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Research paper

Characterization of in vitro antibody-dependent cell-mediated cytotoxicity activity of therapeutic antibodies — Impact of effector cells

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ABSTRACT

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an important mechanism of action implicated in the clinical efficacy of several therapeutic antibodies. In vitro ADCC assays employing effector cells capable of inducing lysis of target cells bound by antibodies are routinely performed to support the research and development of therapeutic antibodies. ADCC assays are commonly performed using peripheral blood mononuclear cells (PBMCs), natural killer (NK) cells or engineered cell lines as effector cells. In this study we evaluated the impact of different effector cell types including primary PBMCs, primary NK cells, engineered NK cell lines, and an engineered reporter cell line, on the in vitro ADCC activity of two glycoforms of a humanized IgG1 antibody. The results of this study show the differential effects on both the efficacy and potency of the antibodies by different effector cells and the finding that both the allotype and the expression level of CD16a affect the potency of effector cells in ADCC assays. Our results also show that engineered NK or reporter cell lines provide reduced variability compared to primary effector cells for in vitro ADCC assays.
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1. Introduction

Monoclonal antibodies (mAbs) are a rapidly growing class of therapeutics, with more than 30 mAbs approved worldwide for the treatment of various diseases, including cancer and autoimmune diseases. The entire clinical pipeline currently includes ~350 mAbs and novel molecules are entering clinical studies at a rate of nearly 40 per year (Nelson et al., 2010; Reichert, 2013). Properly designed in vitro assays are valuable tools that are routinely used to support various research and development activities of mAb therapeutics.

Therapeutic antibodies have been shown to improve overall survival as well as time to disease progression in a variety of human malignancies, including breast, colon and hematological cancers (Griggs and Zinkewich-Peotti, 2009; Shuptrine et al., 2012). One of the mechanisms used by

Abbreviations: AbX, antibody with mostly fucosylated glycans; AbX-AF, antibody with completely afucosylated glycans; ADCC, antibody-dependent cell-mediated cytotoxicity; AICC, antibody-independent cell-mediated cytotoxicity; CV, coefficient of variation; EC_{50}, half maximal effective concentration; ET ratio, effector cell:target cell ratio; FACS, fluorescence activated cell sorting; FBS, fetal bovine serum; Fc, fragment crystallizable; FC, flow cell; FcγR, Fcγ receptor; FF, homozygous CD16a-F158; IgG1, immunoglobulin G1; k_a, association rate constant; k_d, dissociation rate constant; K_D, dissociation equilibrium constant; mAbs, monoclonal antibodies; MEM, minimum essential medium; NK cell, natural killer cell; PBMC, peripheral blood mononuclear cell; PE, phycoerythrin; RFU, relative fluorescence unit; RPMI 1640 medium, Roswell Park Memorial Institute 1640 medium; RT, room temperature; RU, response unit; SPR, surface plasmon resonance; VF, heterozygous CD16a-V/F158; VV, homozygous CD16a-V158.

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antibodies to kill tumor cells is antibody-dependent cell-mediated cytotoxicity (ADCC). In ADCC, an antibody first binds to its antigen on target cells and then the Fc (fragment crystallizable) portion interacts with FcγR (Fcγ receptor) on effector cells. Binding of FcγR to Fc activates the effector cells which subsequently release cytotoxic agents such as perforin and granzymes leading to lysis of target cells (Chavez-Galan et al., 2009). Natural killer (NK) cells expressing CD16a (a.k.a. FcγRIIIa) which is an activating receptor member of the FcγR family are one of the primary effector cells mediating ADCC activity (Moretta et al., 2002). Human FcγR including additional activating receptors such as CD64 (FcγRI) and CD32a (FcγRIIa), as well as the inhibitory receptor CD32b (FcγRIIb) are widely expressed on a range of immune cell populations, including B cells, dendritic cells, macrophages, mast cells, NK cells and neutrophils (Caligiuri, 2008; Nimmerjahn and Ravetch, 2008). By binding to the antibody Fc region, FcγRs provide a link between the specificity of the adaptive immune system and powerful effector functions such as ADCC triggered by innate immune effector cells.

