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Review

Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases

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ABSTRACT

Monoclonal antibodies are being developed as therapeutics to complement drugs and vaccines or to fill the gap where no drugs or vaccines exist. These therapeutic antibodies (ThAb) may be especially important for infectious diseases in which there is antibiotic resistance, toxin-mediated pathogenesis, or for emerging pathogens. The unique structure of antibodies determines the specific nature of the effector function, so when developing ThAb, the desired effector functions need to be considered and integrated into the design and development processes to ensure maximum efficacy and safety. Antibody subclass is a critical consideration, but it is noteworthy that almost all ThAb that are licenced or currently in development utilise an IgG1 backbone. This review outlines the major structural properties that vary across subclasses, how these properties affect functional immunity, and discusses the various approaches used to study subclass responses to infectious diseases. We also review the factors associated with the selection of antibody subclasses when designing ThAb and highlight circumstances where different subclass properties might be beneficial when applied to particular infectious diseases. These approaches are critical to the future design of ThAb and to the study of naturally-acquired and vaccine-induced immunity.

1. Introduction

Emil von Behring's discovery of anti-toxins to diphtheria, tetanus and anthrax in 1901 eventually led to the discovery of antibodies (Gronski et al., 1991). Over a century later, the use of therapeutic antibodies (ThAb) has become one of the fastest growing areas of the pharmaceutical industry, yet ironically, the development of monoclonal ThAb against infectious diseases has been slow compared to most other fields. There remains a significant knowledge gap in identifying the roles for ThAb against specific infectious diseases, and also the exact molecular properties that are required to ensure ThAb induce the desired effector functions, but avoid unwanted responses. It is well known that the main isotypes of human immunoglobulins have unique structural features that allow them to perform specific immune effector functions (Fig. 1) (Nezlin, 1998). In this review, we focus on IgG and its subclasses and highlight the complex structure-function relationship that is critical to designing safer and more effective monoclonal ThAb (Table S1). We place this knowledge into the context of infectious diseases and highlight how studies should now evaluate the best IgG subclasses or molecular properties required for effective treatment of particular infectious diseases.

A wide repertoire of monoclonal ThAb are currently licenced with hundreds more in pre-clinical and clinical development (The Antibody Society, 2014; Wu et al., 2014). These ThAb are administered for a wide range of conditions, although the vast majority are used for cancer, autoimmune disorders and transplantation (Fig. 2A and Table S2). It is interesting to note that despite the clear role antibodies play against many infections,

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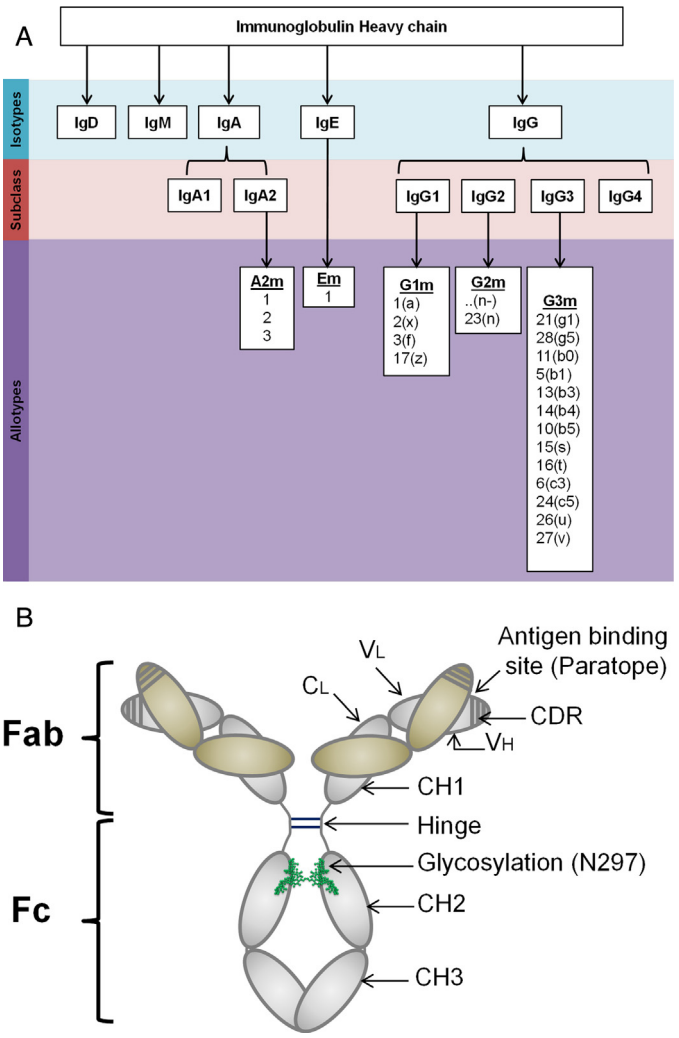

