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ORIGINAL ARTICLE

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Recombinant human monoclonal HLA antibodies of different IgG subclasses recognising the same epitope: Excellent tools to study differential effects of donor-specific antibodies

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In the field of transplantation, the humoural immune response against mismatched HLA antigens of the donor is associated with inferior graft survival, but not in every patient. Donor-specific HLA antibodies (DSA) of different immunoglobulin G (IgG) subclasses may have differential effects on the transplanted organ. Recombinant technology allows for the generation of IgG subclasses of a human monoclonal antibody (mAb), while retaining its epitope specificity. In order to enable studies on the biological function of IgG subclass HLA antibodies, we used recombinant technology to generate recombinant human HLA mAbs from established heterohybridomas. We generated all four IgG subclasses of a human HLA class I and class II mAb and showed that the different subclasses had a comparable affinity, normal human Fc glycosylation, and retained HLA epitope specificity. For both mAbs, the IgG1 and IgG3 isotypes were capable of binding complement component 3d (C3d) and efficient in complement-dependent cell lysis against their specific targets, while the IgG2 and IgG4 subclasses were not able to induce cytotoxicity. Considering the fact that the antibody-binding site and properties

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; BCM, background corrected mean fluorescence intensity; CDC, complement-dependent cytotoxicity; C1q, complement component 1q; C3d, complement component 3d; DNA, deoxyribonucleic acid; DSA, donor-specific antibodies; ELISA, enzyme-linked immunosorbent assay; FcγR, Fc gamma receptors; HLA, human leukocyte antigen; IgG, immunoglobulin G; IgM, immunoglobulin M; IL, interleukin; LB, Lunia-Bertani; mAbs, monoclonal antibodies; MFI, mean fluorescence intensity; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PHA, phytohemagglutinin; RACE, rapid amplification cDNA ends; RNA, ribonucleic acid; SAB, single antigen beads; VH, heavy chain variable domain; VL, light chain variable domain.

remained unaffected, these IgG subclass HLA mAbs are excellent tools to study the function of individual IgG subclass HLA class I and class II-specific antibodies in a controlled fashion.

KEYWORDS

affinity, glycosylation, human leukocyte antigen, IgG subclass, monoclonal antibody, transplantation

1 | INTRODUCTION

In the field of transplantation, the induction of a humoural immune response to mismatched HLA antigens on the donor kidney is associated with graft rejection and inferior graft survival, but only in a subpopulation of patients.[1-3] The various clinical effects may be caused by the (mixture of) immunoglobulin G (IgG) subclass of produced donor-specific antibodies (DSA).[4-7] Indeed, various patterns of IgG subclasses have been observed in sera of transplanted patients that developed *de novo* DSA. However, their relative contribution to graft damage remains elusive, due to conflicting results on their clinical significance.[7,8]

The pathogenicity of an HLA antibody is determined by both the affinity for the HLA epitope recognised by the Fab part and the effector function of the antibody, defined by the Fc part. Indeed, the degree of complement activation and the binding capacity to Fc gamma receptors (FcγR) differs per IgG subclass.[9-11] In renal transplantation, DSA capable of complement activation, for example, IgG1 and IgG3, are associated with allograft loss.[7,12-14] However, other studies have implied that the presence of IgG2 and IgG4 can act either synergistically or inhibitory on complement activation, depending on the epitopes recognised.[15,16] Additionally, HLA IgG antibodies have been associated with graft damage independent of the complement cascade.[17-19] Binding of DSA to endothelial cells can lead to infiltration of macrophages causing antibody-mediated rejection, of which the severity is increased in case of IgG1 and IgG3 antibodies, due to their capacity to bind FcγR.[19] Furthermore, binding and crosslinking of HLA targets on endothelial cells can result in intracellular signalling, resulting in cell proliferation and initiation of coagulation.[18,20,21]

Thus, understanding the underlying mechanisms of IgG HLA antibody-mediated graft damage can contribute to the establishment of risk factors associated with antibody-mediated rejection. Several methodological studies on the effect of HLA antibodies in renal transplantation have been performed using human HLA monoclonal antibodies (mAbs).[16,19,22-25] However, these studies are restricted to the available human HLA mAbs, which are mainly of the IgG1 subclass. Therefore, we adapted a method to recombinantly generate and produce human mAbs of all four IgG subclasses, with the aim to generate HLA class I and class II-specific mAbs of all IgG subclasses recognising the same HLA epitope with the same affinity.

