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A new look at a poorly immunogenic neutralization epitope on cytomegalovirus glycoprotein B. Is there cause for antigen redesign?

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ABSTRACT

The immune response is able to control cytomegalovirus infection in most subjects. However, in some patient groups the virus is not well contained resulting in disease and severe morbidity. The development of efficacious vaccines is therefore a high priority. Antibodies may contribute to protection against disease caused by CMV but the most efficient targets for protective humoral immunity are not precisely known. Glycoprotein B (gB) is a protein that is targeted by virus-neutralizing antibodies. One epitope on gB, AD-2, is poorly immunogenic following natural infection and vaccination. It is consequently not effectively exploited as a target for antibodies by the immune system. However, antibodies specific for this epitope, when they develop, display important functional activities that may play a role in protection against infection. In this study critical features of human antibody recognition of this epitope are re-assessed based on structural and immunochemical data. The analysis suggests that the immune system may only be able to develop an AD-2 specific antibody response through rare, very specific rearrangement events that by chance create a naïve B cell that can be recruited into an AD-2 specific immune response. These results reinvigorate the notion that if we are to be able to effectively exploit AD-2 specific humoral immunity we need to readdress the nature of the antigen incorporated into vaccines so as to more effectively recruit B cells into the response against this epitope.

1. Introduction

Cytomegalovirus (CMV) in an opportunistic pathogen that causes severe morbidity in some patient cohorts. Thus, although the virus is well contained in most immunocompetent individuals it represents a major challenge in immunodeficient individuals and in neonatally infected children. In fact, a substantial part of human immunity is directed towards this viral pathogen (Kern et al., 2002) but the virus has also developed multiple strategies to evade eradication by the immune system (Wiertz et al., 1997). A vaccine that could evoke effective immunity protecting subjects at risk of disease would be very beneficial as CMV-induced malignancies very substantially reduce the quality of life of affected individuals and incur substantial costs to society. Several efforts to develop efficacious vaccines are therefore in progress but they have yet to deliver a product for use in everyday clinical practice (Sung and Schleiss, 2010). To facilitate development of an appropriate vaccine it is critical to understand the nature of the most beneficial immune responses (Krause et al., 2013) and to be able to mount such immunity rapidly and in titres sufficient to limit the infection even in subjects whose immune system is not operating at its full potential.

Although T cell immunity is an important factor that limits the consequences of CMV infection, antibodies may also act on the virus and neutralize its infectious properties (Nigro and Adler, 2013). Much of the early work of CMV-neutralizing antibodies was conducted on fibroblast cells, a fact that may have over-emphasised the importance of certain antibody specificities and missed others that are critical for protection against infection of other cell types such as epithelial and endothelial cells (Gerna et al., 2008). The fact that CMV infects different cell types and may exploit different entry pathways represents a major challenge for vaccine development, as antibody-mediated protection may require induction of antibodies that address different entry routs of infection. Numerous viral proteins are now known to be important for CMV infection, including for instance glycoprotein B (gB), gH, gL, gM, gN as well as UL128/130/131. In summary, this virus represents a target that poses immense difficulties if immunity is to in part rely on efficacious virus-neutralizing antibodies.

Abbreviations: AD-2, antigenic domain; CDR, complementarity determining region(s); CMV, cytomegalovirus; gB, glycoprotein B; H, heavy; L, light.

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Glycoprotein B (gB) was early on identified as a major target for virus neutralizing antibodies (Britt, 1984). However, not all antibodies targeting gB, including those that target its immunodominant epitope, are effective neutralizers of infection resulting in incomplete virus neutralization by polyclonal antibody populations (Speckner et al., 1999).

Antigenic domain, AD-2, on gB was defined more than two decades ago (Meyer et al., 1992) using a human monoclonal antibody, C23 (Matsumoto et al., 1986). Antibodies targeting this epitope, an epitope that is largely conserved (Chou, 1992) in clinical isolates of the virus, are able to neutralize viral infection of both fibroblasts and epithelial cells (Macagno et al., 2010). Interestingly, an AD-2-specific antibody, ITC88 (Ohlin et al., 1993), has also been shown to mediate other functions as well, as it is able to prevent early stages of the anti-apoptotic effect that allows CMV-infected cells to survive after infection (Reeves et al., 2012). Antibodies against gB AD-2 thus have multiple effects that may make them particularly interesting as components of CMV immune responses. Altogether it is of importance to assess the humoral immune response to gB and to focus the ability of a gB-based vaccine so as to mount antibodies that can make a difference in terms of virus-neutralization and protection against disease.

Immunity to AD-2 will not develop effectively following infection. Only a fraction of naturally CMV-seropositive individuals develop such antibodies and those that produce them do so with delayed kinetics (Schoppel et al., 1997) and often at low titres, possible even below the concentration that is able to neutralize infection of fibroblasts in vitro by 50% (Ohlin et al., 1997). Furthermore, experimental vaccines based on the Towne strain or recombinant gB were not very effective in inducing antibodies against gB AD-2 (Axelsson et al., 2007). The immune system thus faces a particular challenge when it comes to development of AD-2 specific humoral immunity.

