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Fc-FUSION PROTEINS; STRUCTURAL MODIFICATIONS AND HORMONAL THERAPY

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ABSTRACT
In the recent years, IgG-based therapeutics and antibodies have gained significant importance for the treatment of a wide range of diseases, including autoimmunity and inflammation, cancers, cardiovascular and infectious diseases and transplantation medicine. Approximately 370 IgG-based therapeutics are approved for clinical use or are under development for many diseases lacking proper treatment options. On the other hand, the Fraction crystallizable (Fc) -based drugs demand additional considerations because interactions between the Fc domain and its receptors have immunological consequences. These interactions raise concerns about the long-term use of these products, which are often employed to treat chronic conditions. Degradation of recombinant proteins in culture is a common and serious problem. Degradation of most proteins, including recombinant monoclonal antibodies and fusion proteins, may be attributed to host cell-derived proteases, which may be very site specific or have a broad substrate range. Heterodimeric Fc fragments, which can present the fusion partner as native-like monomeric or heterodimeric forms, represent a promising scaffold for the next generation of Fc-fused proteins and cytokines. This review article encompasses the challanges for better potential candidates for therapeutics using Fc fusion technology.

KEYWORDS: Fc fusion, antibodies, heterodimeric, protein, therapeutics.

INTRODUCTION
Ever since the development of chimeric, humanized and more recently human antibodies together with antibody engineering technologies has substantially improved the pharmacokinetic, pharmacodynamic and immunologic properties of this class of therapeutics, making IgG-based therapeutics nowadays a highly potent, specific and generally well-tolerated therapeutic approach. IgG based biologic agents also benefit from widely utilized simplified and scalable purification procedures. As such, therapeutic agents that are molecularly engineered to include an Fc-domain i.e Fc fusion proteins or mAbs that are modified to exhibit increased affinity toward FcRn confer upon such agents the benefits of improved pharmacokinetics and potentially pharmacodynamics without compromising the specificity of the therapeutic moiety.[1-2] There has been advancement in the usage of Fc fusion drugs because of monomeric and heterodimeric congeners now available. This review evaluates the recent advances and the challanges posed by using this technology.

Fundamental biology of FcRn-dependent IgG homeostasis
The size of IgG molecules are approximately 150 kDa and composed of two identical light chains and heavy chains. IgG is the most abundant immunoglobulin in the humans and together with albumin accounts for nearly 80% of the protein plasma mass.[3] Remarkably, these two biologically unrelated molecules share an extended plasma half-life of 19–22 days, which by far exceeds the typical half-life of a few minutes to a few days for other known human plasma proteins. Intimately related to the extended half-life of both proteins is a common natural pathway that revolves around FcRn. Functioning as a single broadly distributed and, contrary to what is suggested by its name, life long expressed Fc receptor, it serves as the key homeostatic regulator in this process and thereby exhibits a profound relevance for the basic physiologic processes and plasma homeostasis of these two major proteins.[2]

Improving half lives and impact of post translational changes: The mean and median half-life values for approved therapeutic antibodies are about 12–13 days, about a week short of the approximate values of 21 days. Nevertheless, most of the Fc fusion proteins generated to date have half-life values in humans of approximately 4–5 days. The important exception is abatacept, which has a half-life of about 13–16 days.[4] Belatacept, which has a structure that differs from abatacept by only two amino acid residues, has an average half-life of about 8–9

days.[5] (Refer Table 1). The shorter-half-life values for Fc fusion proteins may be due to lower affinity to FcRn or the higher-order structure provided by Fab arms of normal antibodies. Post translational alterations for e.g glycosylation can modulate the immunogenicity, efficacy, solubility and pharmacokinetic behavior of biopharmaceuticals and was extensively reviewed.[6] Multiple relations were reported between N-glycosylation and the therapeutic efficacy and immunogenicity of therapeutic proteins, while much less is known about the influence of O-glycans mainly attributed to their in homogenous chemical nature.[7] The glycosylations macro- and micro heterogeneity of proteins can significantly influence product efficacy and immunogenicity and is highly dependent on cell culture and manufacturing conditions and thus, the glycosylation of biopharmaceuticals presents an important quality and safety parameter.