2 | MATERIALS AND METHODS

2.1 | B-cell heterohybridomas

Human B-cell heterohybridomas WIM8E5 (IgG1, κ) and RTLK1E2 (IgG1, κ), that had been established from two women who had been immunised during pregnancy by mismatched *HLA-A*11:01* and *HLA-DRB1*03:01*, respectively, were used to generate recombinant human HLA class I and class II-specific mAbs.[25] Heterohybridoma cells were cultured in Iscove's modified Dulbecco's medium supplemented with 100 μ/mL penicillin, 100 μg/mL streptomycin, 10% foetal bovine serum, 200 mM L-glutamine (all Gibco, Invitrogen, Paisley, UK), 50 μM 2 mercapto-ethanol (Sigma-Aldrich, Zwijndrecht, the Netherlands).

2.2 | Generation of human recombinant IgG1 HLA mAbs

RNA was isolated from heterohybridoma cells using RNeasy mini kit (Qiagen, Hilden, Germany). Next, SMART cDNA synthesis was performed using PrimeScript Reverse Transcriptase (Takara, Saint-Germain-en-Laye, France), and variable heavy chain (VH) IgG1, and variable light chain (VL) kappa (κ) or lambda (λ) gene products were amplified by 5′-RACE polymerase chain reaction (PCR). The VH and VL PCR products were purified with QIAquick gel extraction kit (Qiagen) and treated with T4 DNA polymerase (Bioké, Leiden, the Netherlands). Subsequently, the VH and VL products were ligation-independently cloned into pcDNA3.3 expression vectors[26] with the constant domains of the human IgG1 (*IGHG1*03*), κ (IGKC), or λ (*IGLC2*01*). The vectors were used for transformation of One Shot MAX Efficiency DH5α-T1R competent cells (ThermoFisher Scientific, Waltham, Massachusetts) by heat shock. The transformed cells were cultured on LB-agar plates supplemented with 50 μg/mL ampicillin (Sigma-Aldrich) and after overnight incubation at 37°C, multiple single colonies were picked and grown overnight in LB

medium containing ampicillin. From the cultures, plasmids were isolated using either QIAprep Spin Miniprep kit (Qiagen) or NucleoBond Xtra Midi EF (Bioké). The plasmids were sequenced by Sanger sequencing (Macrogen, Amsterdam, the Netherlands) to verify the hybridisation of VH and VL products with the expression vector. All kits were used according to manufacturer's instructions.

2.3 | Generation of human recombinant IgG subclass HLA mAbs

To generate recombinant IgG subclass HLA mAbs, the IgG1 plasmid was double digested with the appropriate restriction enzymes (Bioké). Simultaneously, pMK vectors containing IgG2 (*IGHG2*02*), IgG3 (*IGHG3*01*), or IgG4 (*IGHG4*01*) constant domains (ThermoFisher Scientific) were double digested the same way to obtain the constant domains. Next, the IgG subclass constant domain was ligated with the digested vector by T4 DNA ligase (Bioké). Subsequently, plasmids were generated as described above. Plasmids were sequenced to verify ligation of constant domain with the vector and to check if any mutations had occurred. No adaptation was made to the light chain.

2.4 | Production of human recombinant IgG HLA mAbs

For recombinant mAb production, heavy and light chain containing vectors were used for transient co-transfection of Expi293F cells with ExpiFectamine, Opti-MEM, and Expi293 expression medium (ThermoFisher Scientific) according to the instructions provided by the manufacturer. After 5 days of culture, supernatants containing the recombinant mAbs were harvested and filtered. The presence of IgG was determined by total IgG enzyme-linked immunosorbent assay (ELISA), as previously described.[27] IgG specificity of the different subclasses was confirmed by a human IgG subclass ELISA kit (ThermoFisher Scientific).

2.5 | Purification of recombinant IgG HLA mAbs

The recombinant mAbs were purified using Amicon ProAffinity Concentration Kit Protein G (Merck Millipore, Burlington, Massachusetts). A maximum of 1000 μg mAb was loaded onto 200 μL Protein G resin and incubated for 60 minutes at room temperature on a roller bench. After wash steps, mAb was eluted and neutralised. Next, the buffer was exchanged with phosphate-buffered saline (PBS, B Braun, Melsungen, Germany) using a Slide-a-lyzer 0.5-3 mL dialysis cassette (ThermoFisher Scientific) by incubating the cassette in beaker with PBS for 21 hours at 4°C, PBS was refreshed a couple of times during incubation. The concentrations of purified mAbs were measured using the protein A280 protocol of NanoDrop2000 (ThermoFisher Scientific), and molar concentration were calculated for each mAb.

2.6 | HLA antibody detection

For verification of the IgG subclasses, the supernatants were screened with Lifecodes Lifescreen Deluxe screening kit (Immucor Transplant Diagnostics, Stamford, Connecticut) modified by using anti-IgG1 (10 μg/mL; HP6001), anti-IgG2 (2.5 μg/mL; HP6002), anti-IgG3 (10 μg/mL; HP6050), and anti-IgG4 (2.5 μg/mL; HP6025) PE-conjugated detection antibodies (Southern Biotech, Birmingham, Alabama).

