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IgG Subclasses Shape Cytokine Responses by Human Myeloid Immune Cells through Differential Metabolic Reprogramming

Willianne Hoepel,*,† Sona Allahverdiyeva,*,†,‡ Haneen Harbiye,*,† Steven W. de Taeye,‡,§ Alwin J. van der Ham,¶ Leonie de Boer,‡ Sebastiaan A. J. Zaat,‡ Michel van Weeghel,‖,# Dominique L. P. Baeten,*,† Riekelt H. Houtkooper,‖ Bart Everts,¶ Gestur Vidarsson,§ and Jeroen den Dunnen*,†

IgG Abs are crucial for various immune functions, including neutralization, phagocytosis, and Ab-dependent cellular cytotoxicity. In this study, we identified another function of IgG by showing that IgG immune complexes elicit distinct cytokine profiles by human myeloid immune cells, which are dependent on FcγR activation by the different IgG subclasses. Using monoclonal IgG subclasses with identical Ag specificity, our data demonstrate that the production of Th17-inducing cytokines, such as TNF, IL-1β, and IL-23, is particularly dependent on IgG2, whereas type I IFN responses are controlled by IgG3, and IgG1 is able to regulate both. In addition, we identified that subclass-specific cytokine production is orchestrated at the posttranscriptional level through distinct glycolytic reprogramming of human myeloid immune cells. Combined, these data identify that IgG subclasses provide pathogen- and cell type–specific immunity through differential metabolic reprogramming by FcγRs. These findings may be relevant for future design of Ab-related therapies in the context of infectious diseases, chronic inflammation, and cancer. ***The Journal of Immunology*, 2020, 205: 000–000.**

Antibodies of the IgG isotype are one of the most abundant proteins in human serum. IgG Abs are crucial for various immune functions, such as phagocytosis, degranulation, complement activation, and Ab-dependent cellular cytotoxicity (ADCC) (1). Many of these effector functions are dependent on their interaction with FcγRs, a receptor for the Fc region of IgG (2). IgG Abs can be further subdivided in four subclasses, IgG1, IgG2, IgG3, and IgG4, which are named in order of decreasing abundance in human serum (3). The production of different IgG subclasses is skewed depending on the type of Ag involved. For example, responses to bacterial capsular polysaccharide Ags are mostly restricted to IgG2 (4–7). In contrast, viral infections are generally characterized by Abs of the IgG1 and IgG3 subclass, with IgG3 appearing first during the course of infection (4, 8). In addition, the four IgG subclasses display a different capacity to induce effector responses. In general, IgG3 is considered to have the most pronounced immune-activating effects (1, 9, 10), which is based on the potent ability of IgG3 to induce processes such as phagocytosis, complement activation, and ADCC (3). This proinflammatory reputation of IgG3 is strengthened by its short half-life compared with the other IgG subclasses, which may function to limit excessive inflammatory responses ignited by IgG3 (3).

Although the different capacities of IgG subclasses to induce effector responses such as phagocytosis and complement activation have been well studied, still little is known about IgG subclass–specific differences in induction of cytokine production, another essential effector function of IgG Abs. Although initially underappreciated, it has become clear that, particularly in humans, IgG plays a critical role in orchestrating inflammation by controlling the production of key proinflammatory cytokines through activation of FcγRs (11–14). This IgG-induced up- and downregulation of cytokines serves an important function in host defense by tailoring immune responses to the encountered pathogen. For example, IgG opsonization of bacteria strongly amplifies the production of cytokines by dendritic cells (DCs) that promote Th17 responses, which is required to efficiently counteract bacterial infections (12, 15). Alternatively, IgG opsonization of viruses selectively decreases the production type I and type III

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Abbreviations used in this article: ADCC, Ab-dependent cellular cytotoxicity; CDS, cytosolic DNA sensor; Ct, cycle threshold; DC, dendritic cell; ECAR, extracellular acidification rate; IgG-IC, IgG immune complex; IRF5, IFN regulatory factor 5; Pam3, Pam3CSK4; si-RNA, small interfering RNA.

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IFNs by DCs, which may provide a negative feedback mechanism to prevent collateral damage and promote antiviral CD8 T cell responses (16, 17). However, undesired activation of IgG-induced cytokine production (e.g., by autoantibodies) also drives excessive inflammation, as observed in chronic inflammatory disorders such as rheumatoid arthritis (11, 14) and systemic lupus erythematosus (18–20).

IgG Abs can elicit cytokine production by a variety of human cell types in different tissues, ranging from immune cells such as DCs and macrophages to nonimmune cells such as nasal and lung epithelium (21). Although FcγRIIa is the main cytokine-inducing receptor on myeloid immune cells such as DCs (12, 15), also other members of the FcγR family have been described to elicit cytokine production, including FcγRI (14) and FcγRIII (21). Importantly, the recognition of (unbound) IgG by FcγRs in itself is not sufficient to induce cytokine production. Instead, this only occurs after two additional steps. First, IgG Abs need to bind to their Ag (e.g., pathogens or autoantigens), resulting in the formation of IgG immune complexes (IgG-IC), which activate FcγRs by binding with increased avidity to provide FcγR cross-linking and subsequent signaling (22). Second, FcγRs induce very little cytokine production upon individual stimulation and have to collaborate with other receptors to amplify or inhibit the production of specific cytokines (13). FcγRs can engage in cross-talk with a variety of different receptors, which include pathogen-sensing receptors such as TLRs, NOD-like receptors (NLRs), and cytosolic DNA sensors (CDS) (12, 16) but also cytokine receptors such as IL-1R and IFN-γR (23). Notably, the ultimate cytokine mixture that is induced by IgG-IC is not uniform but instead is shaped by the immunological context (e.g., by the pathogen involved or the cell type on which FcγRs are triggered). However, how IgG Abs are able to generate these pathogen- and cell type–specific responses is still largely unknown.

