced gp120 shedding (57-60), thereby diminishing the utility of sCD4 as a therapeutic agent. In fact, in related HIV-2 and SIV systems, sCD4 has been reported to actually enhance virus infectivity (61). It is currently unclear what role, if any, CD4-induced gp120 shedding plays in HIV-1 Env function (62, 63).
which, as noted above, are unusually long compared with those of other retroviruses. In some cases, these deletions enhanced Env-induced membrane fusion, suggesting that sequences in the gp41 cytoplasmic tail may modulate Env fusogenicity (94, 96, 97, 99, 101, 102).

In gp120, primarily two regions are involved in membrane fusion. A number of studies determined that antibodies to V3 were capable of neutralizing virus infectivity (103–109) without affecting virus binding to CD4 (108, 109). Mutational analyses demonstrated that single amino acid substitutions within the HIV-1 V3 loop, and the analogous domain of HIV-2, blocked Env-induced syncytium formation (83, 110, 111) and virus infectivity (83, 112). More recent studies have also implicated the V1/V2 region in membrane fusion. Mutations within V1/V2 were reported to block syncytium formation without affecting the gp120-gp41 interaction or CD4 binding (113), and the transfer of V2 sequences from syncytium-inducing Env glycoproteins conferred the ability to induce fusion on non-syncytium-inducing Env glycoproteins (114, 115). Consistent with a role for V1/V2 in membrane fusion, antibodies to this region are capable of neutralizing virus infectivity (116, 117).

It has been postulated for a number of years that molecules other than CD4 may be necessary for membrane fusion induced by HIV-1 Env. The following observations suggest that factor(s) provided by human cells are required for HIV-1 Env-induced membrane fusion: (i) expression of human CD4 in murine cells does not confer upon them the ability to support HIV-1 infection (39), (ii) in a cell-fusion reaction, the target cell must be of human origin (113a), and (iii) Env-expressing cell can be of non-human origin (118), and (iii) the formation of some somatic cell hybrids between human cells and CD4-expressing non-human cells can overcome the fusion defect observed in human CD4-expressing non-human cells, suggesting that the inability of CD4+ murine cells to support HIV infection is due to the absence of factor(s) on murine cells, rather than the presence of a mouse cell-specific interfering function (119–121). Although non-CD4 molecules have been reported to serve as alternate HIV-1 receptors on non-human cells (122), no widely accepted CD4 co-receptor has been identified. It was suggested by Callebaut et al. (123) that CD26 (dipeptidyl-peptidase IV) conferred susceptibility to HIV-1 infection upon CD4-expressing murine (NIH 3T3) cells. A number of groups, however, failed to confirm a role for CD26 in HIV-1 infection or syncytium formation (124–128). Recent protease digestion data suggest that the factor(s) provided by human cells may be nonproteinaceous (129).

Tissue Tropism

An additional function of the HIV-1 Env glycoprotein is to determine the cell-type specificity, or tissue tropism, of virus infection. In culture, HIV-1 typically infects either cells of the monocyte/macrophage lineage or immortalized T-cell lines, but rarely both. Primary virus isolates obtained from infected individuals during the early, asymptomatic phase of infection are frequently non-syncytium-inducing and macrophage-tropic, and cells of the monocyte/macrophage lineage are thought to be important targets for virus infection in vivo (for review, see Ref. 130). HIV-1 isolates which are syncytium-inducing and capable of productively infecting T-cell lines tend to arise late in infection after the onset of AIDS-defining symptoms (131). In fact, it has been argued that the evolution in vivo of syncytium-inducing, T-cell line-tropic variants may play a crucial role in disease development (74). As would be predicted for a property determined by Env, the block to infection in nonpermissive cells appears to be primarily at the level of entry, presumably resulting from a defect in membrane fusion (132, 133). Interestingly, both macrophage-tropic and T-cell line-tropic isolates are capable of efficiently infecting primary human CD4+ T-lymphocytes.

Studies conducted in a number of laboratories have concluded that sequences within gp120 are responsible for determining the tissue tropism of HIV-1. The V3 loop, discussed above in the context of membrane fusion, plays a central role in tropism. The introduction of sequences encompassing the V3 loop from macrophage-tropic clones to T-cell line-tropic clones is able to confer macrophage tropism upon certain T-cell line-tropic clones (134–138). It is clear, however, that a combination of sequences both within, and outside, V3 is required for optimal macrophage infec-
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