Preparations of monoclonal antibodies

The advent of hybridoma technology allowed the production of monoclonal antibody (mAb) that could be utilized for targeted therapy, owing to its increased specificity towards a target antigen. mAbs are primarily composed of two major functional units.[8] One is the crystallizable fragment (Fc), and the other is the fragment of antigen binding (Fab). Fab unit contains three hypervariable complementarity determining regions (CDRs), which together form the antigen binding site on the surface of the antibody. The major issue with murine mAbs is their low half-life and immunogenicity, despite achieving specific targeting.[9] In chimeric antibodies, the murine variable domain is fused with the human constant domain. There are different ways by which humanized antibodies are created. One of the most common methods is to graft mouse CDRs onto human acceptor antibody framework. Fully human antibodies, on the other hand, are either naturally obtained, recombinantly expressed, or collected from transgenic mouse, in which the immune repertoire has been replaced by the human genes. Another effective way of producing human antibody is phage display.[10]

General problems faced in anti-body based therapy

Antibodies, like other proteins, are not very stable relative to small molecule drugs; for example, mAbs possess potential for self-association or aggregation.[11] Because of the relatively high dose required for efficacy, they are often prepared at an extremely high concentration, which further enhances self-association, and as a consequence, may lead to high viscosity and difficulty in handling/processing. In addition, their large size and surface-exposed functional groups are a major source of several chemical degradation pathways. These are only a subset of the challenges currently encountered in the development of antibody therapeutics. Studies in mice and human FcgR transgenic mice identified the involvement of various activating FcgRs and the innate immune cells such as the macrophages, monocytes, neutro-phils, basophils, and mast cells in inflammatory diseases and systemic hyperacute anaphylaxis.[12] Administration of therapeutic mAbs carries the risk of immune reactions such as acute anaphylaxis. Fc-fusion drugs can interact with innate cells expressing FcgRs, but there are almost no reports of acute anaphylactic reactions.[13]

Mechanism of action of Anti-body: The primary mode of action for mAb-based therapeutics for the treatment of cancer has been considered to be antibody derived cell-mediated cytotoxicity (ADCC). According to this mechanism, the Fc portion interacts with FCγ-receptors on the surface of the effector immune cells for e.g monocyte and macrophages, while the antibody is bound to the surface antigen of the cancer cell through its Fab region, and results in ADCC response.[14-15] For example, cetuximab, which binds to the epidermal growth factor receptor (EGFR) antigen on the surface of the tumor cells, is known to mediate its anti-tumor activity through ADCC.[16] Another important mode of action is direct interference of specific cell signaling pathways. For example, bevacizumab binds to the soluble vascular endothelial growth factor (VEGF) and thus inhibits angiogenesis, which is important for the maintenance of tumor vasculature and tumor health.[17-18] There are also other mechanisms that are worth mentioning, including induction of phagocytosis, complement activation, and activation of T-cell immunity.

Immunogenecity to Fc fusion drugs

Immunogenicity to therapeutic mAbs and Fc-fusion drugs is mostly triggered by a humoral immune response dependent on CD4 T helper cells and mediated by B cells with crucial involvement of the professional antigen-presenting cells e.g., dendritic cells (DCs) and macrophages. These cells express the activating FcgRI and FcgRIIA and the inhibitory FcgRIIB. Exploiting Fc biology may also offer strategies to engineer the Fc partner to achieve desirable properties in the drug.[19] Examples of such rational protein design include engineering both the active moiety and the Fc partner of the CTLA4–Fc drug to improve functional out-comes and using an anti-IgE fused to a Fc domain that was mutated to enhance targeting to the inhibitory FcgRIIB, to block basophil activation and re-lease of allergy mediators.[20-21]