In this study, we set out to identify the relative role of IgG subclasses in their capacity to induce cytokine production. Strikingly, we identified that the production of proinflammatory cytokines such as TNF, IL-1β, and IL-23 by human myeloid immune cells is dependent on IgG1 and particularly IgG2, whereas IgG3 had very little effect. In contrast, the suppression of type I IFN was mainly induced by IgG1 and IgG3, indicating that IgG induces subclass-specific cytokine profiles. We further demonstrate that the cytokine profile induced by IgG subclasses differs between myeloid cells, indicating IgG subclasses induce cell type–specific immunity. Moreover, we identified that IgG subclass–specific cytokine responses are not regulated at the level of gene transcription or inflammasome activation but result from distinct glycolytic reprogramming of myeloid immune cells. Combined, these data identified that human IgG subclasses tailor cytokine responses to the immunological context through differential metabolic reprogramming by FcγRs.

Materials and Methods

Cells

Cells were isolated from buffy coats (Sanquin Blood Supply). Buffy coats obtained after blood donation are not subjected to informed consent, which is according to the Medical Research Involving Human Subjects Act and Academic Medical Center Ethics Review Committee. All samples were handled anonymously. Ethical review and approval were not required for this study in accordance with the local legislations. DCs and macrophages were obtained by isolating monocytes from buffy coats, using density gradient centrifugation on Lymphoprep (Nycomed) and Percoll (Pharmacia). Then, cells were generated by culturing for 6 d in IMDM (Lonza) containing 5% FBS (Biowest) and 86 μg/ml gentamicin (Life Technologies) with supplemented cytokines. At day 2 or 3, half of the medium was replaced for new medium and cytokines. DCs were differentiated with 20 ng/ml GM-CSF (Invitrogen) and 2 ng/ml IL-4 (Miltenyi Biotec) and macrophages with 50 ng/ml rM-CSF (ImmunoTools). Monocytes were directly isolated from PBMCs after Lymphoprep by MACS isolation using CD14 microbeads (Miltenyi Biotec) and stimulated directly afterward. For silencing, cells were harvested at day 3 and microporated with 500 nM IFN regulatory factor 5 (IRF5) small interfering RNA (si-RNA) or control si-RNA (Dharmacon) and cultured for 3 more d in the indicated cultured medium except gentamicin.

Abs

For the opsonization of *Streptococcus pneumoniae*, IgG-subclass Abs against polysaccharide 6A were used, produced as described before (24, 25). The different IgG subclasses had no effect on the opsonization capacity of *S. pneumoniae*. For the other experiments, IgG was coated on plates to mimic IgG-IC. IgG subclasses 1–4 and allotypes (IGHG3*01/*04/*16/*18) with either a specificity for anti-RhD or anti-TNP were used. Anti-RhD and anti-TNP Abs showed similar observations and were described before (26).

Stimulation

DCs and macrophages were harvested at day 6 by respectively using ice for 30 min or TrypLE Select (Invitrogen). Two micrograms per milliliter IgG subclasses or IgG allotypes were coated on a 96-well, high-affinity plate (Nunc) or a 48-well plate (Costar). High-affinity plates were blocked with 10% FBS in PBS at 37°C 1 h prior to use. Cells were stimulated with 10 μg/ml Pam3CSK4 (Pam3) (InvivoGen) or 20 μg/ml Poly I:C (Sigma-Aldrich). To block glycolysis, 10 mM 2-deoxy-D-glucose (Sigma-Aldrich) was added to the cells simultaneously with the stimuli. For the bacteria experiment, *S. pneumoniae* were preopsonized with 250 μg/ml IgG in PBS for 45 min. After washing, bacteria were diluted in X-VIVO Media supplemented with gentamicin (Life Technologies) and stimulated with DCs afterward (multiplicity of infection 1:10) in a round-bottom plate (Costar) for 24 h.

ELISA

To determine cytokine production, supernatants were harvested after 24 h of stimulation and stored at −20°C. Cytokines levels were measured using Ab pairs for TNF (eBioscience), IL-1β, IL-6, IL-23 (U-CyTech Biosciences), and IL-10 (BD Biosciences). IFN-β was detected using an IFN-β ELISA kit (PBL Assay Science).

Quantitative RT-PCR

Cells were stimulated and lysed at indicated time points, and mRNA was extracted using RNeasy Mini Kit (QIAGEN). cDNA was synthesized using RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Quantitative RT-PCR was performed (StepOnePlus Real-Time PCR System; Applied Biosystems) using the following mixture and TaqMan primers (Thermo Fisher Scientific): GAPDH (4310884E), TNF (Hs00174128_m1), IL-1β (Hs00174097_m1), IFN-β (Hs01077958_s1), IFN-λ1 (Hs00601677_g1), and IRF5 (Hs00158114_m1). mRNA levels were normalized to the geometric mean of the cycle threshold (Ct) values of housekeeping gene GAPDH [$2^{Ct(housekeeping)-Ct(target)}$], and folds were calculated.

Caspase-1 activation

DCs (120,000–150,000 per well) were stimulated for indicated time points. Caspase-1 activity was determined according to manufactures protocol, using caspase-1–binding compound FAM-YVAD-FMK (FAM-FLICA Caspase-1 Assay Kit; ImmunoChemistry Technologies).