The HLA specificities of the recombinant mAbs were determined by screening the recombinant mAbs with Lifecodes HLA class I or II single antigen beads (SAB) using goat anti-human Pan-IgG PE-conjugated on a Luminex platform (Immucor). The ability of recombinant mAbs to bind complement component 3d (C3d) was tested with Lifedcodes C3d detection (Immucor). Both Lifecodes kits were used according to manufacturer's instructions. The data were analysed with Match It! Antibody software version 1.3.0 (Immucor).

2.7 | Bio-layer interferometry

Affinity of antibody to antigen was determined via bio-layer interferometry (BLI) using the Octet RED96 system (FortéBio, Fremont, California). HLA IgG subclasses from WIM8E5 were immobilised to anti-human IgG Fc kinetic biosensors with a response threshold of 0.6 nm. To determine the association phase, parallel sensors were dipped into wells containing soluble, recombinant *HLA-A*11:01* in a 2-fold titration from 200 to 6.25 nM for 300 seconds so an equilibrium could be reached. Next, sensors were placed into buffer alone-containing wells for a further 1000 seconds to determine the dissociation phase. Affinity values (K_D) were calculated via steady-state analysis, where the response equilibrium (R_{eq}) was plotted against the HLA analyte concentration for each sensor and K_D values were measured as the HLA concentration of 50% of the overall calculated maximum response (R_{max}). All experiments were carried out using standard kinetic buffer (1× PBS, 0.1% bovine serum albumin, 0.02% Tween-20), at a temperature of 30°C and a constant plate shake speed of 1000 rpm.

2.8 | Fc domain glycosylation profiling

Of WIM8E5 and RTLK1E2 IgG subclasses, 2 μg sample was added to a final volume of 20 μL PBS and affinity

captured with ProtG beads. After desalting, the mAbs were eluted with 100 µL 100 mM formic acid and subsequently vacuum dried at 60°C. The dried samples were resuspended in 40 µL digestion solution consisting of 25 mM ammonium bicarbonate and 5 ng/µL sequencing grade trypsin and followed by overnight digestion at 37°C to obtain tryptic glycopeptides. Fc glycosylation was measured by nano liquid chromatography-mass spectrometry of glycopeptides followed by data processing using LaCyTools as previously described.[28] From the relative abundances of the glycopeptides, the levels hybrid-type, high-mannose, and complex-type Fc *N*-glycans as well as the level of galactosylation, fucosylation, bisection, and sialylation were calculated.

2.9 | Cells

HLA-typed peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from healthy donors after informed consent (Sanquin Blood Supply, Amsterdam, the Netherlands). PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation and cryopreserved until further use. For the cytotoxicity experiment with RTLK1E2 mAbs, B cells were magnetically isolated from PBMCs using EasySep direct HLA cross-match B-cell isolation kit (Stemcell Technologies, Köln, Germany) with a purity of >90%.

2.10 | Complement-dependent cytotoxicity assay

Terasaki plates (Greiner) were oiled and filled with 1 µL of supernatant containing the mAb of interest in triplicate. Then, 3000 HLA typed PBMCs or B cells were added to each well and incubated for 60 minutes at 20°C. Next, 5 µL rabbit complement (Inno-train, Kronberg, Germany) was added and incubated for 60 minutes at 20°C. To visualise cytotoxicity, 5 µL propidium iodide ink was added to each well, and after 15 minutes incubation in the dark the plates were analysed with Patimed (Leica Microsystems, Amsterdam, the Netherlands).

2.11 | Statistical analysis

The Kruskal-Wallis test was used for unpaired analysis and the Friedman test was used for paired analysis. Statistical level of significance was defined as $P < .05$, and analyses were performed with GraphPad Prism, version 7.02 (GraphPad Software, La Jolla, California).

3 | RESULTS

3.1 | Recombinant human IgG subclass HLA mAbs

Genes encoding the variable heavy chain and light chain domains were cloned into expression vectors, after which recombinant antibodies can be expressed by transient co-transfection of both vectors.[29-31] Here, we generated recombinant human HLA class I and class II mAbs, WIM8E5, and RTLK1E2, respectively, of all four IgG subclasses. To verify IgG subclass, the supernatant of all four IgG subclass mAbs were screened with IgG subclass ELISA and a modified Luminex screening assay using detection antibodies specific for each IgG subclass. As shown in Figure 1, the specific IgG constant domains were recognised by the correct detection antibody, indicating that mAbs of all four IgG subclasses were produced.