Novel hormone congeners with Fc Fusion proteins: The rhGH-Fc is a novel candidate for long-acting rhGH therapy with more convenient weekly administration, as it reduces glomerular filtration and receptor-mediated clearance while allowing for the rapid reversal of potential adverse events.[22] The effect of two injections of rhGH-Fc separated by 1 week was comparable to that of the same dose of 14 daily injections of rhGH.[23] The invention relates to fusion proteins of heterodimeric follicle stimulating hormone (FSH) where in the alpha and beta subunits of FSH are each conjugated to an FcRn binding partner or to an Fc fragment. In one embodiment, the invention provides fusion proteins

having two polypeptide chains, one chain having at least αFSH, linked directly or indirectly through an optional linker to an Fc fragment of an immunoglobulin, and the second chain having β FSH, also linked directly or indirectly through an optional linker to an Fc fragment of an immunoglobulin. By way of these fusion proteins, the invention provides methods for increasing the half-life of FSH and, therefore, further provides an effective means for increasing a subject's fertility with reduced dosing frequency and/or treating a disease state responsive to FSH therapy. FcRn can facilitate bidirectional passage of Fc-bearing molecules across mucosal surfaces and can reach the circulation when given orally or by inhalation. Successful delivery of Fc-fusion proteins via the respiratory tract by inhalation has been described for erythropoietin. FcRn-mediated delivery via the oral route of Fc– follicle-stimulating hormone (FSH) in rats led to an increase in ovarian and testicular weight in females and males respectively and prolonged the half-life of FSH compared with unfused FSH. Fc-bearing molecules given systemically (iv) can reach mucosal surfaces and provide effective therapy.[24] Endogenous peptides like the Atrial natriuretic peptide (ANP) may be a useful molecule for the treatment of cardiovascular diseases due to its potent natriuretic effects. In an effort to prolong the short in vivo half-life of ANP, fusions of the peptide to the Fc domain of IgG have been generated using a semisynthetic methodology. Synthetic ANP peptides were synthesized with thioesters at either the N- or C-termini of the peptide and subsequently linked to the N-terminus of recombinantly expressed Fc using native chemical ligation.[25] The pharmacokinetics of several constructs were evaluated in experimental rats, and the half-life of the ANP-Fc's were found to be approximately 2 orders of magnitude longer than that of the unconjugated peptide. These novel ANP-Fc fusion constructs hold promise for future therapeutic application in the treatment of cardiovascular diseases. Heterodimeric Fc fragments can also be a good scaffold to harber the ANP peptide. In subsequent studies using cynomolgus monkeys, pulmonary delivery of a human Epo Fc-fusion protein (EpoFc) as a dimer or as a monomer was successfully accomplished when the respective fusion proteins were aerosolized with a particle size of 4–6 mm to target the upper airways.[26] In both mice and monkeys, the FcRn-dependence of this transepithelial transport was confirmed by demonstrating that an EpoFc-fusion, which was mutated in three amino acids in the Fc domain critical for FcRn binding (I253A, H310A and H435A), was only poorly absorbed when administered either to the upper airways or deep lung. Experimental confirmation that this pathway functions similarly in humans was obtained from the successful completion of a phase I clinical trial assessing the efficacy of delivery of an EpoFc-fusion protein into the bloodstream of normal healthy volunteers in which transport of the EpoFc-fusion proteins in the upper airways was shown to occur in a dose-dependent fashion and induce a reticulocytosis.[27]

CONCLUSIONS

The Fc-fusion platform technology has had an impressive track record in terms of generating successful drug products. Nevertheless, there continues to be considerable potential for extending the serum half-life of Fc fusion proteins. In recent times there are attempts to engineer the Fc partner for enhanced binding to FcRn are already underway both in experimental systems and in drug-development programs. Apart from this, Fc biology is fostering the development of therapies using Fc proteins alone. Translating these concepts and results into Fc-fusion drugs rationally designed for specific functional outcomes require validated assays, reagents, and animal models for proof-of-concept and pre-clinical studies. Fc–FcR interactions are species specific and experimental systems must use either chimeric fusion proteins with the species-specific Fc or 'humanized' mice with the appropriate receptors. Recent progress in basic science bodes well for the development of a new generation of Fc fusion protein therapies and this has significant potential.

Table 1: Monoclonal antibodies and years of regulatory approvals

Year	Brand Name	Molecule name	Target Indication	Primary Indication	Antibody type
1986	Orthoclone	Muromonab	CD 3	Acute Transplant rejection	Murine IgG2a
1997	Rituxan	Rituximab	CD 20	Non-Hodgkin's Lymphoma	Chimeric IgG 1
1998	Enbrel	Etanercept	TNF-receptor	Rheumatoid Arthritis	TNFR2Fc fusion
2002	Humira	Adalimumab	TNF alpha	Rheumatoid Arthritis	Human IgG1

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