Metabolism assays

Glycolysis in DCs was determined by real-time analysis of the extracellular acidification rate (ECAR) (XF-96e Extracellular Flux Analyzer; Seahorse Bioscience). An XF-96 culture plate was coated with 4 μg/ml IgG subclasses and 30,000 DCs were plated per well in unbuffered RPMI 1640 (Sigma-Aldrich), which was filtered through a 0.22-μm filter system (Corning, Amsterdam, the Netherlands); the pH was set to 7.4 using 37% HCl and was supplemented with 5% FCS. Cells were rested at 37°C in 0% CO_2 for 1 h. ECAR was monitored in response to glucose (10 mM; port A; Sigma-Aldrich) and 30 min later to 10 μg/ml Pam3 or 20 μg/ml Poly I:C (port B). ECAR values as shown in the graphs represent the increase in ECAR as determined 45 min after injection of port B over the baseline ECAR values before port A injection.

Results

IgG-induced proinflammatory cytokine production is dependent on IgG1 and IgG2

An important function of human IgG Abs is to direct immune responses through the induction of proinflammatory cytokines.

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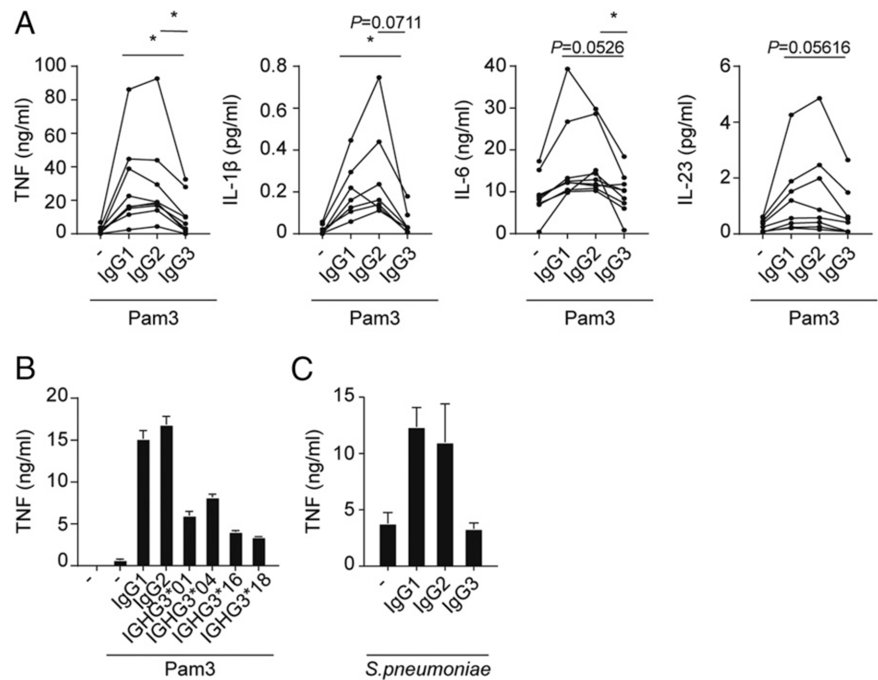

FIGURE 1. IgG-induced proinflammatory cytokine production is dependent on IgG1 and IgG2. (**A** and **B**) Human monocyte-derived DCs were stimulated for 24 h with 10 μg/ml Pam3 and IgG-IC. (A) Seven to nine independent experiments performed in triplicate, with different donors. *$p < 0.05$, one-way ANOVA with Bonferroni multiple comparisons test. (B) Representative example of three independent experiment with different donors performed in triplicate (mean ± SEM). (**C**) DCs were incubated for 24 h with *S. pneumoniae* (multiplicity of infection = 10) with were preopsonized or not with 250 ng/ml of the different IgG subclasses (IgG1–3) for 45 min and washed. Representative example of three independent experiments performed in triplicate (mean ± SEM). (A–C) Cytokine production in supernatant was determined by ELISA.

In this study, we set out to identify which of the IgG subclasses are responsible for this Ab-induced inflammation. Previous studies revealed that IgG-induced inflammation critically depends on two events. First, IgG needs to form IgG-ICs, as occurs by binding to (auto)antigens, which enables binding to low-affinity FcγRs (27). Second, IgG-IC activation of FcγRs requires concomitant stimulation with other receptors such as TLRs, leading to a strong and synergistic amplification of particular proinflammatory cytokines (11–14). To assess the relative role of IgG subclasses, we used human monocyte-derived DCs, which we previously showed have stable FcγR expression with little donor-to-donor differences (12, 23). We stimulated cells for 24 h with IgG-IC in combination with TLR2 ligand Pam3 a bacterial mimic, and determined proinflammatory cytokine production in the supernatant. As for IgG-IC, we used plate-bound IgG, which we have previously optimized to generate a standardized and highly reproducible stimulus that is comparable to IgG-IC generated in other ways, including opsonized bacteria and viruses, coated beads, and heat-aggregated IgG (12, 16).

Strikingly, TLR costimulation with IgG1 and IgG2 strongly amplified the production of proinflammatory cytokines TNF, IL-1β, and IL-23, whereas IgG3 had very little effect on these cytokines (representative donor Supplemental Fig. 1A, multiple donors Fig. 1A). Corroborating previous findings, IgG-IC costimulation had little effect on IL-6 production (Fig. 1A, Supplemental Fig. 1A). These data demonstrate that IgG-induced proinflammatory cytokine production is mainly amplified by IgG1 and IgG2 and, to a far lesser extent, by IgG3. TLR costimulation with IgG4, which generally has lowered interaction with FcγRs (3), did induce TNF production but not to the same extend as IgG1 and IgG2 (Supplemental Fig. 1B).

