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Research paper

Fucose removal from complex-type oligosaccharide enhances the antibody-dependent cellular cytotoxicity of single-gene-encoded antibody comprising a single-chain antibody linked the antibody constant region

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Abstract

Fucose removal from complex-type oligosaccharide of human IgG_1-type antibody results in a great enhancement of antibody-dependent cellular cytotoxicity (ADCC). The aim of this study was to clarify the effect of fucose removal on effector functions of a single-gene-encoded antibody with an scFv used as the binding domain. We generated both a fucose-negative anti-tumor associated glycoprotein (TAG)-72 scFv-Fc using α-1,6-fucosyltransferase knock-out CHO cells and a highly fucosylated scFv-Fc from parental CHO cells. Expression, assembly and antigen binding activity of the scFv-Fcs were not influenced by fucose removal. The scFv-Fc lacking fucose exhibited significantly more potent FcγRIIIa binding and ADCC compared to highly fucosylated scFv-Fc. These results prove that ADCC enhancement by fucose-removal is effective in not only whole IgG_1, but also scFv-Fc, and thus increases the potential of Fc-fusion proteins as therapeutic candidates.

Keywords: Antibody-dependent cellular cytotoxicity; Fc receptor; Single-chain Fv; Fc-fusion

1. Introduction

Antibody-dependent cellular cytotoxicity (ADCC) is a lytic attack on antibody-targeted cells that is triggered by the binding of lymphocyte receptors (FcγRs) to the antibody constant region (Fc). ADCC is considered to be one of the major effector functions of therapeutic antibodies.

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; Fv, variable domains of immunoglobulin; scFv, single-chain Fv; VH, variable domain of immunoglobulin heavy chain; VL, variable domain of immunoglobulin light chain; Fc, constant domain including hinge, second constant domain CH2 and third constant domain CH3 of immunoglobulin; TAG-72, tumor-associated glycoprotein-72.

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FcγRIIIa, the FcγR predominantly expressed on natural killer cells and responsible for ADCC activation, has two isoforms, 158Val and 158Phe. The FcγRIIIa/158V allele shows higher binding affinity for IgG_1 antibody compared to the FcγRIIIa/158F isoform (Wu et al., 1997; Shields et al., 2001). Importantly, Cartron et al. (2002) have reported recently that the anti-CD20 chimeric IgG_1 antibody Rituxan was more effective for follicular non-Hodgkin lymphoma patients with FcγRIIIa/158Val than patients with FcγRIIIa/158Phe. Similar results have been reported by Anolik et al. (2003) for Phase I/II trials of Rituxan treatment of systemic lupus erythematosus. These reports underscore the importance of ADCC of IgG_1 in the clinic and highlight the therapeutic value of enhancing ADCC effector function.

Several groups have reported that ADCC enhancement can be achieved by manipulating human IgG_1 subclass antibody oligosaccharides. ADCC requires the presence of oligosaccharides in the Fc region and is sensitive to change in the oligosaccharide structure (Nose and Wigzell, 1983; Wright and Morrison, 1997; Jefferis et al., 1998). One IgG molecule contains two asparagine (*N*)-linked oligosaccharide sites in its Fc region (Rademacher et al., 1986). The general structure of IgG *N*-linked oligosaccharides is complex-type, characterized by a mannosylchitobiose core with or without bisecting *N*-acetylglucosamine (GlcNac)/L-fucose and other chain variants including the presence or absence of galactose and sialic acid. Among all of the sugar components in the oligosaccharide, galactose (Kumpel et al., 1994, 1995), bisecting-GlcNac (Umana et al., 1999; Davies et al., 2001), and fucose (Shields et al., 2002; Shinkawa et al., 2003) have been reported to affect ADCC.

We have clarified previously the critical importance of fucose among these sugar components. Removal of fucose from humanized anti-interleukin 5 receptor antibody or chimeric anti-CD20 antibody enhanced their ADCC >50-fold (Shinkawa et al., 2003). Compared with fucose manipulation, the role of bisecting-GlcNac in ADCC was minimal, and galactose modifications did not contribute significantly to ADCC enhancements (Shinkawa et al., 2003). The influence of non-fucosylated oligosaccharide on ADCC has also been reported by Shields et al. (2002) using humanized anti-HER2 IgG_1 and humanized anti-IgE IgG_1. They showed that the reduction of fucose on IgG_1s improved both ADCC and binding to FcγRIIIa. Fucose removal from human IgG_1-type antibody is, thus far, one of the most powerful ways to improve antibody effector function. Further, we have demonstrated the superiority of antibodies with enhanced ADCC in vivo. Low fucose chimeric anti-CCR4 IgG_1 antibody showed significantly higher anti-tumor activity than the highly fucosylated antibody in a murine xenograft model employing a CC chemokine receptor 4 (CCR4)-positive T-cell lymphoma and human peripheral blood mononuclear cells (PBMCs) (Niwa et al., 2004).

Some cell lines, such as YB2/0 (Shinkawa et al., 2003), had been used to produce IgG molecules with reduced fucose by 30–90%. To generate a new host cell producing a homogeneous product with optimal ADCC enhancement and more appropriate for large-scale manufacture, we disrupted both *FUT8* alleles in a Chinese hamster ovary (CHO) cell line by sequential homologous recombination (Yamane-Ohnuki et al., 2004). This cell line is incapable of adding fucose to antibodies as *FUT8* encodes an α-1,6-fucosyltransferase that is the only enzyme to catalyze the transfer of fucose from GDP-fucose to *N*-acetylglucosamine (GlcNac) in an α-1,6 linkage. Importantly, from a manufacturing standpoint, the $FUT8^{-/-}$ cell lines (CHO/$FUT8^{-/-}$) have morphology and growth kinetics similar to those of the parent, and produce completely fucose-negative recombinant antibodies.

Several groups reported that Fc-fusion molecules, which consist of target binding domains and Fc portion (hinge, CH2 and CH3 domain) of human IgG antibody, have ADCC activity (Shu et al., 1993; Cooper et al., 2003; Heuser et al., 2003; Lorenzo et al., 2004). Single-gene-encoded scFv-Fc is a type of Fc-fusions which contains single-chain Fv (scFv) as a target binding domain (Fig. 1). scFvs are the Fvs designed as single-peptide molecules which consists of the variable region of antibody heavy chain (VH), the variable region of antibody light chain (VL) and peptide-linker. Because of its small size (110 kDa), scFv-Fc molecules are considered to have higher penetration into bulky tumors than whole IgG molecules (150 kDa). Another merit of scFv-Fcs is that they retain immune effector functions mediated by Fc domains. For example, Shu et