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Research paper

Qualification of a homogeneous cell-based neonatal Fc receptor (FcRn) binding assay and its application to studies on Fc functionality of IgG-based therapeutics

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ABSTRACT

The Fc region of IgG-based molecules plays an important role in determining their in vivo pharmacokinetic profile by its pH-dependent binding to the neonatal Fc receptor (FcRn) which is expressed on the endothelial cells lining blood vessels. By virtue of this pH-specific interaction with IgG-Fc, FcRn mediates IgG homeostasis in human adults by maintaining serum IgG levels, and also transfers maternal IgGs from mother to fetus via the placenta. The Fc–FcRn interaction is also critical for keeping IgG-based therapeutic molecules in circulation thereby enhancing their serum half life. A homogeneous cell-based flow cytometric FcRn binding assay was established to characterize the Fc–FcRn interaction of therapeutic IgG-based molecules. It is a competition-based assay, wherein the IgG-Fc containing test molecule competes with a fixed concentration of fluorescently-labeled IgG-Fc moiety in solution for binding to the cell-expressed FcRn. The cell-bound fluorescence is read on a flow cytometer. Response of the test sample is analyzed relative to the standard sample and the results are reported as % relative binding. The assay is robust and meets the qualification criteria for specificity, method linearity, accuracy and precision over the relative binding range of 60%–160%. This assay was shown to effectively characterize altered Fc–FcRn interactions for photo-stressed, heat-stressed, oxidized, and Fc mutant samples. It was observed that the relative binding of the IgG-Fc to the cell-surface-expressed FcRn in the assay varies across different molecules, even within the same IgG subclass. This indicates that the Fc–FcRn binding can be influenced by the antigen-binding region of the molecules in addition to the IgG subclass. Overall, this assay is reflective of the in vivo mechanism of immunoglobulin binding to membrane-bound FcRn, and can be used as an analytical tool for assessing lot-to-lot consistency and stability testing across different batches of the same molecule. Additionally, the assay can be used as an effective tool for elucidating the amino acids in the IgG-Fc domain that are critical for FcRn binding and also for comparing the binding of different IgG-Fc containing molecules to FcRn.

1. Introduction

With the recent advances in genetic engineering and recombinant DNA techniques, significant amount of interest has been generated in the development of IgG-based therapeutics with improved properties. Accordingly, numerous therapeutic antibodies have been approved for human use and many more are in the pipeline under development (Chan and Carter, 2010). From monoclonal antibodies to Fc-fusion

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proteins (including peptibodies that are molecules with small peptides fused to Fc (Sutherland, 2004)), and now to the newer modalities like bispecific antibodies, the science of these molecules has advanced rapidly. These molecules are already being administered in humans for a variety of indications including cancer, inflammation and autoimmune diseases (Kim et al., 2005).

These IgG-based molecules typically have two functional domains: the Fab (fragment of antigen binding)/peptide region, and the Fc (fragment crystallizable) region. The Fab/peptide region is responsible for antigen recognition and is usually the "business" or target specific end of a therapeutic IgG molecule. The Fc region, depending on the subclass, may be involved in the immune effector function pathways against pathogenic cells wherein it binds to a specialized class of Fc receptors, FcγR, and/or some proteins of the complement system and induces pathogen-cell death. The Fc region is also known to bind to the neonatal Fc receptor (FcRn). The binding of the Fc domain of IgG-based molecules (both endogenous and therapeutic) to the FcRn is known to influence their in vivo pharmacokinetic profile by maintaining them in circulation for longer periods and thereby prolonging their serum half life (Raghavan and Bjorkman, 1996; Roopenian and Akilesh, 2007; Presta, 2008). Alterations in Fc binding affinity for FcRn have previously been shown to impact the pharmacokinetic profile and half life of human IgG antibodies. For instance, Dall'Acqua et al. (2006) showed that substitutions of three residues in the Fc region of anti-RSV IgG (MEDI-524 YTE) resulted in a 10-fold increase in its binding to both cynomolgus monkey and human FcRn at pH 6.0 and an approximately 4-fold increase in serum half life in cynomolgus monkeys when compared to the native non-engineered form of the antibody (MEDI-524). In another study by Zalevsky et al. (2010), two substitutions in the Fc domain (M428L/N434S) of bevacizumab led to an 11-fold improvement in FcRn affinity at pH 6.0 with a 3.5-fold increase in half life in cynomolgus monkeys. These studies demonstrate that enhanced FcRn binding has a strong correlation with the improved half life of an engineered therapeutic.