To corroborate that HLA specificities remained unaffected by the recombinant technology, original hybridoma-generated mAbs and recombinant human IgG subclass mAbs were screened with HLA class I or II SAB Luminex assay. Upon comparison of the background corrected mean fluorescence intensity (BCM) values of both WIM8E5 (Figure 2A) and RTLK1E2 (Figure 2B) mAbs, no difference in HLA specificities was observed with the original hybridoma-generated mAb for both recombinant IgG subclass HLA mAbs.

3.2 | Affinity and Fc domain glycosylation is similar between IgG subclasses

As the recombinant IgG subclass HLA mAbs have the same HLA specificity, we next questioned whether these mAbs have the same affinity for the immunising HLA allele. Therefore, the recombinant IgG subclass WIM8E5 mAbs were tested with bio-layer interferometry (BLI). The affinity values (K_D) observed for the target *HLA-A*11:01* were in the range of 25 to 32 nM for all four IgG subclass WIM8E5 mAbs (Figure 3).

For mAb production Expi293F cells are used, so we wanted to determine if the correct human glycosylation was present on the Fc part of the generated mAbs. The glycosylation characterisation of the generated recombinant IgG subclass HLA class I and class II mAbs showed that the IgG subclasses have a similar profile (Figure 4). In addition, the observed glycosylation traits of the mAbs are in accordance with those found on IgG in human serum using the same method.[32] However, the relative levels of bisection (the presence of a bisecting *N*-acetylglucosamine) and sialylation are lower on the mAbs, as compared to what is generally found on IgG in human serum, while the abundance of high mannose-type species is higher.[32] For IgG3, we detected

