Structural characteristics and biological functions of the HIV-1 gp120 V3 region*

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Abstract Recent studies demonstrate that the V3 loop of HIV-1 gp120 plays an important role in the attachment of HIV-1 to the target cells. Several amino acids in this domain are involved in the interaction of gp120 with the co-receptors. The V3 loop elicits one of the earliest antiviral antibody responses in HIV-1 infection and has been identified as the principal neutralizing determinant (PND). A subset of antibodies to V3 loop show a broad range of neutralizing activity. Unfortunately, this loop undergoes broad mutation and is one of the hypervariable regions. Mutations of some amino acids in this PND could affect syncytium formation, virus infectivity and neutralization. Knowing the structural characteristics and biological functions of the V3 region could help us to understand mechanism of HIV infection and to develop new strategy against HIV-1. In this review, the structural characteristics, variation and biological functions of the V3 loop as well as immunological responses to the V3 loop are discussed.

Keywords: HIV-1, V3 loop, neutralizing epitope, structure, biological function.

Acquired immunodeficiency syndrome (AIDS) is one of the grievous diseases in the world and is mainly caused by the human immunodeficiency virus type 1 (HIV-1). The virion attaches the host cells, mainly CD4-positive T lymphocytes and macrophages, through the interaction of its envelope protein with the CD4 molecule as well as co-receptor of the target cells. The infection involves a complicated process that has not been well understood. The third hypervariable region (V3) of the HIV-1 surface glycoprotein gp120 plays an important role in this process. The V3 loop, and maybe together with other domains of gp120, contacts to the coreceptor, mainly CCR5 or CXCR4, following the binding event between gp120 and CD4 molecules on the target cell surface[1]. In this review, the structural characteristics, variation and biological functions of the V3 loop as well as immunological responses to the V3 loop are discussed.

1 Structural characteristics and variation of the V3 region

The V3 region typically consists of about 35 to 36 amino acids, and one cysteine at each terminus of this domain. These two cysteines formed a disulphide bridge. Even this disulphide-linked "loop" is not exactly a loop because of its long sequence and non-regular secondary structure in globular proteins, the "V3 loop" is commonly used[2]. At the top of the V3 loop, the RGPG motif forms a tight β-turn between two twisted anti-parallel β-sheet side regions, each of which encodes the more variable sequence, and at the C-terminal end of the V3 loop peptide, a prominent amphipathic α helix from Thr23 to Gln32 has been well defined. The central glycine and proline residues of the turn are linked by a *cis* peptide bond[3~5]. Ghiara et al. for the first time determined the crystal structure of a complex between a partial sequence of the HIV-1 gp120 V3 loop and the Fab fragment of a broadly neutralizing antibody[6]. Recently, the crystal structure of a complex of gp120, a two-domain fragment of human CD4 and an antigen-binding fragment of a neutralizing antibody has been obtained[7~9]. Based on this structure, though the truncated gp120 does not contain any variable loops, the variable V3 loop is predicted to be close to a conserved area in gp120 that is believed to interact with the coreceptors[7~9].

Though as a most variable region of the envelope protein of HIV virion, the V3 loop has some recommendable structural features. Close to the N-terminus

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of the V3 loop, there is an Asn-linked glycosylation site in most sequenced isolates. It is reported that the N-glycosylation can protect HIV-1 from neutralization antibodies. Removal of this site conferred various degrees of enhanced sensitivity to neutralization by monoclonal antibodies (mAbs) directed against the CD4 binding site, which suggests that the conserved carbohydrate side chain of the V3 loop may serve as a shield to protect the CD4-binding domain in gp120[10,11]. But it is unclear whether this structure is necessary for the HIV-1 infectivity. Some studies found that the induced mutation in this glycosylation site neither impairs nor improves the ability of mutant virus to replicate in permissive cells[10,12]. However, other researchers demonstrated that the elimination of this particular glycan could reduce the ability of T-tropic HIV-1 to bind to CXCR4 and hence its ability to infect T cell lines[13]. Besides, mutations in the V3 loop sequence can eliminate or reduce syncytia formation mediated by recombinant HIV-1 envelope proteins[14].

2 Biological functions of the V3 region

HIV-1 entry is initiated by interaction of the envelope protein gp120 with a set of at least two receptors: CD4 and one of the coreceptors which are members of the chemokine receptor family. The usage of a coreceptor is related to the cell tropism of the HIV-1 strains. CXCR4 serves as a coreceptor for T cell line tropic (T-tropic) HIV-1[15], while CCR5 serves for macrophage tropic (M-tropic) HIV-1[16]. Thus, the T- and M-tropic viruses were designated X4 and R5 viruses, respectively[17]. Recent studies showed that the V3 region contains the determinants for the cell tropism and coreceptor usage[18]. The gp120 V3 loop of the T-tropic viruses contains more positively charged amino acid residues than that of the M-tropic viruses, and the extracellular domains of CXCR4 have more complementary negatively charged residues than those of CCR5. This may explain why T-tropic viruses preferentially bind to CXCR4[18]. Deletion of the V3 residues could abolish CCR5 interaction. Amino acid substitutions introduced to two V3 residues recently were found to affect CCR5 interaction. One of these was a highly conserved arginine residue located adjacent to the N-terminus of V3. The other was the hydrophobic residue phenylalanine located adjacent to the crest of V3[19,20]. Some other studies demonstrated that several mAbs directed against V3 could block the ability of gp120 to interfere with the interaction between CCR5 and its natural ligands[21,22]. It now becomes clearer that six V3 residues are important for CCR5 utilization by the R5 viruses. Two of them (Arg303 and Ala333) are located in the base of V3 and are highly conserved by R5 viruses of all HIV-1 subtypes. The other four residues (Lys310, Ile312, Arg318 and Phe320) are located in two β-strands flanking the crest of V3[23]. In some other research, the role of V3 loop in the interaction of T-tropic virus envelope protein with the chemokine receptor CXCR4 was discussed[24]. The interactions between sgp120 and CXCR4 are globally similar to those previously observed between sgp120 and CCR5, with some apparent differences in the strength of the sgp120-CXCR4 interactions and their dependence on CD4[25]. Single amino acid modifications in V3 can dramatically modify coreceptor usage. Moreover, linear V3 loop peptides can compete with intact cell surface-expressed gp120/gp41 for CCR5 or CXCR4 interaction[26]. Interaction of the T-tropic HIV-1 V3 loop with CXCR4 is independent of the V1/V2 regions of gp120 or cellular CD4[27]. In addition, the prerequisite of T-tropic V3 loop in the infection of CXCR4-positive colonic HT-29 epithelial cells has been investigated[28]. All these findings and other related studies showed that the V3 loop, together with other domains of envelope protein, plays an important role in the interaction of HIV-1 particles with the host cells.

