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Mutations increasing exposure of a receptor binding site epitope in the soluble and oligomeric forms of the caprine arthritis-encephalitis lentivirus envelope glycoprotein

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Abstract

The caprine arthritis-encephalitis (CAEV) and ovine maedi-visna (MVV) viruses are resistant to antibody neutralization, a feature shared with all other lentiviruses. Whether the CAEV gp135 receptor binding site(s) (RBS) in the functional surface envelope glycoprotein (Env) is protected from antibody binding, allowing the virus to resist neutralization, is not known. Two CAEV gp135 regions were identified by extrapolating a gp135 structural model that could affect binding of antibodies to the RBS: the V1 region and a short sequence analogous in position to the human immunodeficiency virus type 1 gp120 loop B postulated to be located between two major domains of CAEV gp135. Mutation of isoleucine-166 to alanine in the putative loop B of gp135 increased the affinity of soluble gp135 for the CAEV receptor(s) and goat monoclonal antibody (Mab) F7-299 which recognizes an epitope overlapping the gp135 RBS. The I166A mutation also stabilized or exposed the F7-299 epitope in anionic detergent buffers, indicating that the I166A mutation induces conformational changes and stabilizes the RBS of soluble gp135 and enhances Mab F7-299 binding. In contrast, the affinity of a V1 deletion mutant of gp135 for the receptor and Mab F7-299 and its structural stability did not differ from that of the wild-type gp135. However, both the I166A mutation and the V1 deletion of gp135 increased cell-to-cell fusion activity and binding of Mab F7-299 to the oligomeric Env. Therefore, the CAEV gp135 RBS is protected from antibody binding by mechanisms both dependent and independent of Env oligomerization which are disrupted by the V1 deletion and the I166A mutation, respectively. In addition, we found a correlation between side-chain β-branching at amino acid position 166 and binding of Mab F7-299 to oligomeric Env and cell-to-cell fusion, suggesting local secondary structure constraints in the region around isoleucine-166 as one determinant of gp135 RBS exposure and antibody binding.

Keywords: Lentivirus; Glycoprotein; Receptor; CAEV; HIV-1; Rotamer; Conformation; Entropy; Neutralization; Neutralization-resistance

Introduction

The caprine arthritis-encephalitis virus (CAEV) is a macrophage-tropic lentivirus of goats that causes chronic progressive connective tissue disease characterized by arthritis of synovial joints, interstitial mastitis and, more rarely, pneumonia and encephalitis (Cheevers and McGuire, 1988; Crawford et al., 1980; Narayan et al., 1993). CAEV is closely related to the ovine maedi-visna virus (MVV) and, more distantly, to the human immunodeficiency virus type 1 (HIV-1) and other lentiviruses (Cheevers and McGuire, 1988; Narayan et al., 1993). A hallmark of lentiviruses is their intrinsic resistance to neutralization mediated by antibody, which probably contributes to virus persistence in vivo. In this regard, most goats infected with CAEV and sheep infected with field MVV strains produce low or undetectable levels neutralizing antibody against the infecting strain (Cheevers et al., 1991, 1993; Narayan et al., 1984). This intrinsic resistance to neutralization seems to be determined at least in part by the virus as one MVV strain, the Icelandic MVV-K1514, consistently induces higher

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levels of neutralizing antibodies in both sheep and goats (Narayan et al., 1984). The viral factors that determine this phenotype in CAEV and MVV have not been defined but most likely include structural features of the surface envelope glycoprotein.

The envelope glycoprotein of small ruminant lentiviruses (SRLV) has the canonical retroviral surface (SU) and transmembrane (TM) glycoprotein organization with the functional form on the surface of infected cells and virions probably forming an oligomeric complex of SU/TM heterodimers. The lack of covalent binding between CAEV SU (gp135) and TM makes the oligomeric structure unstable and gp135 is readily shed in soluble form from the surface of infected cells and this soluble gp135 is capable of binding receptors and antibodies against conformational epitopes (Hullinger et al., 1993; Özyörük et al., 2001). The secondary/tertiary structure of SRLV gp135 is not known. More critically, the gp135 receptor and/or coreceptor binding site(s), the obvious target(s) for neutralizing immune responses, have not been identified. To initiate the structural characterization of SRLV gp135, we developed a model based on partial sequence similarities between gp135 and HIV-1 gp120, for which the core structure has been described (Hötzel and Cheevers, 2000, 2001; Kwong et al., 1998, 2000; Wyatt et al., 1998). In this gp135 model, the inner-proximal domain of gp120 facing TM comprised by the C2 region between β-strands 4 and 8 and β-strand 25 in the C5 region is structurally conserved in gp135. Consistent with this model, mutations of amino acids between the putative CAEV gp135 β4 and β5 and in or just downstream from putative β-strand 25 impair stable interactions between gp135 and TM (Hötzel and Cheevers, 2003). Furthermore, as predicted by the model, epitope mapping results indicate that putative β-strands 5 and 25 of CAEV gp135 are located close to each other in the folded glycoprotein despite their distance in the primary structure (Hötzel and Cheevers, 2003). Therefore, the proposed structural model is a useful tool to resolve some aspects of the gp135 structure and function.

