(Macdougall et al., 2012). Prior to patent expiry, the innovator company (Amgen) developed an improved EPO and obtained new patent protection. Improvement was achieved by the introduction of two extra *N*-linked glycosylation sites, resulting in increased sialylation and enhanced biological activity and *in vivo* half-life (Elliott et al., 2004; Sinclair, 2013). Interestingly, while EPO cells secreted from CHO cells is heterogeneously glycosylated when expressed as a transmembrane protein, on CHO cells, it is homogeneously glycosylated and fully sialylated; the membrane bound EPO can be released to yield a fully active EPO product (Singh et al., 2015).

The "hallmark" of an antibody is perceived to be its specificity for a target pathogen (antigen); however, protection against invading microorganisms requires activation of a cascade of downstream biological mechanisms, resulting in the killing and elimination of pathogenic organisms or pathologic targets. These biologic mechanisms are triggered following the binding of immune complexes (ICs) to one or more multiple soluble or cell-bound IgG-Fc effector molecules (Jefferis, 2012; Jefferis and Lefranc, 2009; Vidarsson et al., 2014). It is established that *N*-linked glycosylation of asparagine 297 (N297; Eu numbering) is essential for optimal activation of the effector molecules. Analysis of oligosaccharides released from normal polyclonal human IgG reveals a considerable heterogeneity of diantennary oligosaccharide structures. Similar analysis of monoclonal human IgG proteins, produced by neoplastic plasma cells (multiple myeloma), also reveals a restricted glycoform profile that is characteristic for each protein—that is, each malignant plasma cell population (patient).

The generation of homogeneous antibody glycoforms has allowed each to be evaluated for its functional profile, and these studies have informed the production of selective glycoforms tailored to the disease entity being addressed (Jefferis, 2012; Raju and Jordan, 2012; Raju and Lang, 2014). While the IgG-Fc glycoform can have a profound impact on function, sensitive physical studies have failed to demonstrate a comparable structural difference between different glycoforms. It is evident that each oligosaccharide structure has a subtle but unique impact on the tertiary/quaternary conformation of the IgG-Fc, and hence functions. Before discussing the functional consequences for individual IgG-Fc glycoform(s) of normal, myeloma, and recombinant IgG, an overview of IgG-Fc structure is appropriate.

4.12 QUATERNARY STRUCTURE OF IgG-Fc: THE PROTEIN MOIETY

The first crystal structure of IgG-Fc, resolved at 2.9-Å, was published by Deisenhofer (1981). It was reported that interpretable electron density was not obtained for residues 223–237, which comprise most of the core and lower hinge region, or the C-terminal residues 444–446. It was not known at this time that the C_H3 exon codes for a C-terminal (K, 447) lysine residue is removed by an endogenous serum carboxypeptidase B. Crystal structures have been reported for human, rabbit, and mouse IgG-Fc and chicken IgY-Fc fragments and for human IgG-Fc alone or in complex with Staphylococcal protein A (SpA), Streptococcal protein G (SpG), rheumatoid factor (RF), and soluble (s) recombinant forms of human sFcγRIIa, sFcγRIIIb, and sFcγRIIIa (Corper et al., 1997; Frank et al., 2014; Jefferis, 2012; Padlan, 1990; Radaev et al., 2001; Ramsland et al., 2011; Sauer-Eriksson et al., 1995;