physicochemical properties of an antibody molecule and consequently pharmacokinetics (Lim et al., 2008; Millward et al., 2008), solubility (Wu et al., 2010), aggregation, and so on. While V_H glycosylation of a human IgG antibody was shown to have the same pharmacokinetics as the V_H deglycosylated molecule in a mouse model (Lim et al., 2008), introduction of a glycosylation site within bispecific single-chain diabodies resulted in a significant increase in serum half-lives (Stork et al., 2008). An antibody with specificity for IL-13 was generated that included a glycosylation sequon (53NSS55) within the heavy chain CDR2 (Wu et al., 2010). Initially, this site was engineered out by replacing N53 with an aspartic acid residue; however, the product exhibited very limited solubility (~13 mg/mL) and consequent aggregation. By contrast, reintroduction of N53, together with engineering within V_L, resulted in a V_H glycosylated antibody product with a solubility >110 mg/mL (Wu et al., 2010).

4.21 CAVEAT: ONE CANNOT EXTRAPOLATE FROM *IN VITRO/EX VIVO* TO *IN VIVO* BIOLOGICAL ACTIVITIES

The impact of the expression/production platform on product characteristics has been emphasized in the foregoing; however, it should equally be emphasized that when reporting functional activities, the parameters of the assay system employed determine the results obtained. A clear example is the determination/reporting of complement mediated lysis and binding. Early studies employed hamster or rabbit serum as source, each being more stable than human complement. However, there are significant structural and functional nuances between these species' proteins. Another parameter is the epitope density expressed by the target cell (Rojko et al., 2014; Voice and Lachmann, 1997; Zhang et al., 1995). An important parameter in contemporary effector function studies is the source and/or expression of Fcγ receptors. Binding studies conducted, *in vitro*, have employed the soluble external domain of FcγR (sFcγR) produced in a variety of cell types, mostly sourced from commercial companies. The external domain of sFcγRIIIa bears five *N*-linked glycosylation sites, all occupied, one of which makes direct contacts with the IgG-Fc and determines binding affinity; therefore, the glycoform of the receptor is of equal importance. The impact of glycoform on binding to the other sFγR has not been reported. Similarly, when conducting ADCC studies, the FcγR expression level on the effector cell and the epitope density on the target cell and the sensitivity of the readout impact the apparent outcome. In the context of this chapter, it is relevant to include the following section emphasizing FcγR heterogeneities.

4.22 FcγR RECEPTORS MEDIATING ADCC AND/OR THE REMOVAL AND DESTRUCTION OF IgG/ANTIGEN IMMUNE COMPLEXES

Three types or classes of membrane bound human FcγR have been defined by immunochemical, biochemical, and gene sequencing studies: FcγRI [CD64], FcγRII [CD32], FcγRIII [CD16], with an additional six subtypes: FcγRIIA, FcγRIIB1, FcγRIIB2, FcγRIIC, FcγRIIIA, FcγRIIIB (Anthony et al., 2012; Cartron et al., 2002;