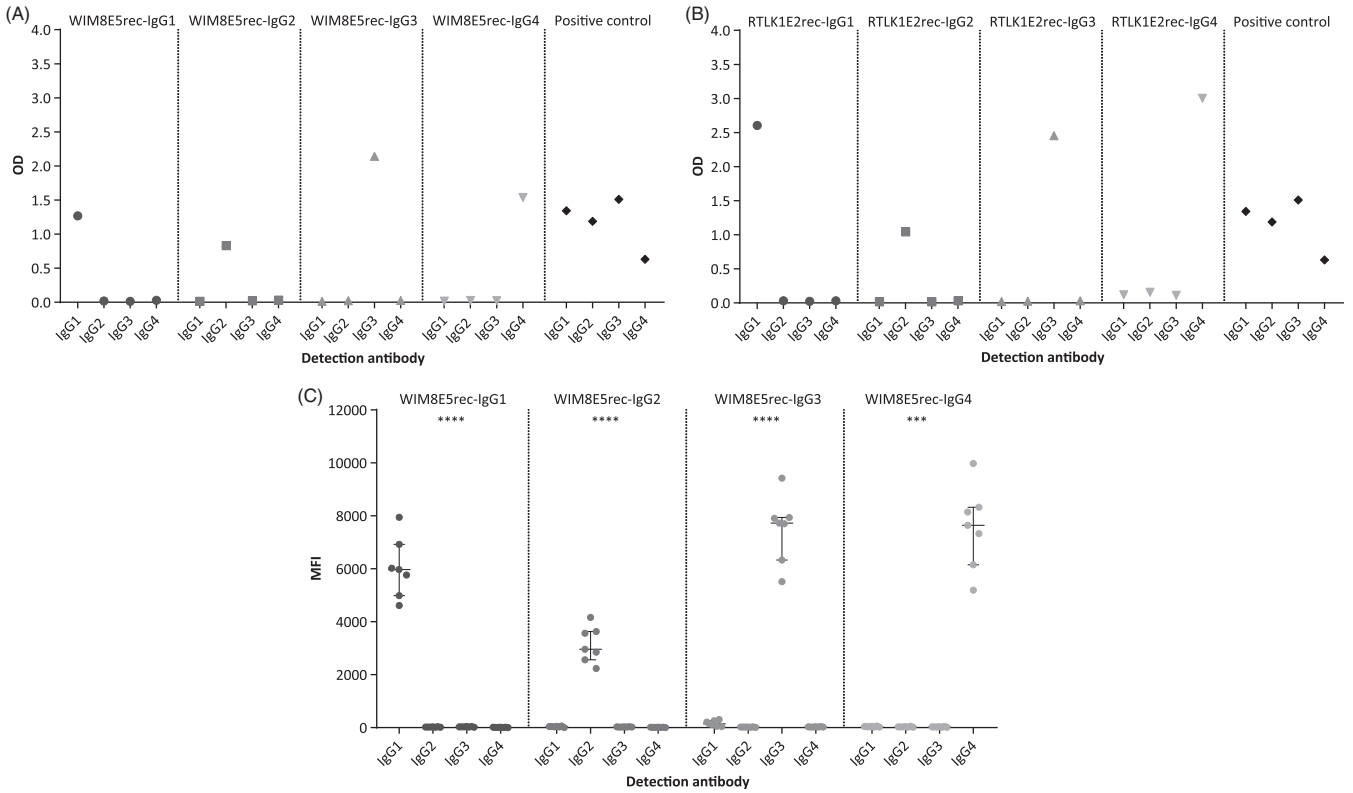

FIGURE 1 Recombinant IgG subclass HLA monoclonal antibodies (mAbs) could be detected by the corresponding IgG-specific detection antibody. IgG subclass could be detected with IgG subclass enzyme-linked immunosorbent assay (ELISA) kit for both WIM8E5rec-IgG mAbs (A) and RLTK1E2rec-IgG mAbs (B). Positive control is a human serum. (C) Similar findings were observed when screening recombinant WIM8E5 IgG subclass mAbs with Lifecodes Lifescreen Deluxe kit. The kit contains seven groups of HLA class I beads and each data point represents a single bead group. Kruskal-Wallis test was used to compare median of all four detection antibodies per IgG subclass mAb. Error bars represent median ± interquartile range. MFI is mean fluorescence intensity. OD is optical density. ***$P < .001$, ****$P < .0001$

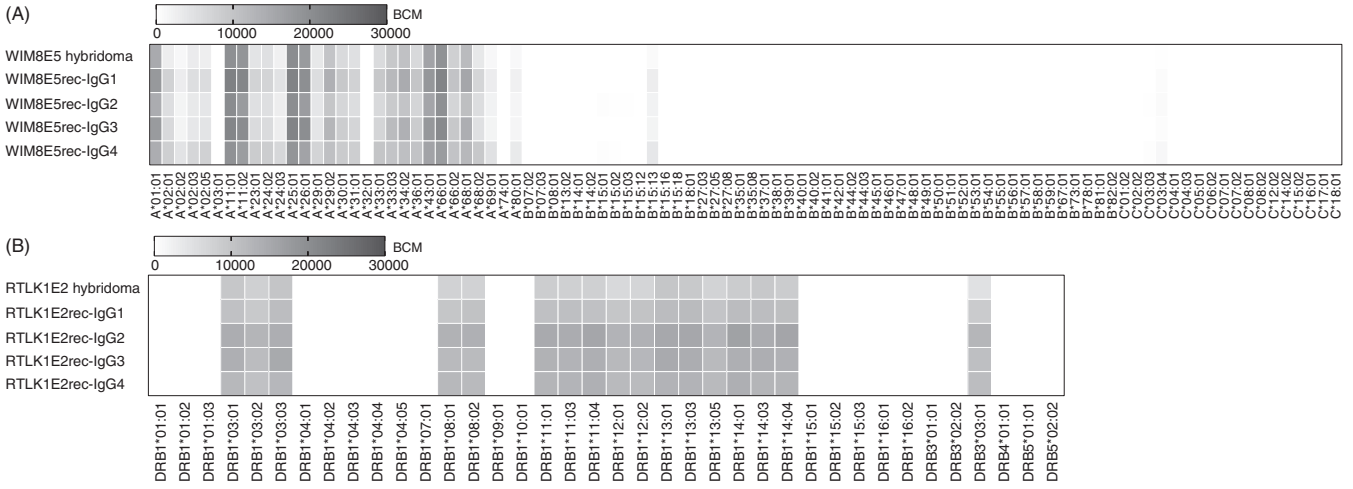

FIGURE 2 The same HLA epitope is recognised by the recombinant IgG subclass HLA monoclonal antibodies (mAbs). HLA specificities of recombinant IgG1, IgG2, IgG3, and IgG4 of WIM8E5 mAb (A) and RTLK1E2 mAb (B, only DRB1/3/4/5 beads are shown as all other loci were negative) as detected by Luminex SAB assay. Purified recombinant mAb concentration tested was 62.5 nM. BCM is background corrected mean fluorescence intensity

