ACS MEETING

THE IMPORTANCE OF GLYCOFORM HETEROGENEITY

WASHINGTON, D.C.—It is becoming increasingly obvious that protein glycosylation patterns are important—if not vital—to their therapeutic efficacy. The oligosaccharides can influence a protein's clearance rate, its specific activity, and its immunogenicity. And the amount of microheterogeneity can be quite significant. But what is not yet totally obvious is how to control this variability.

Speaking at the American Chemical Society meeting here in late August, Stanford University's (Stanford, CA) Timothy J. Hahn emphasized how cell culture conditions can affect glycosylation patterns. He notes that, for human transferrin—an iron binding/transporting protein secreted by a hepatoma cell line—there are two N-linked glycosylation sites, and three main glycoforms (two, one, and no biantennary [branched] structures). But “the longer the cells grow, the more glycoforms appear.” The differences in processing are associated with the growth phase of the cells. Hahn and his colleagues hypothesize that this microheterogeneity results from up- and down-regulation of the level of competing glycosyltransferases.

Culture conditions also affect antibody glycosylation and pharmacokinetics, claims Cetus' (Emeryville, CA) Brian Maiorella. He and his collaborators have examined the pharmacokinetics of several human IgMs. They have measured the plasma concentration of antibody remaining in the circulation at various times after injecting antibodies into rat tail veins. The half-life is generally in the 8–13 hour range. But, he says, they found one unusual antibody, IgM1, that has a very rapid clearance, with a half-life of three minutes. “Is this rapid clearance an inherent property, or can it be influenced by culture conditions?”

To answer this question, the scientists grew the cells in serum-free medium in an airlift reactor, in a perfusion hollow-fiber bioreactor with one percent fetal bovine serum, or in nude mouse ascites fluid. They found “a tremendous difference in the clearance rates of IgM1 produced under different culture conditions...In ascites, the antibody has a half-life of 13 hours; from serum-supplemented perfusion culture, it has a half-life of 18 minutes, and from serum-free culture, it is three minutes.”

What about the glycosylation patterns? IgM is a large molecule with 21 peptide chains linked by 98 disulfide

bridges, and at least 50 glycosylation sites. There are five conserved glycosylation sites in the mu heavy chain constant region, and no sites in the light chain constant region. Oxford Glycosystems' (Abingdon, U.K.) analysis of the monosaccharides showed that the IgM1 produced in the airlift culture, like the control pooled serum IgM, has no neutral nonsialylated

structures, whereas the IgM1 produced in ascites fluid has 16 percent complex and neutral oligosaccharides. “The IgM1s produced in ascites and cell culture differ significantly with respect to their pharmacokinetics and oligosaccharide structure...however, we don't know if those differences are related,” concludes Maiorella. —Jennifer Van Brunt

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