Structurally, FcRn is a heterodimer composed of a transmembrane α-chain homologous to major histocompatibility complex (MHC) class-I like molecules and a soluble light chain, β2 microglobulin. Typically, it is expressed on the surface of endothelial cells lining blood vessels (Roopenian and Akilesh, 2007). It binds to the interface between the C_H2 and the C_H3 domains of heavy chains in the Fc region of IgG molecules (distinct from the FcγR binding sites) under mildly acidic conditions (pH ~6–6.5) and releases the IgG at neutral-to-basic pH (~7–7.4). By this highly pH-dependent interaction, FcRn mediates IgG homeostasis in human adults by maintaining serum IgG levels, and also imparts humoral immunity to the newborn by transferring the maternal gamma globulins (IgGs) antenatally from mother to fetus via placenta (Leach et al., 1996; Simister et al., 1996). FcRn–Fc co-crystal structure has identified several key amino acids in the C_H2 and the C_H3 domains that are critical for this pH-dependent interaction (Burmeister et al., 1994; Martin et al., 2001; Kuo et al., 2010). The pH dependence is induced by the protonation of histidine residues (primarily at positions 310 and 435), located at the interface of C_H2 and C_H3 domains of the Fc region of IgG, at acidic pH that aides in the formation of salt bridges with acidic residues (Glutamic acid 117, Glutamic acid 132, Glutamic acid 135, Aspartic acid 137) on FcRn surface. Formation of these salt bridges is instrumental in stabilizing the hydrophobic interaction between Fc and FcRn at acidic pH. The hydrophobic isoleucine at position 253 of an IgG molecule is another residue that is critical to FcRn binding.

It is important to characterize the Fc–FcRn interaction of IgG-based therapeutics due to its potential impact on their in vivo pharmacokinetic profile. The pharmacokinetic profile (including the half life) of an IgG-based therapeutic is important in view of its direct impact on the drug administration regimen that is one of the key attributes defining the quality of the drug. Thus, it would be useful to establish a convenient, robust, accurate and precise FcRn binding assay that can ensure that the Fc domain of an IgG-based therapeutic is intact, functional, and can bind to FcRn. Such an assay could also potentially be useful to allow for the detection of any subtle structural changes that may occur in the Fc domain of an IgG-based therapeutic.

Non-cell-based assays using surface plasmon resonance (SPR) (Ober et al., 2001; Pan et al., 2009; Bertolotti-Ciarlet et al., 2009; Wang et al., 2011a,b) and AlphaScreen® technologies (Chamberlain et al., 2007 — patent) have routinely been used for studying the binding of the Fc domain of IgG molecules to the soluble form of FcRn. These methods evaluate the non-physiological presentation of FcRn in soluble form. A cell-based assay, wherein the Fc region of IgG binds to the cell-surface-expressed FcRn, is more appropriate for measuring FcRn binding as the cell-bound form closely represents the way FcRn is presented physiologically. In view of that, a few other formats utilizing engineered cell lines expressing human FcRn on the cell surface have also been reported in the literature (Ober et al., 2001; Hinton et al., 2004, 2006; Lu et al., 2011). However, most of the previously reported FcRn binding methods did not contain information addressing method qualification or validation.

To characterize the Fc–FcRn interaction of therapeutic IgG-based molecules for the purpose of assessing lot-to-lot consistency and stability of Fc domain, a homogeneous cell-based flow cytometric binding assay was established and qualified. It is a competition-based assay, wherein the Fc domain of an IgG test molecule competes with a fixed concentration of fluorescently-labeled Fc domain for binding to cell-expressed FcRn under mildly acidic conditions (pH 6). Cell-bound fluorescence is read on a flow cytometer. Response of the test sample is analyzed relative to a reference standard and the results are reported as % relative binding (%RelBind). The method was qualified for accuracy, precision, linearity and specificity as per the validation design described in the Guidelines of the International Conference on Harmonization (ICH) (Federal registers 60 and 62, 1995 and 1997), and was shown to be stability indicating and robust.

In this paper, we describe the robustness assessment and the qualification of the cell-based FcRn binding assay, and present a few key results that have implications in designing the IgG-based therapeutics. This assay characterizes the binding of the Fc domain of an IgG to the membrane-expressed FcRn similar to the in vivo setting and can be used as an effective analytical tool for comparing FcRn binding across different batches of the same product and also across different molecules.