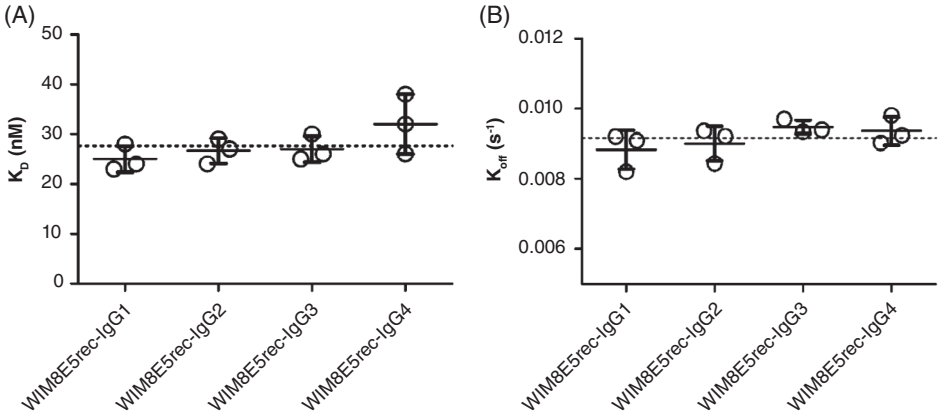

FIGURE 3 Recombinant IgG subclass HLA monoclonal antibodies (mAbs) have similar affinity. The affinity (A) and dissociation rates (B) of recombinant IgG subclass WIM8E5 mAbs were determined via bio-layer interferometry. Calculated values are consistently similar across all IgG subclasses against the target *HLA-A*11:01*. The dotted lines represent the affinity (A) and dissociation constant (B) average across all four IgG subclasses. Error bars represent the mean ± SD of three experiments

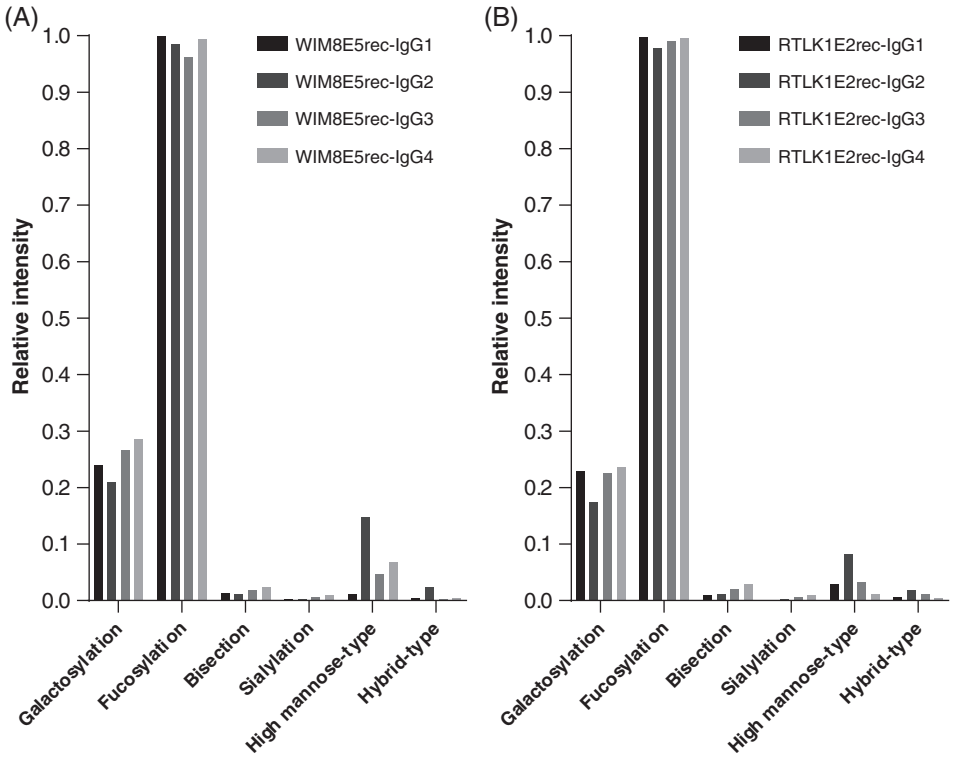

FIGURE 4 The glycosylation profile of recombinant IgG subclass HLA monoclonal antibodies (mAbs) is similar. Relative intensity values of derived traits for the recombinant IgG1, IgG2, IgG3, and IgG4 of WIM8E5 mAb (A) and RLTK1E2 mAbs (B) are shown

partial occupancy of the *O*-glycosylation sites in the hinge region (data not shown), which is in line with the hinge region *O*-glycosylation of IgG3 from the human circulation.[33]

3.3 | Cytotoxicity of recombinant human IgG subclass HLA mAbs

To determine whether the recombinantly generated IgG subclass HLA-specific mAbs showed the anticipated cytotoxicity patterns, we performed complement-dependent cytotoxicity (CDC) assays. Incubation of WIM8E5 recombinant IgG subclass mAbs with PBMCs expressing HLA antigens recognised by WIM8E5 (HLA-A11, -A1) showed that WIM8E5rec-IgG1 and -IgG3 mAbs were highly cytotoxic in a dose-dependent manner (Figure 5A). Both IgG2 and IgG4 subclasses did not show cytotoxicity. For the HLA class II mAb RTLK1E2, a CDC using purified B cells (HLA-DR17, -DR13) was performed. Both RTLK1E2rec-IgG1 and -IgG3 were highly cytotoxic (Figure 5B). While CDC with rabbit complement is standard practice in transplantation, it does not show if mAbs can also activate human complement.