IgG subclasses can be further divided into different allotypes, of which, particularly for IgG3, there are many variations (3). To determine whether the lack of cytokine induction by IgG3 is dependent on the allotype, we stimulated DCs with four different IgG3 allotypes that have major differences in hinge length (IGHG3*01 = IGHG3*16 > IGHG3*18 > IGHG3*04) and in their affinity for FcγRIIa (IGHG3*01 = IGHG3*04 > IGHG3*16 > IGHG3*18), the main cytokine-inducing FcγR on human DCs (3). Although we observed some differences between IgG3 allotypes in their capacity to promote proinflammatory cytokine production, all IgG3 allotypes induced substantially less TNF compared with IgG1 and IgG2 (Fig. 1B). These data indicate that the capacity to induce proinflammatory cytokine is mainly dependent on the IgG subclass, and not the IgG allotypes.

To further confirm that the IgG isotypes differ in their capacity to promote cytokine production in a more physiological manner, we opsonized *S. pneumoniae* bacteria with an identical monoclonal IgG Ab against polysaccharide 6A that only differed in subclass (24, 25). Similar to the IgG-IC subclasses, opsonization of *S. pneumoniae* with IgG1 or IgG2 amplified TNF production, whereas IgG3 did not (Fig. 1C).

Combined, these data indicate that IgG-induced proinflammatory cytokine production is dependent on the isotype and is mainly induced by IgG1 and IgG2, but not IgG3.

IgG subclasses induce cell type–specific cytokine responses by human myeloid immune cells

In addition to DCs, IgG-IC (generated from purified pooled serum) have been shown to promote cytokine production by various other human myeloid immune cells, including monocytes (23) and macrophages (11, 14). Yet, although the amplification of TNF and IL-1β is universal, other cytokines such as IL-6 and IL-10 are modulated by IgG-IC in a cell type–specific manner. For example, although IgG-IC increase IL-10 production by DCs and macrophages, IgG-IC suppress IL-10 production by human monocytes (23). Therefore, in this study, we set out to determine whether IgG subclasses also contribute to induction of cell type–specific inflammatory responses. Similar to DCs, in human monocytes, TNF and IL-1β were mainly amplified by IgG1 and IgG2, whereas IgG3 had little effect (Supplemental Fig. 1C for representative donor, Fig. 2A for combined data of multiple donors). However, in monocytes, we observed that IL-6 production was specifically amplified by IgG3, but not by IgG1 and IgG2 (Fig. 2A, Supplemental Fig. 1C). In turn, the suppression of IL-10 in monocytes was mainly dependent on IgG1 and IgG2 (Fig. 2A, Supplemental Fig. 1C). In macrophages, the cytokine profile induced by the IgG subclasses was comparable to that in DCs (Supplemental Fig. 1D for representative donor, Fig. 2B for

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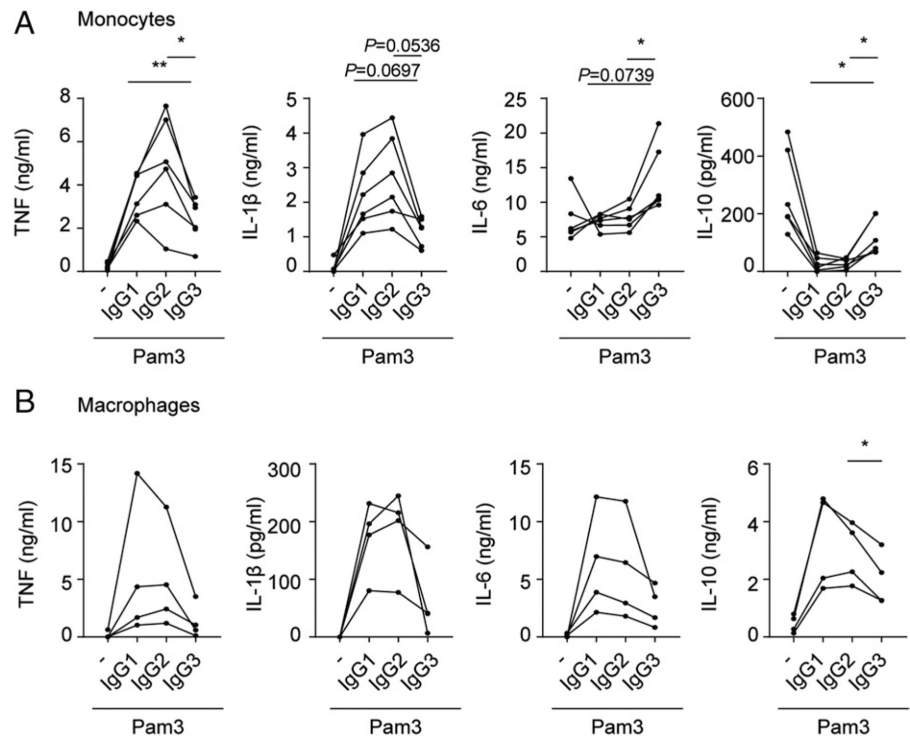

FIGURE 2. IgG subclasses induce cell type–specific cytokine responses by human myeloid immune cells. (**A**) Human monocytes or (**B**) human monocyte-derived macrophages were stimulated for 24 h with 10 μg/ml Pam3 and IgG-IC. (A and B) Cytokine production in supernatant was determined by ELISA. Experiments were performed in triplicate with six (A) and four (B) independent donors. $*p < 0.05$, $**p < 0.01$, one-way ANOVA with Bonferroni multiple comparisons test.

combined data of multiple donors). Combined, these data indicate that IgG subclasses also contribute to the induction of cell type–specific cytokine responses.