Fig. 1. Antibody structure and nomenclature. (A) The potential isotypes, subclasses and allotypes of immunoglobulins in humans. Both the letter and number code are provided for each allotype (Jefferis and Lefranc, 2009). (B) Schematic depicting the basic structure of an antibody and associated nomenclature. The light chain (shown in brown) consists of a variable (VL) and a constant (CL) domain while the heavy chain (shown in grey) consists of a variable (VH) domain and three constant domains (CH1, CH2 and CH3). Interchain disulfide bonds within the hinge region stabilise overall antibody structure. The complementarity determining regions (CDR, shown as striped lines) determine antigen specificity. The glycosylation patterns can also affect function (shown in green).

there are only two licenced monoclonal ThAb that target infectious agents (Palivizumab against human Respiratory Syncytial Virus and Raxibacumab against *Bacillus anthracis*) (Table 1). The majority of licenced ThAb are full length rather than Fab fragments (Fig. 2B). The benefits of using full length ThAb include longer serum half-life as a result of interaction with FcRn, improved effector function *via* engagement with a range of Fc receptors, and in some cases, more effective neutralisation when compared to the corresponding Fab fragment (Abboud et al., 2010; Bournazos et al., 2014; DiLillo et al., 2014; Halper-Stromberg et al., 2014). Most of the approved ThAb are either humanised or fully human IgG antibodies (Figs. 2C and S1). Murine or chimeric antibodies carry an increased risk of adverse anti-murine reactions in patients, and there has been a general move towards using a fully human scaffold in the development of potential ThAb. While using intact IgG allows for the selection of antibody IgG subclass to elicit specific effector functions, this is not reflected in the current range of licenced antibodies in which the majority are IgG1 (Fig. 2D and Table S2). This is likely due to IgG1 displaying potent effector functions, being the most predominant serum subclass, and was the backbone used in early approved ThAb. IgG2 or IgG4 has been used when a lack of specific cellular activity is desirable. Interestingly, there are no approved IgG3 ThAb, with suggestions that this may be because of (i) an increased likelihood for proteolysis due to an extensive hinge region (Carter, 2006), (ii) the many IgG3 allotypes across populations, (iii) IgG3 cannot be purified with protein A, or (iv) the reduced serum half-life of IgG3 compared to other subclasses (Table S1). In this review, we provide a concise overview of the known human IgG effector and subclass properties (Section 2) and discuss how this information could be used when designing ThAb to infectious diseases (Section 3).

2. Molecular properties of IgG subclasses relevant to therapeutic antibodies

IgG consists of two heavy chains and two light chains with the main molecular features described in Fig. 1B (Liu and May, 2012; Padlan, 1994; Ramsland and Farrugia, 2002). Within IgG, the fragment antigen binding (Fab) region contains the paratope, and can exert direct effects through binding interactions with antigen (*e.g.*, blocking a host recognition protein or inhibiting a toxin/enzyme of a pathogen). Meanwhile, the fragment crystallisable (Fc) region interacts with a variety of accessory molecules to mediate indirect effector functions such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) (Peipp et al., 2008) (Fig. 3A and B). These Fc mediated effector functions are especially important against infectious diseases where cellular and complement mediated responses are important for efficient pathogen clearance.

The structural and functional properties of the IgG subclasses vary, as do their response profiles to different infectious diseases, and these differences can be utilised in the development of effective ThAb (Carter, 2006; Jefferis, 2012). Although the heavy chains share greater than 90% sequence identity across IgG subclasses (Rispens and Vidarsson, 2014), there are differences in surface-exposed residues on the constant (CH1, CH2 and CH3) domains, as well as substantial variation within the hinge region (Fig.3C, D and Table S1). It is the hinge structure that confers many of the unique properties to each IgG subclass such as stability, flexibility and distances spanned by the two Fabs and the attendant Fc (Liu and May, 2012; Roux et al., 1997; Tian et al., 2014). Importantly, some areas of the Fc and the hinge that differ between IgG subclasses clearly overlap with residues known to be involved with binding to both activating and inhibitory Fcγ receptors (FcγR), the neonatal receptor for IgG (FcRn) and complement component C1q (Fig. 3A and B). The occurrence of key amino acid differences within the binding sites of these effector molecules helps explain the observed differences in the effector properties of the IgG subclasses (Table S1). This structural and molecular information is important when choosing a subclass backbone for a therapeutic antibody or introducing changes in key amino acids to tailor antibodies for a specific purpose.

2.1. Binding site on IgG-Fc for activating and inhibitory FcγRs

The Fc of IgG interacts with several cellular FcγRs to stimulate and regulate downstream effector mechanisms (Guilliams et al., 2014). There are five activating receptors, namely FcγRI (CD64), FcγRIIa (CD32a), FcγRIIc (CD32c), FcγRIIIa (CD16a) and FcγRIIIb (CD16b), and one inhibitory receptor FcγRIIb (CD32b) (Hogarth and Pietersz, 2012; Pincetic et al., 2014). IgG subclasses vary in their ability to bind to FcγR and this differential binding determines

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