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Research paper

Robust recombinant FcRn production in mammalian cells enabling oriented immobilization for IgG binding studies

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ABSTRACT

The MHC class-I related receptor or neonatal Fc receptor (FcRn) protects IgG and albumin from degradation by rescuing them in endothelial cells in a pH dependent fashion and consequently increases their respective half-lives. Monoclonal antibody-based therapies are of increasing interest and characterizing the interaction with FcRn is important for the development of an antibody candidate. In order to facilitate the production of soluble FcRn suitable for interaction studies, we generated semi-stable pools co-expressing FcRn α-chain, β2-microglobulin, biotin ligase and EGFP using a dual promoter, multi-cistronic vector. Human and mouse FcRn were purified in the mg/L range of culture medium and a single purification step was sufficient to reach a high level of purity. The receptors were characterized by ELISA, flow cytometry and surface plasmon resonance and shown to be functional. The single site biotinylation facilitated the directional immobilization of FcRn on the sensor chip and significantly increased the response level of the surface compared to amine coupling used in previous studies. Using this system, the affinity constants of seven IgGs, from various species and isotypes, were determined for human and mouse FcRn, including two hamster isotypes. These results confirm the higher selectivity of the human receptor and the promiscuous binding of mFcRn to IgGs from different species.

1. Introduction

The neonatal Fc receptor (FcRn) is a transmembrane heterodimer which comprises an MHC class-I like α heavy chain glycoprotein (FcRnα) non-covalently associated to soluble β2-microglobulin (β2m), with apparent molecular weights of 40–45 kDa and 14 kDa, respectively (Andersen et al., 2008a;Datta-Mannan et al., 2007;Leitner et al., 2006; Szlauer et al., 2009;Simister and Mostov, 1989). FcRn was first isolated in placental syncytiotrophoblasts where it is involved in the maternofetal transport of IgG from the mother to the fetus and confers short-term passive immunity (Andersen et al., 2008a;Antohe et al., 2001;Leach et al., 1996;Morphis and Gitlin, 1970;Rodewald and Kraehenbuhl, 1984;Simister and Mostov, 1989). Study of perinatal IgG transport in FcRnα or β2m deficient mice demonstrated the role of FcRn in immunity transfer from mother to newborn, deficient mice being unable to absorb IgG from maternal milk (Israel et al., 1995;Roopenian et al., 2003). This work also supports the fundamental role of β2m in association with the FcRn α-chain for the functionality of the receptor (Roopenian et al., 2003). FcRn is an important mediator of immunity in adults, particularly in the gut which is exposed to a large number of infectious agents (Baker et al., 2009). FcRn vehicles IgG across the intestinal epithelium into the lumen where binding of antigen to IgG can occur. The antigen–antibody complex can then be transported across the intestinal barrier for antigen presentation to APCs in the lamina propria (Mi et al., 2008;Yoshida et al., 2004). In addition, FcRn is crucial for the regulation of IgG

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and albumin serum levels as their binding to FcRn is pH dependent. Interaction occurs below pH 7.0 and albumin and IgG are released at physiological pH as the affinity of IgG for FcRn is more than 100-fold higher at pH 6.0 than at pH 7.4 (Raghavan et al., 1995). The acidic pH of the endosome allows IgG binding to FcRn and prevents IgG degradation in the lysosomal compartment. IgG are then released when the recycling endosome fuses with the cellular membrane due to the re-equilibration to neutral pH in the extracellular milieu. This salvage process protects IgG and albumin and increases their respective half-lives (Anderson et al., 2006;Baker et al., 2009; Chaudhury et al., 2003;Dall'Acqua et al., 2002). FcRn bears two independent binding sites for IgG and for albumin and can bind both proteins simultaneously (Chaudhury et al., 2003;Chaudhury et al., 2006). The importance of this endocytic rescue process is further supported by clinical observations of humans with deficient FcRn expression presenting low serum IgG levels due to their increased degradation (Wani et al., 2006).

FcRn homologues are present in several species including human, rat, mouse, cattle and chicken but their tissue distribution and specificity vary across species (Baker et al., 2009). For instance human FcRn binds to human, rabbit and guinea pig IgG but not to rat, bovine, sheep or mouse IgG (except mouse IgG2b) whereas mouse FcRn binds to all of these IgG (Baker et al., 2009;Ober et al., 2001). Differences in binding affinity constants were described for IgG subclasses from various species on human and mouse FcRn (Dall'Acqua et al., 2002;Mi et al., 2008;Wani et al., 2006;Yoshida et al., 2004).

