Methods xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Humanized FcRn mouse models for evaluating pharmacokinetics of human IgG antibodies

Gabriele Proetzel *, Derry C. Roopenian

The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Neonatal Fc receptor
Humanized mice
Monoclonal antibody
Serum half-life
Pharmacokinetics
Efficacy

ABSTRACT

A key element for the successful development of novel therapeutic antibodies is to fully understand their pharmacokinetic and pharmacodynamic behavior before performing clinical trials. While many in vitro modeling approaches exist, these simply cannot substitute for data obtained from appropriate animal models. It was established quite early that the unusual long serum half-life of immunoglobulin G's (IgGs) and Fc domains are due to their rescue and recycling by the neonatal Fc receptor (FcRn). The diverse roles of FcRn became apparent after isolation and cloning. Interesting are the significant species differences between rodent and human FcRn reactivity, rendering wild type rodents an inadequate model for studying IgG serum half-life. With the advance of genetic engineering mouse models have been established expressing human FcRn, and lacking mouse FcRn protein. These models have become highly relevant tools for serum half-life analysis of Fc-containing compounds.

1. Introduction

Paul Ehrlich created the term of a "magic bullet" to illustrate how a therapeutic compound would specifically target and eliminate a disease-causing agent. With the breakthroughs of hybridoma technology and monoclonal antibody (mAb) development in 1975 [1], antibodies with their target-specific binding specificities could now be envisioned as treatment for of a wide range of diseases, potentially realizing the idea of a magic bullet. This vision is being increasingly realized by the current success of therapeutic mAbs, with over thirty FDA approved mAbs, spanning a range of disorders, including cancer, autoimmune disorders, infectious diseases, neurodegenerative diseases, macular degeneration, osteoporosis and transplant rejection.

Compared to other serum proteins, mAbs are unique in several features, sharing a common structural framework, while exhibiting a unique epitope specific binding site. Compared to other serum proteins, mAbs are unique in several features, sharing a common structural framework, while exhibiting a unique epitope specific binding site. The Fab (fragment of antigen binding) fragment confers high antigen specificity while the Fragment crystallizable (Fc) region equips immunoglobulin G (IgG) with three key features: (a) immune effector functions, (b) persistence in circulation and (c) transport across cellular barriers. The overall versatility of the IgG framework is immense and open to engineering approaches. With cloning and the ease of genetic engineering property of mAb can now be optimized in line with its anticipated use. The phenomenon of long IgG half-life in circulation was investigated early on. In 1965, Spiegelberg and Weigle found that IgG molecules serum half-life was dependent on the presence of the Fc fragment [2]. The half-life of IgGs is typically 7–22 days, while other antibody classes like IgM, IgA, IgD and IgE half-life is shorter, between 2 and 6 days [3]. All other serum proteins, with the exemption of serum albumin, have a very short serum half-life in the range of only minutes to hours. This remarkable serum half-life of IgG was hypothesized to be the result of a receptor that engages the Fc fragment rescuing IgG from catabolic elimination. This putative Fc receptor eventually proved to be the neonatal Fc receptor (FcRn) which was isolated from rat in 1989 [4]. However, as the name implies, FcRn was first characterized in the context of its transporter functions. It was known that IgG is readily transported across the materno-fetal barrier transfer providing the newborn with passive immunity before its own immune system develops [5,6]. In rodents, but not humans, FcRn additionally transports IgG from maternal colostrum across the neonatal intestine [7]. It was only later that FcRn was shown to be operative throughout

Abbreviations: B2M, beta-2-microglobulin; EGFR, epidermal growth factor receptor; ES, embryonic stem; Fab, fragment of antigen binding; Fc, fragment crystallizable; FCGRT, fc receptor IgG alpha chain transporter; FcRn, neonatal Fc receptor; HSA, human serum albumin; Ig, immunoglobulin; IP, intraperitoneal; IV, intravenous; mAb, monoclonal antibody; MDCK, madin–darby canine kidney; MHC, major histocompatibility complex; MSA, mouse serum albumin; p, probability value; PK, pharmacokinetics; VEGF, vascular endothelial growth factor.