Suppression of type I IFNs is dependent on IgG1 and IgG3

Although IgG-IC amplify proinflammatory cytokines induced by bacteria-sensing pattern-recognition receptors such as TLR2, TLR4, and TLR5, IgG-IC (from pooled IgG) has recently been shown to suppress the production of type I and type III IFNs upon costimulation with virus-sensing pattern-recognition receptors such as TLR3, RIG-I–like receptors (RLRs), and CDS (16, 20, 28). We therefore set out to determine whether IgG subclasses also differently control type I IFN suppression. Interestingly, although Poly I:C (TLR3)–induced production of IFN-β by human DCs was suppressed upon costimulation with IgG1 and IgG3, costimulation with IgG2 had very little effect on IFN-β production (representative example Supplemental Fig. 2A, multiple donors Supplemental Fig. 2B). These data indicate that in contrast to proinflammatory cytokines, the modulation of type I IFN is mainly dependent on IgG1 and IgG3.

IgG subclass–specific cytokine production is not regulated by gene transcription and caspase-1 activation

IgG-IC costimulation is known to change immune responses by modulating cytokine production by human myeloid immune cells at three different levels. These are 1) altered cytokine gene transcription (12); 2) activation of caspase-1, which promotes IL-1β production by cleaving pro–IL-1β into its functional form (12, 14); and 3) increased cytokine gene translation that is dependent on glycolytic reprogramming (29, 30). To determine at which level IgG subclasses regulate their distinct cytokine profiles, we first assessed cytokine gene transcription upon costimulation with IgG1, IgG2, or IgG3. Notably, all subclasses amplified gene transcription of proinflammatory cytokines *TNF* and *IL1B* in a similar manner (Fig. 3A). Similarly, all subclasses also equally suppressed the transcription of *IFNB* and *IFNL* (encoding IFN-λ1) (Fig. 3B). Next, we set out to investigate whether IgG subclasses display a different capacity to activate caspase-1. However, no differences in caspase-1 activation were observed upon costimulation with the different IgG subclasses (Fig. 3C). Combined, these data show that cytokine gene transcription and caspase-1 activation are modulated by IgG subclasses in a similar manner, suggesting that a mechanism other than gene transcription or caspase-1 activation drives the differences in proinflammatory cytokine and type I/III IFN production by IgG subclasses.

IgG subclasses induce distinct metabolic reprogramming

Because no differences between IgG subclasses were observed in cytokine gene transcription and caspase-1 activation, we next studied IgG-IC–induced metabolic reprogramming. IgG-IC (from pooled IgG) induces metabolic reprogramming by increasing the glycolytic rate, which is essential for posttranslational amplification of (particularly) proinflammatory cytokine production (30). To determine whether the subclass-specific cytokine production is dependent on differences in glycolytic reprogramming, we stimulated human DCs and analyzed them for changes in ECAR as a measure of lactate production (a proxy for the glycolytic rate). Strikingly, although costimulation of Pam3 with IgG1 and IgG2 amplified the glycolytic rate, costimulation with IgG3 had very little effect on glycolysis (Fig. 4A).

Metabolic reprogramming of myeloid immune cells by IgG-IC has been described to be dependent on the transcription factor IRF5 (30, 31). Yet, because these prior experiments have been performed using pooled IgG that mostly contains IgG1, it is still unclear whether IgG2, which in this study, we identified is the most potent inducer of proinflammatory cytokines, also induces metabolic reprogramming through IRF5. We therefore silenced IRF5 (silencing efficiency of 70%) and assessed the glycolytic rate upon stimulation with Pam3 and/or IgG2. As shown in Fig. 4B, costimulation with IgG2 strongly increased the glycolysis, which was prevented by silencing of IRF5, indicating that IgG2-induced glycolytic reprogramming is indeed dependent on IRF5. To ascertain that the production of proinflammatory cytokines by IgG subclasses is dependent on glycolysis, we used glycolytic inhibitor

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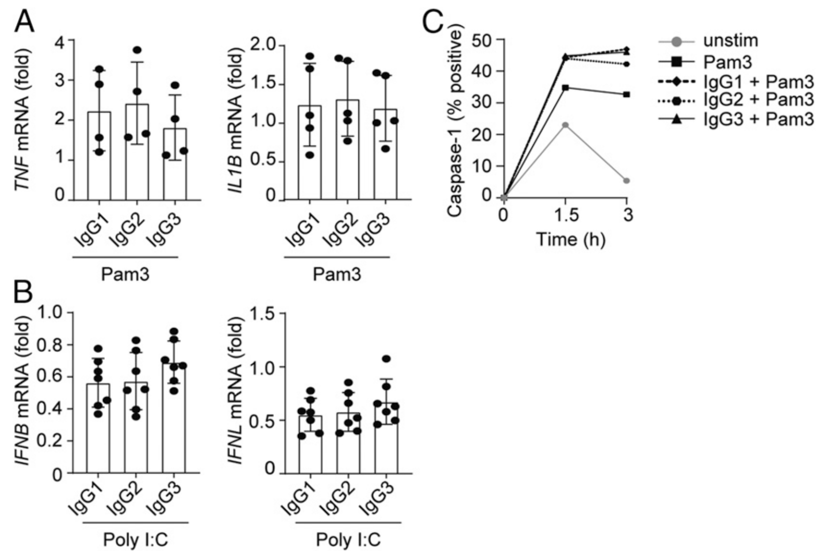

FIGURE 3. IgG subclass–specific cytokine production is not regulated by gene transcription and caspase-1 activation. (**A** and **B**) DCs were stimulated for 3 h with 10 μg/ml Pam3, 20 μg/ml Poly I:C, or in combination with IgG-IC from different subclasses, and *TNF*, *IL-1B*, *IFNB*, and *IFNL* mRNA transcription was measured by quantitative RT-PCR (qPCR) (normalized to housekeeping gene expression). Data of (A) five and (B) seven independent experiments are shown as fold increase for each donor after costimulation, normalized to mRNA expression when stimulated with Pam3 or Poly I:C alone (set to 1) (mean ± SEM). (**C**) Caspase-1 activity was determined by stimulating DCs at indicated time points with 10 μg/ml Pam3 or in combination with IgG-IC from different subclasses. After stimulation, cells were incubated for 1 h with the caspase-1–binding compound FAM-YVAD-FMK, and fluorescence was measured by flow cytometry. Representative example of three independent experiments.