Monoclonal antibodies (mAbs) have become an important and quickly expanding class of therapeutic molecules. A particularly attractive feature of mAbs is their long serum half-life of about 21 days (Keizer et al., 2010). Most mAbs currently in development are based on unmodified IgG formats of different isotypes. However, multiple strategies, such as the introduction of mutations into different regions of the Fc as well as modifications of glycosylation pattern, can be applied to modify the Fc receptor binding properties of a mAb (Presta, 2008). These approaches can be used to reduce or increase binding to different subsets of Fc receptors and modify effector functions as well as pharmacokinetic properties of mAbs by altering their binding properties to FcRn (Roopenian and Akilesh, 2007). Thus characterizing IgG binding to FcRn is important for the development of therapeutic mAb candidates (Datta-Mannan et al., 2007;Oganesyan et al., 2009;Yeung et al., 2009). As recombinant human FcRn is not commercially available, several methods using bacterial, yeast, insect and mammalian cells, have been described for the expression and purification of soluble FcRn (Andersen et al., 2008a;Dall'Acqua et al., 2002;Berntzen et al., 2005;Lee et al., 2010;Gastinel et al., 1992;Popov et al., 1996). Mammalian expression is preferred as it provides proper folding and post-translational modifications that are important for FcRn function but leads to relatively low expression yields.

In this study, we produced large amounts of FcRn using a semi-stable expression system in mammalian cells, leading to FcRn production in the mg/L range (Magistrelli et al., 2010). Full-length and soluble form of human and mouse FcRn were produced with a dual promoter vector for FcRnα and β2m co-expression. We also used the multicistronic design of this vector to co-express (i) the biotin ligase enzyme (LsBirA) for site specific in vivo biotinylation of soluble FcRn using the AviTag™ and (ii) the enhanced green fluorescent protein (EGFP) as a reporter gene to easily monitor the transfection process. A single chromatography step allowed the purification of FcRn from concentrated serum-containing supernatant. Functionality of FcRn and pH-dependence of FcRn–IgG interaction was confirmed by ELISA, flow cytometry and SPR. The purified FcRn complex could be easily immobilized on streptavidin-coated surfaces and showed increased functionality compared to direct immobilization methods. We determined the affinity constants for seven IgG on human and mouse FcRn: human IgG1 and IgG4, mouse IgG1 and IgG2b, rat IgG1, hamster IgG1 and IgG2.

2. Materials and methods

2.1. Construction of pEAK8 dual promoter EF1/SRα vector

The Srα promoter and polyA sequences were amplified by polymerase chain reaction (PCR) from the vector pSRα puro (30 cycles with 95 °C for 15 s, 56 °C for 20 s, and 68 °C for 75 s with a final extension for 7 min at 68 °C) using AccuPrime™ Pfx kit (Invitrogen, Basel, Switzerland). After a nested PCR to introduce the polylinker sites *BstX*I–*Nhe*I–*Xba*I *Kpn*I–*Sap*I–*Sca*I, an assembled PCR was performed. The resulting fragment was digested with *Spe*I and cloned into the original pEAK8 vector (Edge Biosystems, Gaithersburg, MD) digested with *Spe*I, dephosphorylated and purified on agarose gel.

2.2. Molecular cloning

The cDNAs encoding the full-length and the extracellular domain of human FcRn α-chain (GenBank accession no. NM004107, residues 1–297aa), mouse FcRn α-chain (GenBank accession no. BC003786, residues 1–297aa), human β2-microglobulin (GenBank accession no. NM004048, residues 1–119aa) and mouse β2-microglobulin (GenBank accession no. NM009735, residues 1–119aa) were amplified by PCR from cDNA libraries generated with mRNA isolated from peripheral blood mononuclear cells. After a subsequent PCR step, cDNAs were cloned into modified pEAK8 dual promoter vector, and a hexahistidine tag and a Biotin AviTag™ (Avidity, Denver, CO) were introduced at the C-terminus of the FcRnα coding sequence. Full-length and soluble forms of FcRn coding sequences were placed under the control of the EF1 promoter and β2-microglobulin under the control of the Srα promoter for the expression in mammalian cells. The FcRnα coding sequence was followed by a viral internal ribosome entry site (IRES) and a second or third cistron for the co-expression of the biotin ligase (BirA) and EGFP. The pEAK8 vector contains the puromycin resistance gene, the Epstein–Barr virus nuclear antigen 1 (EBNA-1) and the *oriP* origin of replication. EBNA-1 and *oriP* are required for propagation of the pEAK8 vector as episomal DNA in human cells and for generation of stable transfectants. All constructs were sequenced to ensure the absence of any undesirable mutation (Fasteris, Geneva, Switzerland).