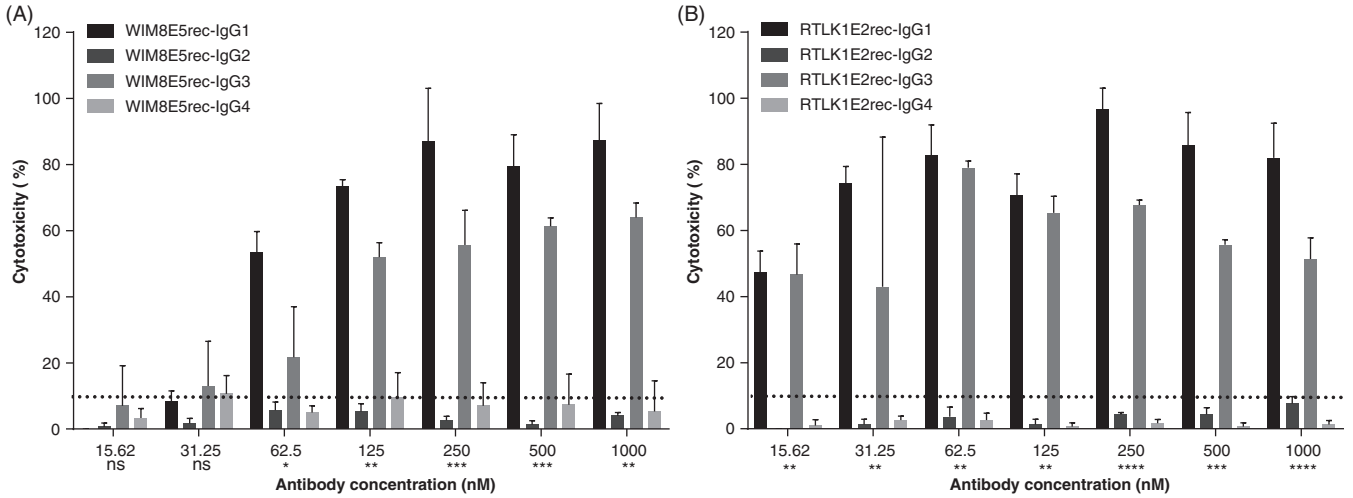

FIGURE 5 Recombinant IgG1 and IgG3 HLA monoclonal antibodies (mAbs) are cytotoxic. (A) Recombinant IgG subclass WIM8E5 mAbs were incubated with PBMC expressing HLA-A1, -A11, -B8, -B55, -Cw3, and -Cw7. WIM8E5rec-IgG1 and -IgG3 induced cell lysis (>60%), while IgG2 and IgG4 were unable to induce complement cytotoxicity. (B) Recombinant IgG subclass RTLK1E2 mAbs were incubated with B cells expressing HLA-DR17, -DR13, and -DR52. RTLK1E2rec-IgG1 (>80%) and -IgG3 (>60%) induced cell lysis, while no cell lysis was observed for RLTK1E2rec-IgG2 and RTKL1E2rec-IgG4. mAbs were added in various concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.62 nM). Error bars represent the mean ± SD of triplicate wells. The Kruskal-Wallis test was used per dilution to compare the IgG subclass HLA mAbs. Dotted line indicates background. ****$P < .0001$ ***$P < .001$, **$P < .01$, *$P < .05$

Testing the recombinant IgG subclass HLA mAbs with C3d SAB assay showed that only IgG1 and IgG3 mAbs are capable of binding human C3d (Figure S1), suggesting that these mAbs can activate human complement.

4 | DISCUSSION

In this study, we show the generation and production of recombinant human HLA class I and class II-specific mAbs of all four IgG subclasses from established B-cell heterohybridomas. The generated recombinant HLA mAbs of all four IgG subclasses recognise the same HLA epitope with the same binding affinity. Currently, we were only able to determine the affinity for the HLA class I mAbs, due to lack of recombinant HLA class II. Furthermore, we show that all recombinant IgG subclass HLA mAbs have human-type Fc glycosylation and the glycosylation profiles were similar between the mAbs. The conserved *N*-glycans located at asparagine 297 of the Fc part play a role in the function of an antibody and the different levels of specific glycosylation traits could have pronounced effects on complement activation and FcγR binding.[34,35] Both IgG1 and IgG3 HLA class I and class II mAbs are capable of complement activation, while a weak or no cytotoxicity was observed for the IgG2 and IgG4 mAbs. Preliminary data suggest that IgG1 and IgG3 can induce antibody-dependent cell-mediated cytotoxicity (ADCC), but only a low percentage of cell lysis was observed (Figure S2). This can be explained by the high levels of fucosylation (>96%) on the recombinant IgG subclass HLA mAbs, as it has been shown that high levels of fucosylation on IgG negatively influences ADCC activity.[36] Glyco-engineering of our recombinant IgG subclass HLA mAbs may further allow altering their functional properties.[37]