2-deoxyglucose, which inhibits hexokinase activity (32). Blocking of glycolysis indeed abolished the amplification of TNF, IL-1β, and IL-23 production by all subclasses, including the very moderate amplification by IgG3 (Supplemental Fig. 3).

Interestingly, the nature of modulation of cytokine production by IgG-IC is dependent on the coreceptor involved. In general, IgG-IC costimulation with bacteria-sensing receptors such as TLR2, 4, or 5 or NLRs leads to amplification of proinflammatory cytokines TNF, IL-1β, and IL-23 (12, 14), whereas costimulation with virus-sensing receptors such as TLR3, RLRs, and CDS particularly suppresses type I IFNs, type III IFNs, and ISGs (16, 20). To determine whether IgG-IC also induce subclass-specific glycolytic changes upon costimulation with virus-sensing receptors, we measured the ECAR upon costimulation with TLR3 ligand Poly I:C. TLR3 stimulation, similar to TLR2 stimulation, increased the glycolysis (Fig. 4C). Yet, surprisingly, costimulation with IgG1 and IgG2 lowered TLR3-induced glycolysis, whereas IgG3 had little effect (Fig. 4C). These data indicate that the nature of metabolic reprogramming by IgG-IC is strongly dependent on the coreceptor involved.

Combined, these data indicate that IgG subclass–specific cytokine production is associated with distinct metabolic reprogramming of myeloid immune cells, which is diversified even further depending on the coreceptor involved (Fig. 5).

Discussion

IgG Abs are crucial for various immune functions, including neutralization, phagocytosis, and ADCC. In this study, we identified another function of IgG, namely that human IgG tailors responses to the immunological context by orchestrating subclass-specific proinflammatory cytokine profiles by human myeloid immune cells (schematically summarized in Fig. 5). Although IgG2 strongly promoted the production of Th17-inducing proinflammatory cytokines such as IL-1β, IL-23, and TNF, IgG3 suppressed the production of type I and III IFNs, and IgG1 mediated both. Moreover, we identified that this isotype-specific cytokine production is particularly controlled at the posttranscriptional level, which was associated with distinct metabolic reprogramming of immune cells.

Although IgG subclasses are more than 90% identical at the amino acid level, each subclass has a unique profile with respect to its capacity to induce different effector responses. The prevailing concept regarding human IgG subclasses is that IgG3 is the most proinflammatory subclass (1), whereas IgG2 is sometimes even referred to as anti-inflammatory (10). However, this categorization into pro- or anti-inflammatory subclasses is mainly based on a selection of Ab effector functions, such as Ab-dependent phagocytosis and complement activation. In contrast, the capacity of IgG subclasses to elicit cytokines and chemokines, which is important for both host defense and (chronic) inflammation (13, 33), has long remained unexplored. In stark contrast to other effector functions, our data in this study suggest that IgG2 is the most potent inducer of key proinflammatory cytokines such as TNF, IL-1β, and IL-23, whereas IgG3 has very little effect on these cytokines. These data suggest that the current paradigm of pro- and anti-inflammatory IgG isotypes is likely to be an over-simplification. Instead, overall assessment of the different properties supports the concept of a "division of labor" by human IgG subclasses (Fig. 5, top panel). Although IgG3 Abs are equipped to strongly induce phagocytosis, ADCC, and complement activation, IgG2 is the main subclass that controls proinflammatory cytokine production. Interestingly, IgG1 appears to be in the middle by being able to induce both the IgG2 (cytokine production) and IgG3 (phagocytosis/ADCC/complement) effector functions, although this may come at the cost of being able to induce all these effector functions at a slightly lower level. Somewhat surprisingly, we also observed

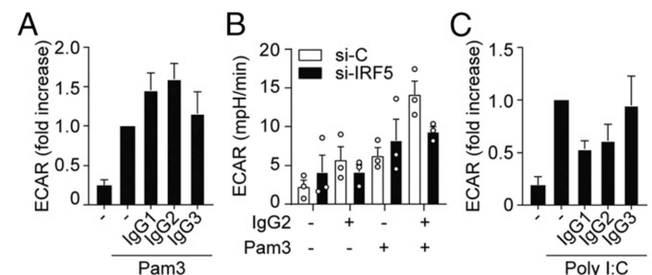

FIGURE 4. IgG subclasses induce distinct metabolic reprogramming. (**A** and **C**) DCs were stimulated with Pam3 or Poly I:C in combination with the different IgG subclasses, and ECAR was determined. Values are shown as fold increase after Pam3 or Poly I:C stimulation alone (set to 1). Mean ± SEM of six independent donors. (**B**) IRF5 in DCs was silenced (70% silencing efficiency) using control or IRF5 si-RNA, and the cells were subsequently stimulated with Pam3, IgG2, or the combination, during which the ECAR was determined. Experiments were performed in triplicate (mean ± SEM).

