Department för Laboratoriemedicin

The effect of Galα(1,3)Gal glycopeptides-conjugates and human serum on HIV-1 infection and replication

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The first step in the entry of HIV-1 is the binding of the viral envelope glycoprotein (gp120) to the CD4 molecule expressed mainly on T helper cells. Upon binding, gp120 undergoes a series of conformational changes that expose inner epitopes, which in turn facilitate binding to the viral co-receptors (CCR5 or CXCR4). Further rearrangement of gp120 enables the fusion peptide of gp41 to reach out towards the cell membrane and facilitate fusion. It is thus evident that antibodies blocking any of these events could successfully inhibit viral entry. However, the high variability and glycosylation of gp120 makes of it a very poor and evasive immunogen. Thus all strategies directed towards this molecule have so far proved less than satisfactory.

In this thesis I present evidence for a new approach that could block viral entry and viral spread. We used peptides corresponding to the region of CD4 that binds to gp120 and we tested them for their capability to inhibit HIV-1 infection by means of their competition with the actual receptor. Moreover, in order to increase their immunogenicity and possibly to increase their biodynamics, we coupled them to the galα(1,3)gal disaccharide. This sugar residue attracts naturally occurring antibodies present in the serum of all humans and launches an innate immune response. The hypothesis proposed was that the glycopeptides would function as adapters between the natural antibodies and the virus particles and/or virus infected cells. We were able to show that the CD4 derived-galα(1,3)gal-coupled glycopeptides could neutralize HIV-1 and that in the presence of human serum (containing the anti-gal antibodies), the infection was further inhibited owing to the activation of proteins of the complement system and to the role of NK cells in mediating lysis of infected cells (ADCC). This was first tested in in vitro assays using the T cell adapted virus HIV$_{IIIb}$ (paper I). Following these studies we tested the efficacy of the glycopeptides on patient isolates in the context of PBMC infection in an attempt to mimic more closely an in vivo situation (paper II). We used six different primary isolates, belonging to different subtypes of HIV-1 and also with different co-receptor specificity. We found that now ~100 times higher concentrations of the glycopeptides were needed to neutralize the different isolates and yet the inhibition was only modest. We therefore designed new glycopeptides with the aim of increasing their binding affinity and we also synthesized three glycopeptides corresponding to the extracellular regions of CCR5. Again the neutralization capacity of the single glycopeptides was not satisfactory but the combination of CD4 and CCR5-derived glycopeptides proved to be a useful strategy.

While in the process of testing the efficacy of the glycopeptides, we found that the human serum, used as a source of the anti-gal antibodies, increased the viral production in a dose dependent manner (paper III). This enhancement was reproducible in different cell lines, as well as, on freshly isolated PBMCs. We used two single replication assays, TZM-bl and ACH-2 cells, to try to dissect the step in the viral replication cycle that was affected by human serum. We showed that a protein (~ 250-300 kDa) was responsible for the enhancement of infectivity. It was found to activate three members of the AP-1 family of transcription factors, which in turn can promote viral transcription at the LTR level. Point mutations in the AP-1 binding sites of the LTR confirmed the specific role of these proteins in the human serum induced- enhanced replication of HIV-1. Further studies on the effect of human serum on in vitro cultured TZM-bl cells, showed also a dose dependent increase in CD4 expression that would impact viral infection and cytopathicity in these cells (paper IV). We showed that human serum influences the steady state levels of CD4 by increasing its recycling to the cell surface from early endosomes.