Both CD4 and the HIV-1 gp120 coreceptors bind elements of the inner and outer domains of gp120 (Kwong et al., 1998; Rizzuto et al., 1998). Therefore, domain organization is critical for receptor binding by gp120. Although the current gp135 structural model does not make any predictions regarding the location of the gp135 receptor binding site(s) (RBS), extrapolation of this model could be used to predict gp135 regions that affect receptor binding and binding of antibodies to the RBS. One of the structural features extrapolated from the model is the domain organization of gp135. The gp120 outer domain sequence is included entirely between inner domain β-strands 8 and 25, suggesting that the long and heavily glycosylated region between the corresponding putative strands of gp135 also forms an "outer domain" participating in receptor binding (Hötzel and Cheevers, 2000, 2001). Two chain regions link the inner and outer domains of gp120, one of these being loop B located just downstream from β-strand 8 (Kwong et al., 1998). The HIV-1 loop B has been postulated to change conformation upon binding of gp120 to CD4 (Kwong et al., 1998). The structure of a simian immunodeficiency virus (SIV) gp120 not bound to receptors has been recently described and, in fact, the region homologous to the HIV-1 gp120 loop B assumes a α-helical and β-strand conformation (Chen et al., 2005), supporting the interpretation that this region changes secondary structure upon receptor binding. Thus, the gp135 chain region located just downstream from the putative gp135 β-strand 8 (Hötzel and Cheevers, 2000) may constitute an analogue of the gp120 loop B in gp135, forming a hinge between the two major putative gp135 domains that changes secondary structure to allow interdomain shifts for RBS formation and binding of receptors and RBS epitope formation.

A second region important in modulating binding of receptors and antibodies to gp120 is the V1/V2 loop structure. Deletion of the gp120 V1/V2 structure increases accessibility of the CD4 and coreceptor-binding sites to antibodies and in some circumstances may allow gp120 to bind coreceptors in the absence of CD4 (Cao et al., 1997; Wyatt et al., 1995). Therefore, the V1/V2 loop constitutes a variable structure shielding the more conserved receptor-binding regions of gp120. The V1/V2 loop emanates from the gp120 inner domain and is located in the primary structure just upstream from the C2 region conserved in gp135 (Kwong et al., 1998). Therefore, the upstream boundary of the conserved region may be used to locate a gp135 region similar to the gp120 V1/V2 loop. In fact, the first variable region (V1) of CAEV gp135 is located in the same position relative to β-strand 4 as the gp120 V1/V2 loop (Fig. 1). Most residues in the gp135 V1 region have charged side-chains despite its sequence variability (Valas et al., 2000), suggesting that this region forms a short surface loop structure. It is not known whether the V1 region interacts with the gp135 RBS.

The identification of two regions potentially affecting RBS structure and exposure, the putative loop B and V1 regions of gp135, respectively, provides an opportunity to

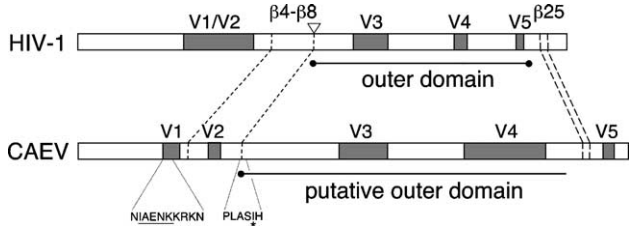

Fig. 1. Positions of mutations introduced in CAEV gp135. Gray boxes represent regions of HIV-1 gp120 and CAEV gp135 variable between strains. The sequences forming the gp120 outer domain and the putative gp135 outer domain are indicated (Hötzel and Cheevers, 2001). Dashed lines show the positions of gp120 β-strands 4 through 8 and 25 and the corresponding gp135 regions (Hötzel and Cheevers, 2000, 2001). The white arrowhead shows the position of gp120 loop B. Amino acid residues deleted in the gp135 V1 region are underlined. The asterisk indicates the isoleucine-166 residue.