Others and we have put substantial efforts into developing and studying human antibodies against CMV gB AD-2. To date, seven independently developed sets of antibodies have been described, and sequence information is available to describe, in molecular detail, six of these antibody sets. Importantly, Thomson et al. (2008) published and analyzed a first structure illustrating the interaction of one such antibody (8F9) with the glycoprotein as represented by an oligopeptide. Just recently, a structure of another human antibody (KE5) in complex with this peptide, established by the same research team, became publically accessible (PDB: 4HHA). The availability of two such structures now prompts me to revise old data in attempts to develop our understanding of the human immune response to this potentially important target on human CMV. Such knowledge provides the foundation for an appropriate decision-making process relevant for future gB-based vaccine designs.

2. Methods

The heavy (H) and light (L) chain encoding gene sequences (Supplementary Table 1) of AD-2 specific antibodies were obtained from GenBank. Gene sequence analysis was performed using the IMGT V-QUEST web tool (http://imgt.org/IMGT_vquest/vquest) (Brochet et al., 2008). Coordinate files describing crystal structures of an AD-2 peptide in complex with antibody fragments of human IgG antibodies 8F9 (PDB: 3EYF) (Thomson et al., 2008) and KE5 (PDB: 4HHA) were downloaded from the RCSB Protein Data Bank (http://www.pdb.org). The unit cell of the structure of 8F9 contains two antibody fragment-peptide complexes that are treated separately (referred to as ABE and CDF, respectively). Structures were visualized and analyzed using the MacPyMOL Molecular Graphics System software, Version 1.3 (Schrödinger, LLC, Cambridge, MA, USA). Amino acid residues are numbered in accordance with the IMGT sequence numbering system (Lefranc, 2011). Standard IUPAC one-letter abbreviations of amino acid names are used throughout this text.

3. Results and discussion

3.1. Sequence similarity between antibodies that bind the AD-2 epitope on CMV gB

Past efforts defined sequence similarity within a set of human AD-2 specific antibodies with origin in three clonally different rearrangements (McLean et al., 2005). Three additional independent rearrangement have recently been described (Macagno et al., 2010; Kauvar et al., 2011) but not extensively analyzed in this context. In total six clonally different rearrangements are thus available for analysis of the nature of AD-2 specific binders. These sequences are indeed all very similar (Fig. 1). These similarities include a common origin of the H and L chain gene rearrangement in the IGHV3-30 (or the closely related IGHV3-30-3) germline gene and the IGKV3-11 germline gene, respectively. Furthermore, the L chain gene rearrangements all encode L chain complementarity determining region (CDR3) with a length of 10 residues, a length that is found in only about one fifth of kappa L chain sequences (Tomlinson et al., 1995). All sequences carry conserved mutations in at least one of two adjacent residues (mostly SS ≥ GG) in CDR1 of the L chain. The CDR3 of the H chain, a sequence often critical for antibody specificity, have a length of either 16 or 18 residues, lengths that are found in only about 8% and 6% of human antibody rearrangements, respectively (Wu et al., 2012). The H chain CDR3 also carry highly conserved sequence motifs in both its N and C-terminal parts, as further outlined in Section 3.6. Altogether specific sequence features appear to define antibodies targeting AD-2 of CMV gB, several of which, as shown in Section 3.7, are not encoded in the germline itself. As the epitope is also poorly immunogenic it is hypothesized that there might be only a single major path towards development of a binder targeting this epitope following natural infection in vivo, but also that the immune system experiences great difficulties in following this path.

3.2. Polar interactions define AD-2 peptide interaction

With access to two structures of independently rearranged antibody fragments in complex with a peptide, one of which has been described in great detail by Thomson et al., (2008), it is now possible to look for common principles in antibody recognition of this epitope. In both structures, multiple polar interactions appear to be formed between the AD-2 peptide and the paratopes of both 8F9 and KE5. Furthermore several of these proposed polar interactions are conserved between the structures. Of eight conserved interactions, six occur between polypeptide backbone atoms of the H chain CDR3 and the antigen while two occur between atoms of conserved antibody side chains (R107 and W113) of L chain CDR3 and the antigen (Table 1).

3.3. Critical antigen side chains in close proximity to paratope

Structural analysis may be supported by the specificity profile of antibodies, for instance as determined by epitope scanning mutagenesis. Mapping of the AD-2 specificity as represented by a monoclonal antibody (ITC88) and by antibodies found in polyclonal sera obtained from naturally seroconverted individuals has been performed using peptides modified by alanine scanning. Four residues of gB (Y72, T75, L76 and Y78) have thus been pinpointed as critical for the antibody–gB interaction of polyclonal sera as well as of the human monoclonal antibody ITC88 (Axelsson et al., 2007;