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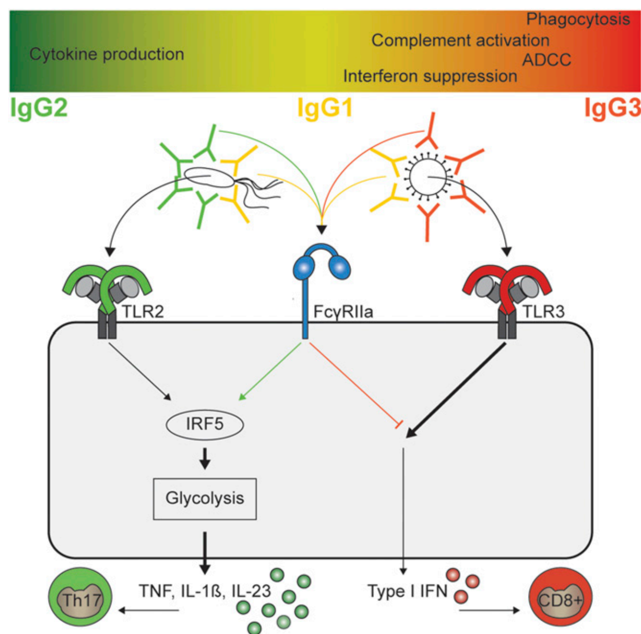

FIGURE 5. Proposed model of division of labor by IgG subclasses. Top panel, IgG2 is the main subclass that controls proinflammatory cytokine production, whereas IgG3 Abs are able to strongly induce phagocytosis and ADCC and complement activation. IgG1 appears to be in the middle. Bottom panel, FcγRIIa-TLR2 costimulation (mimicking bacterial infections) strongly upregulates the production of proinflammatory cytokines TNF, IL-1β, and IL-23 via IRF5-mediated glycolytic changes. In contrast, FcγRIIa-TLR3 costimulation (mimicking viral infections) induces suppression of type I IFN responses. IgG1 can mediate both effects.

cytokine induction by IgG4, the IgG subclass that is generally considered to have the least functional potency and little interaction with FcγRs (1, 10). Yet, a direct comparison in our assay demonstrated that IgG4 induces more proinflammatory cytokine production by myeloid immune cells than IgG3. This further underlines the concept of subclass-specific effector functions of human IgG Abs, instead of the current paradigm that divides IgG in either pro- or anti-inflammatory subclasses.

The main physiological function of IgG-induced cytokine production is to tailor inflammatory responses to the immunological context, such as the pathogen and the tissue involved (13, 33). For example, IgG opsonization of bacteria promotes the production of antibacterial Th17-inducing cytokines by DCs (12, 15), whereas IgG opsonization of viruses has very little effect of Th17-inducing cytokines and mainly affects type I IFN production (16, 20). Yet how IgG orchestrates such diverse responses has long remained enigmatic. In this study, our data indicate that IgG subclasses play a role in generating these pathogen-specific immune responses. IgG2 is the main subclass that is produced in response to bacterial Ags (3), which we now show is also the most potent inducer of human Th17-inducing cytokines IL-1β, IL-23, and TNF. Alternatively, IgG3 is the first subclass that is produced in response to viruses (3), which we, in this study, identified to suppress type I IFN production, which is important to prevent collateral damage induced by prolonged IFN production and to counteract viral infections by promoting antiviral CD8 T cell and B cell responses (16, 34–36).

In addition to pathogen-specific immunity, IgG subclasses also induced cell type–specific responses, as illustrated by differences in cytokine profile between DCs/macrophages and monocytes. Because these cell types are located in different tissues (e.g., skin versus blood), these cell type–specific responses by IgG subclasses may contribute to the generation of tissue/organ-specific immune responses, although more research would be required to confirm this. For future research, it would be very interesting to determine the IgG subclass–specific cytokine profile on other cells because IgG-IC elicit cytokine production by various different cell types, including plasmacytoid DCs (18), intestinal DCs (29), synovial macrophages (14), and nasal and lung epithelial cells (30).

IgG-IC can modulate cytokine production at three levels (i.e., gene transcription, caspase-1 activation, and gene translation) (13, 30). Interestingly, we identified that IgG subclass–specific cytokine production is regulated posttranscriptionally. Yet our data also show that all IgG subclasses are capable of modulating cytokine gene transcription and caspase-1 activation, which they do in a comparable manner. This suggests that there is a common pathway induced by all subclasses (modulating gene transcription and caspase-1 activation) as well as subclass-specific pathways (modulating only gene translation). When focusing on the subclass-specific pathways, IgG2 induces signaling by the kinase Syk and the transcription factor IRF5. This leads to an increased glycolytic rate, which in myeloid immune cells, is associated with increased inflammatory responses by promoting de novo synthesis of fatty acids for the expansion of endoplasmic reticulum required for cytokine gene translation (29, 30, 37). How FcγRIIa (co) stimulation increases the glycolytic rate and subsequent translation of selected cytokines is still unknown, but recent findings provide several potential mechanisms. One possibility is that IgG subclasses differentially activate TBK1/IKKε, which in DCs, is essential for hexokinase activity and glycolysis (37) and is also required for FcγR-induced cytokine production (30). In addition, TBK1 could induce metabolic reprogramming through STAT3 activation, which promotes proinflammatory cytokine production by inducing glycolytic and mitochondrial reprogramming (38). Another option could be differential activation of Akt2 by IRF5, which enhances the transcription of glycolytic genes (31). How metabolic reprogramming specifically amplifies particular cytokines is still unclear, but similar effects have been observed upon stimulation of FcαRI (29) and other receptors, which show that loading of mRNA with ribosomes is gene specific, with particular upregulation of genes such as *TNF* and *IL23A* (39).