* Corresponding author.
E-mail addresses: gproetzel@earthlink.net, Gabriele.Proetzel@jax.org (G. Proetzel), Derry.Roopenian@jax.org (D.C. Roopenian).

1046-2023/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ymeth.2013.07.005

lifespan, being responsible for the extended serum half-life of IgG and also serum albumin, and for the transport across endothelial and epithelial barriers, increasing the overall bioavailability of IgG and serum albumin [8–12]. The cellular trafficking mechanisms by which FcRn rescues, transports and recycles IgG is based on pH-dependent interactions and has been reviewed in detail and are not further described here [9,10,13–16].

FcRn forms a heterodimer consisting of the alpha-chain and beta-2-microglobulin (B2M) light chain. The alpha chain, also referred to as heavy chain, is a major histocompatibility complex (MHC) class I-like molecule, with the official gene name the "Fc receptor, IgG, alpha chain transporter" (FCGRT). As is common for all MHC class I proteins, FCGRT must complex with B2M light chain to exit from the endoplasmic reticulum, and for efficient pH-dependent binding of IgG [17]. B2M is ubiquitously expressed and associates with the alpha chain of all conventional MHC class I molecules, and MHC class I-like molecules including CD1, Azgp1 (alpha-2-glycoprotein 1, zinc-binding), Procr (protein C receptor, endothelial) and HFE (hemochromatosis) protein. B2M is a highly conserved protein with amino acid identity between human and mouse of 68.1% (81/119 amino acids). Highest conservation is detected in the functional domains, e.g. transmembrane helices (95% identical) and cytoplasmic loops (93% identical), indicating that B2M may function across species and may be capable to heterodimerize with alpha chain proteins from other species.

While in silico and in vitro modeling is of great value in early stage drug development, alone it is not necessarily predictive of pharmacokinetic (PK) behavior nor is it sufficient to select the best candidates for further clinical development [18]. Especially, in the case of therapeutic mAbs PK analysis is quite complex, requiring careful analysis of a multitude of factors. Such factors comprise of the overall protein structure, glycosylation status, post-translational modifications, isoelectric point (pI), immunogenicity, FcRn binding affinity and interactions with the antigen target; each and all of which are important, potentially confounding factors. Absorption, distribution, metabolism and excretion of administered antibodies are all critical considerations and require in vivo testing. In general, the mouse is a well-established and preferred model for in vivo validation, disease modeling and preclinical pharmacokinetic (PK) analysis. Factors in its favor include ease of handling, cost, availability of numerous disease models, and accessibility of genetically defined animals.

However, conventional inbred or outbred mice, have proven to be inadequate in studying the PK of human Fc-based compounds. In 2001, Ober et al. described significant species differences for FcRn which greatly impact model selection for preclinical testing and development of mAbs [19]. They compared the binding affinities between mouse and human by immobilizing IgG from different species (human, mouse, rat, rabbit, guinea pig, bovine and sheep) to CM5 sensor chips in a Biacore assay, and injected purified human or mouse recombinant FcRn proteins at pH 6.0. In these assays mouse FcRn binds to all IgGs tested, including human and mouse IgG1, while human FcRn shows no effective binding to mouse or rat IgG1. Human FcRn binds besides human IgG1, only rabbit IgG and guinea pig IgG2. Recently, Andersen et al. confirmed these observed differences between rodent and human FcRn using a complementary Biacore assay where FcRn was immobilized and IgG was injected. In addition, Andersen et al. also demonstrated a cross-species difference for albumin binding to the FcRn receptor [20]. Species differences between rodent and primate FcRn receptor are therefore highly relevant in preclinical evaluation of human mAbs and other humanized Fc-containing as well as albumin-based compounds in rodent models.