Soon after HIV-1 was affirmed as the pathogen of AIDS, the receptor for HIV-1 virion on the target cells was identified to be CD4 molecule[29]. However, several lines of cells lacking CD4 molecule can also be infected by HIV-1[30,31]. Galactosyl ceramide (GalC) has been ratified commonly as the receptor for HIV-1 on the surface of CD4-negative cells, though other molecules may also be involved in the virion-cell interaction[32]. Further studies have demonstrated that V3, V4 and V5 domains may be involved in the binding of HIV-1 envelope protein to the GalC. V3 domain that interacts with the receptor on the CD4-negative brain cell and mucosal epithelial cells was also identified[33,34]. Recently, several proteins other than chemokine receptors, such as Nucleolin, PHAP II and PHAP I, have been identified as potential receptors[35] and attracted much attention for their capacity to interact with the V3 loop. Although the details of this interaction have not been clearly understood, it deserves more attention.

A main feature of HIV-1 pathogenesis is the

death of CD4 + T cells due to apoptosis. Some studies found that, in the previously activated cells, gp120 cross-linking results in apoptosis. The apoptotic response to gp120 is almost completely inhibited by soluble CD4 and anti-gp120 antibodies[36~38]. The V3 loop may be involved in the apoptosis induced by gp120 cross-linking since a single point mutation in this loop can inhibit the induction of apoptosis in CD4 + T cells[39]. More recently, it is reported that the semi-conserved domain of the V3 region (LAI strain, aa307~321, RKSIRIQRGPGRAFY) can induce an activation-apoptosis in memory CD4 + cells obtained from healthy individuals[40]. Whether this interaction involves other domain of gp120 needs to be further studied. On the other hand, it was also reported that the apoptosis of CD8 + T cells also involves HIV-1 gp120[41], but whether the V3 domain plays any role in this interaction is unclear.

Other biological functions of the V3 loop have also been investigated. For example, it was reported that when the V3 region with a loop structure binds to the surface molecules of the IL-2-dependent T cells, it leads to the suppression of IL-2 induced T cell growth by affecting the intracellular IL-2 signaling[42]. Incubation of the V3 peptide (HIV-1IIIB aa307~330) with human sperms resulted in the induction of a number of head-to-head binding sperms[43]. Since human semen is the main vehicle for the transmission of HIV-1, further studies in this field may provide useful information.

3 Immunological responses to the V3 region

The V3 loop has been identified as one of the most remarkable regions in the HIV-1 glycoprotein that can induce very broad immune response. Escape of HIV-1 from the immune system is associated with the lack of specific anti-V3 antibodies *in vivo*[44]. The V3 loop elicits one of the earliest antiviral antibody responses in HIV-1 infection, and a subset of anti-HIV-1 antibodies specific for this domain showed a broad range of neutralizing activity[45,46]. Neutralization of a variety of HIV-1 strains has been shown with serum obtained after immunization with antigens containing V3 peptides of different strains[47]. The synthetic V3 peptides were found to induce antibodies that had neutralizing and cell-fusion-inhibiting activities[48]. Lack of a highly conserved glycosylation site at amino acid 301 within the N-terminus of the gp120 V3 loop of a T-cell-tropic SIV-HIV hybrid virus ($SHIV_{SF33}$) rendered the virus highly susceptible to neutralization by polyclonal antisera directed against autologous $SHIV_{SF33}$ as well as heterologous HIV-1 strains[49]. Antibody binding most likely involves an induced fit of the peptide and the gp120 V3 loop is probably conformationally heterogeneous[50]. The central part of the V3 loop (aa308~322) is the important domain for antibody neutralization. The neutralizing epitope GPGRAFY is located on the crest of the V3 loop. Recently, we prepared a multi-epitope-vaccine that induced high levels of antibodies against GPGRAFY-epitope and two mutated epitopes GPGQTFY and GPGQAWY in rabbits. These epitope-specific antibodies all recognized the neutralizing epitope and mutated epitopes, respectively, but did not show any cross-reaction[51], indicating that the mutation of the neutralization epitope might be important for the escape of HIV-1 from the immune system and suggesting a possible anti-HIV strategy[52].

4 Conclusion

The V3 loop involved in the interaction of gp120 with the coreceptors plays an important role in the attachment of HIV to the target cells. This loop undergoes broad mutation and is one of the hypervariable regions. Mutations of some amino acids could affect syncytium formation, virus infectivity and neutralization. The V3 loop elicits the earliest antiviral antibody responses, and a subset of antibodies to V3 loop showed a broad range of neutralizing activity. Exploring the complicate correlation between mutation and biological functions of the V3 region could help to understand the mechanism of HIV-1 infection and to develop new strategies against HIV-1.

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