Genetic analyses of CD16a polymorphisms in cancer patients have demonstrated that ADCC is one of the major mechanisms responsible for the clinical efficacy of several therapeutic antibodies (Cartron et al., 2002; Rodríguez et al., 2012). In CD16a, a point mutation at nucleotide 559 results in the substitution of valine (V) by phenylalanine (F) at amino acid 158. The CD16a-V158 allotype has a higher affinity for IgG1, IgG3 and IgG4 than the CD16a-F158 allotype (Koene et al., 1997). Given the differences in binding affinities, patients carrying homozygous CD16a-V158 would be expected to mount a more vigorous ADCC antitumor response upon mAb treatment. This is indeed demonstrated for a number of therapeutic antibodies, that patients carrying the high-affinity CD16a allotype (CD16a-V158) achieved superior clinical responses compared to those carrying the low-affinity heterozygous CD16a-V/F158 or homozygous CD16a-F158 genotype (Cartron et al., 2002; Weng and Levy, 2003; Rodríguez et al., 2012). Thus, the importance of ADCC for the clinical efficacy of therapeutic antibodies is now widely recognized.

Since ADCC is a pivotal mechanism in the anti-cancer activities of many therapeutic mAbs, increasing ADCC activity is often a goal in developing therapeutic antibodies for oncology indications. The commonly used approach to improve the ADCC activity of antibody therapeutics is to improve the CD16a binding activity through either modification of Fc primary sequences involved in interaction with CD16a (Shields et al., 2001; Lazar et al., 2006; Zalevsky et al., 2009) or the removal of core-fucose in the Fc N-glycan by glycoengineering (Shields et al., 2002; Yamane-Ohnuki et al., 2004; Umana et al., 1999). By improving the binding affinity of antibodies for CD16a, which is the sole Fcγ receptor expressed on most NK cells, both approaches result in enhanced ADCC activity of the antibodies.

Effector functions, including ADCC are routinely characterized and monitored during the research and development of therapeutic antibodies. In vitro ADCC assays are frequently performed to support screening of candidate molecules for desired ADCC activity and to provide biological characterization of clinical candidate for regulatory filings (Jiang et al., 2011; Desjarlais et al., 2007). An effective in vitro ADCC assay requires biologically relevant target cells expressing the antigen of interest at their surface, as well as effector cells that can induce lysis of target cells bound to the test antibody. The lysis of antibody-bound target cells can be measured using various detection methods based on either spectrophotometry or flow cytometry. Whereas freshly prepared PBMCs and NK cells are the most commonly used effector cells in ADCC assays, recent advances in cell engineering have led to development of NK cell lines and reporter cell lines suitable for use in such assays to measure lysis of target cells or alternatively to measure transcriptional activation leading to ADCC (Binyamin et al., 2008; Schnueriger et al., 2011; Clemenceau et al., 2006; Parekh et al., 2012). Different effector cells may behave differently in the same assay system due to differences in the biological properties and expression profiles of different activating and inhibitory receptors. As a result, ADCC activity of antibodies could vary greatly depending on effector cells used in the assay.

In this study, we evaluated the impact of different effector cells expressing varying CD16a allotypes on in vitro ADCC activity of therapeutic antibodies. Two glycoforms of a humanized anti-CD20 IgG1 antibody, one with mostly fucosylated glycans (AbX) and the other with completely afucosylated glycans (AbX-AF), were tested for CD16a binding and ADCC activity using different effector cells including PBMCs, NK cells, engineered NK cell lines, and an engineered reporter cell line. The results show differential effects on both the efficacy and potency of antibodies by different effector cells. They also confirm that both the allotype and expression level of CD16a impact the potency of effector cells in ADCC assays. Our results also show that engineered NK or reporter cell lines can provide reduced variability compared to primary effector cells for in vitro ADCC assays.

2. Material and methods

2.1. Test antibodies and cell lines

AbX is a humanized anti-CD20 antibody based on IgG1 heavy chains and kappa light chains. AbX was produced from the CHO DP12 cell line and contained greater than 98% fucosylated N-glycans. AbX-AF, a completely afucosylated AbX, was produced from a CHO cell line deficient of the alpha-(1,6)-fucosyltransferase (FUT8). Both materials showed comparable physicochemical properties including purity, solubility and low levels of high mannose glycoform or aggregation (data not shown). Additional samples were generated by mixing AbX with AbX-AF; the resulting sample set contains 2%, 5%, 10%, 20%, 50%, and 75% of the AbX-AF with AbX added to make up the final content.

WIL2-S, a human B-lymphoblastoid cell line, was obtained from the American Type Culture Collection (Manassas, VA). Cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen; Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Hyclone; Logan, Utah), 100 U/mL penicillin, 100 μg/mL streptomycin and 292 mg/mL L-glutamine.

NKL and YT-CD16 cells (Deaglio et al., 2002) were cultured in RPMI-1640 medium with 10% heat-inactivated FBS, 50 mg/mL gentamicin, 100 U/mL penicillin and 100 μg/mL streptomycin (all from Invitrogen). The NKL cells required the addition of human recombinant interleukin (IL) 2 (200 IU/mL;

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