We have addressed these differences by developing a series of humanized transgenic mouse models designed to study the PK of human Fc-based compounds. These mouse lines are transgenic for human FCGRT and in addition engineered for a deletion in mouse Fcgrt, i.e. they do not express mouse FcRn, but express human FcRn protein. This collection of transgenic lines express human FcRn either under the control of a ubiquitous CAG promoter or human regulatory elements of FcRn [21,22]. The applications and considerations in the use of these humanized mouse models are discussed below.

2. Materials and methods

2.1. Mice

Mice described here are available from The Jackson Laboratory, Bar Harbor, Maine, USA.

B6.Cg-Fcgrt<tm1Dcr> Tg(CAG-FCGRT)276Dcr/DcrJ (Tg276 mice), stock number 004919, express a human FCGRT cDNA under the control of the ubiquitous CAG promoter,

B6.Cg-Fcgrt<tm1Dcr> Tg(FCGRT)32Dcr/DcrJ (Tg32 mice), stock number 0014565, carry a human FCGRT gene by insertion of a 33 kb cosmid clone containing the complete FCGRT gene of approximately 11 kb, as well as 10 kb of 5′ and 3′ flanking sequences [22–24].

B6.Cg-Rag1<tm1Mom> Fcgrt<tm1Dcr >Tg(CAG-FCGRT)276Dcr/DcrJ; (Tg276-Rag1-null mice), stock number 016919, developed by backcrossing Tg276 mice to Rag1-null mice (stock number 002216).

B6.Cg-Fcgrt<tm1Dcr> Prkdc<scid> Tg(CAG-FCGRT)276Dcr/DcrJ (Tg276-SCID mice), stock number 021146, developed by backcrossing Tg276 mice to B6.CB17-Prkdc<scid>/SzJ mice (stock number 001913).

B6.Cg-Fcgrt<tm1Dcr> Prkdc<scid> Tg(FCGRT)32Dcr/DcrJ (Tg32-SCID mice), stock number 018441, developed by backcrossing Tg276 mice to B6.CB17-Prkdc<scid>/SzJ mice (stock number 001913).

B6.Cg-Fcgrt<tm1Dcr> Tg(FCGRT)32Dcr Tg(B2M)55Hpl/Dcr (Tg32/hB2M) mice were created by intercrossing Tg32 mice with hB2M transgenic mice. The hB2M transgenic mice are described in Krimpenfort et al. [25].

As controls wild type C57BL/6J mice (abbreviated as B6), stock number 000664 and B6.129X1-Fcgrt<tm1Dcr>/DcrJ (FcRn-null mice), stock number 003982 were used [21], see also Table 1.

For in vivo studies performed at The Jackson Laboratory, all mice were maintained under specific pathogen-free conditions

Table 1
Humanized FcRn mouse models.

Stock number	Mouse strain name	Short name	References
003982	B6.129X1-Fcgrt<tm1Dcr>/DcrJ	FcRn-null	[21]
004919	B6.Cg-Fcgrt<tm1Dcr> Tg(CAG-FCGRT)276Dcr/DcrJ	Tg276	[22–24,27]
014565	B6.Cg-Fcgrt<tm1Dcr> Tg(FCGRT)32Dcr/DcrJ	Tg32	[22–24]
016919	B6.Cg-Rag1<tm1Mom> Fcgrt<tm1Dcr>Tg(CAG-FCGRT)276Dcr/DcrJ	Tg276-Rag1-null	[27]
021146	B6.Cg-Fcgrt<tm1Dcr> Prkdc<scid>Tg(CAG-FCGRT)276Dcr/DcrJ	Tg276-SCID	
018441	B6.Cg-Fcgrt<tm1Dcr> Prkdc<scid>Tg(FCGRT)32Dcr/DcrJ	Tg32-SCID	