Currently available human HLA mAbs are mainly derived from multiparous women by Epstein-Barr virus transformation and electrofusion using mouse myeloma cell line, are primarily directed against HLA class I, and are restricted to an IgM or IgG1 isotype.[25,38,39] These human HLA mAbs have been widely used in various applications, such as flow cytometry assays,[40-42] B-cell ELISPOT assays,[43-45] blocking assays,[46] assays to determine HLA expression levels,[47-49] or functional assays of HLA antibodies.[16,19,22-24] Commercial chimeric IgG subclass HLA mAbs are available, but those have a mouse variable part, while W6/32 and F3.3 recognise all HLA class I molecules and majority of HLA class II molecules, respectively.[50]

Recently, Gu et al[51] elegantly characterised an HLA class I mAb generated by a germline phage display from a non-sensitised individual, resulting in an antibody that likely can be generated during an alloimmune response. In contrast, we have produced human recombinant HLA mAbs generated from heterohybridomas that were derived from B cells of immunised individuals. Therefore, the mAbs we produced are truly representative of HLA antibodies produced through alloimmunisation. In addition, we generated both human HLA class I and class II mAbs of all four IgG subclasses. Especially the latter is unique, as there are only a limited

number of HLA class II mAbs available and those are mainly IgG1.

Antibody effector function is determined by its isotype. As mentioned, complement binding HLA DSA is associated with graft loss, but in sera of renal transplant patients both complement binding, IgG1 and IgG3, as well as non-complement binding, IgG2 and IgG4 HLA antibodies are observed.[8,12-14] Previous studies have shown by mixing IgG subclass mAbs that IgG2 and/or IgG4 can inhibit complement activation of IgG1 and/or IgG3 when recognising the same epitope.[15,51] Others performed mixing experiments with human HLA mAbs directed against different epitopes of same HLA antigens and showed that combining these HLA mAbs promote complement activation, while individually the mAbs had no effect.[16] Additional mixture studies with human mAbs directed to HLA class I and class II molecules of different IgG subclasses, comparing different specificities and avidities, should be performed as this will allow greater understanding of the interaction of antibodies of different IgG subclasses recognising different epitopes on the same HLA molecules.

In addition, HLA mAbs have been used for functional assays to study HLA-antibody-induced graft damage independent of the complement cascade. HLA class I antibodies can cause crosslinking on endothelial and smooth muscle cells inducing intracellular signalling, resulting in inflammatory activation, and leukocyte recruitment such as P-selectin-induced monocyte adhesion.[17-19,52-54] Although crosslinking is irrespective of the IgG subclass, the level of P-selectin on endothelial cells and the monocyte recruitment via FcγR mechanism are increased with IgG1 and IgG3 antibodies. The levels of P-selectin and FcγR-dependent monocyte recruitment have been well studied with human IgG1 HLA mAb,[19] but due to lack of IgG3, IgG2, and IgG4 HLA mAbs, the exact influence of antibodies with these isotypes, especially the latter two, is not fully clear.

Human HLA mAb do not represent the polyclonal response observed in sera. However, due to the mixture of antibody specificities, concentrations and isotypes of HLA antibodies present in sera it is difficult to study the role and function of HLA antibodies. By using human HLA mAbs, mixture experiments can be performed in a controlled manner, even with normal human serum as matrix. For future studies, it is essential to extend the specificities of the available mAbs, since especially mAbs for HLA class II are currently lacking.

In conclusion, we show here that recombinant human HLA class I and class II mAbs of all four IgG subclasses recognising the same HLA epitope can be generated from established B-cell heterohybridomas. This method enables us to generate more IgG subclass HLA mAbs recognising different epitopes on the same HLA antigen, which can be used in mixing experiments to study the role and function of HLA DSA of different IgG subclasses in a controlled fashion. These IgG subclass HLA class I and class II mAbs can provide mechanistic insights into the role of DSA in renal transplantation and in other clinical settings.

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CONFLICT OF INTEREST

C. C.C. Z.-v. d. O. and H. J. R. are Genmab employees and own Genmab stocks.

DATA ACCESSIBILITY

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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