A computationally designed HIV neutralization molecule with significant glycosylation

John Saeger – July 1, 2016

The inspiration to design a new HIV microbicide came from the observation that lectins like griffithsin (Emau et al. 2007) have received significant interest as potential HIV microbicides where in principle, binding the HIV particle's putatively dense glycan coat with lectins should act to prevent infection, but my fairly naive understanding of HIV pathogenesis led me to ask a question relating to macrophages. Can't a macrophage eat just about anything? Could this not lead to a productive infection? It turns out that I wasn't the first person to ask this question and there is some suggestion that this indeed may be possible (Marechal et al. 2001). Is there anything we can do to discourage this mode of HIV entry?

What first occurred to me was simply to pegylate the lectin, a procedure that might result in a reduction of macropinocytosis (Qie et al. 2016). So the initial design idea was to get the lysines where we want them and functionalize the lectin. As I studied the glycobiology of HIV however, it quickly became apparent that the HIV glycan coat is variable (Pantophlet 2014). Although on the average, we know that the HIV glycan coat is significantly dense, we have no idea how variable the glycan coat is on an HIV particle to HIV particle basis. This led me to the thought that the glycan coat might not be a very reliable foundation on which to base a microbicide strategy. Furthermore, griffithsin and some other lectins that have been or are being considered as HIV neutralizers bind mannose moieties. What if these mannose moieties exist in the mucosa? Would not the neutralizer molecules bind inappropriate targets, rendering the neutralizer molecules useless before they even get a chance to bind HIV? That this is not an idle concern is supported by the observation that all female reproductive tract epithelia express the mucin MUC1 (Gipson et al. 1997), and MUC1 has N-linked glycosylation that results in a glycan coat which analysis has indicated may have mannose moieties (Parry 2006).

Thus we have two reasons for concluding that we might like something that binds HIV specifically. But how can we be the most rational about the design? The specificity of Env for the CCR5 coreceptor is the strongest phenotypic signature that has been associated with HIV-1 transmission to date (Pantophlet 2014). So we need to attach our neutralizing protein to Env in such a way that CCR5 binding is likely to be inhibited. Besides CCR5 utilization, the strongest phenotypic properties of Env linked to sexual transmission are shorter hypervariable domains and fewer potential N-linked glycosylation sites (Pantophlet 2014). This suggests that adding some N-linked glycosylation sites to our HIV binding molecule might be the right idea. We note that with respect to macropinocytosis, glycosylation may be similar enough to pegylation in discouraging it, in that both pegylation and glycosylation result in a largely hydrophilic "cloud" in the vicinity of the protein molecule.

The detailed design strategy is actually not very different from the strategy used for the recently described computationally designed influenza neutralizer (Koday et al. 2016). Find an accessible and conserved protein patch on HIV's Env protein and design a binding molecule to match. Such a protein patch may exist in the form of the so-called HIV fusion peptide (Kong et al. 2016). I realize that a glycosylated protein pushes the boundaries of what's been done so far in computational protein design, but it may not be such a huge leap. We need a very

stable platform on which to build the binding site so that the presence or absence of designed-in glycosylation will not significantly impact the binding. Then, the design may proceed in a similar fashion to what has been done previously. Constraining the space of design candidates to include potential N-linked glycosylation sites positioned on the protein surface away from the binding site should not be a huge deal. Other than that we may need to pay attention to pH as the target environment may be acidic.

To select the best binding site design I really like the yeast display method used previously (Koday et al. 2016). The final version of the glycosylated HIV neutralization protein can also be produced in yeast. Since we are working on a microbicide and not a treatment, the simple glycosylation that yeast can do by default may be sufficient, however it may be wise to consider humanized glycosylation (Laurent et al. 2016) from the beginning. For preliminary testing with HIV, assays using MT-4 cells may be used (Emau et al. 2007). More sophisticated testing with more sophisticated model systems may also be desireable (Hladik et al. 2007).

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