In contrast to IgG2, IgG3-induced signaling for the suppression of type I IFNs is not dependent on Syk and IRF5 (16) and did not affect the glycolytic rate in these myeloid immune cells. Still little is known about the molecular mechanisms that underlie this type I IFN suppression by IgG, although it could be related to decreased IRF1 expression (16) and/or differences in fatty acid oxidation (40). Intriguingly, the two distinct signaling/metabolic pathways that are activated by IgG2 or IgG3 are induced through the same receptor (i.e., FcγRIIa) because, in human DCs, both the amplification of proinflammatory cytokines and the suppression of type I IFNs is fully dependent on FcγRIIa (12, 15, 16). This is reminiscent of other receptors such as the C-type lectin DC-SIGN, which induces pathogen-specific immunity through distinct signaling upon recognition of either mannosylated or fucosylated structures (41). What is driving this IgG subclass–specific signaling by FcγRIIa is still unclear, but it could be related to differences in affinity for the receptor (IgG3 > IgG1 > IgG2) or differences in glycosylation pattern, similar to what has recently been described for IgA subclasses (42). Interestingly, we identified even more diversity in IgG-induced signaling by showing that the activation of FcγRIIa by the same subclass can lead to the activation of different intracellular pathways because IgG2 enhanced the glycolytic rate upon costimulation with TLR2 but lowered the glycolysis upon costimulation with TLR3. Yet, although blocking of FcγRIIa completely abrogated IgG-induced cytokine responses,

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a role for nonclassical Fc receptors such as C-type lectin receptors cannot be ruled out because these receptors simultaneously recognize the Abs and are also capable of inducing signaling and metabolic pathways (43). Particularly, dectin-1 is an interesting candidate in this regard because it is expressed by myeloid immune cells, is known to synergize with TLR activation (44), and can also signal through Syk and IRF5 (45). This would add another layer of complexity to the mechanism by which IgG Abs are able to shape immune responses to the pathogen involved.

Taken together, these results indicate that human IgG subclasses shape cytokine responses through distinct metabolic reprogramming of human myeloid immune cells. Although the physiological function of this phenomenon most likely is to provide pathogen- and cell type–specific immunity, aberrancies in this inflammatory mechanism may also lead to pathology. For example, in myeloid immune cells of lupus nephritis patients, a shift in balance is observed between the two FcγRIIa-induced signaling pathways, leading to overaction of the Syk-dependent pathway (which promotes proinflammatory cytokines) and inactivation of the Syk-independent pathway (which suppresses type I IFN production) (20), thereby promoting local kidney inflammation. In addition, these findings may be relevant for future design of Ab-related therapies. Currently, the overwhelming majority of therapeutic Abs is of the IgG1 subclass (46), whereas the use of other subclasses may help to skew immune responses depending on the infection of disease in question (e.g., as previously observed in the context of cancer therapy) (47).

Disclosures

D.L.P.B. is an employee of Union Chimique Belge. The other authors have no financial conflicts of interest.

References

1. Lu, L. L., T. J. Suscovich, S. M. Fortune, and G. Alter. 2018. Beyond binding: antibody effector functions in infectious diseases. *Nat. Rev. Immunol.* 18: 46–61.
2. Nimmerjahn, F., and J. V. Ravetch. 2008. Fcgamma receptors as regulators of immune responses. *Nat. Rev. Immunol.* 8: 34–47.
3. Vidarsson, G., G. Dekkers, and T. Rispens. 2014. IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol.* 5: 520.
4. Ferrante, A., L. J. Beard, and R. G. Feldman. 1990. IgG subclass distribution of antibodies to bacterial and viral antigens. *Pediatr. Infect. Dis. J.* 9(8 Suppl.): S16–S24.
5. Siber, G. R., P. H. Schur, A. C. Aisenberg, S. A. Weitzman, and G. Schiffman. 1980. Correlation between serum IgG-2 concentrations and the antibody response to bacterial polysaccharide antigens. *N. Engl. J. Med.* 303: 178–182.
6. Barrett, D. J., and E. M. Ayoub. 1986. IgG2 subclass restriction of antibody to pneumococcal polysaccharides. *Clin. Exp. Immunol.* 63: 127–134.
7. Schauer, U., F. Stemberg, C. H. Rieger, W. Büttner, M. Borte, S. Schubert, H. Möllers, F. Riedel, U. Herz, H. Renz, and W. Herzog. 2003. Levels of antibodies specific to tetanus toxoid, *Haemophilus influenzae* type b, and pneumococcal capsular polysaccharide in healthy children and adults. *Clin. Diagn. Lab. Immunol.* 10: 202–207.
8. Walker, M. R., A. A. Eltahla, M. M. Mina, H. Li, A. R. Lloyd, and R. A. Bull. 2020. Envelope-specific IgG3 and IgG1 responses are associated with clearance of acute hepatitis C virus infection. *Viruses* 12: 75.
9. Damelang, T., S. J. Rogerson, S. J. Kent, and A. W. Chung. 2019. Role of IgG3 in infectious diseases. *Trends Immunol.* 40: 197–211.
10. Chen, K., G. Magri, E. K. Grasset, and A. Cerutti. 2020. Rethinking mucosal antibody responses: IgM, IgG and IgD join IgA. *Nat. Rev. Immunol.* 20: 427–441.
11. Sokolove, J., X. Zhao, P. E. Chandra, and W. H. Robinson. 2011. Immune complexes containing citrullinated fibrinogen costimulate macrophages via toll-like receptor 4 and Fcγ receptor. *Arthritis Rheum.* 63: 53–62.
12. den Dunnen, J., L. T. Vogelpoel, T. Wypych, F. J. Muller, L. de Boer, T. W. Kuijpers, S. A. Zaat, M. L. Kapsenberg, and E. C. de Jong. 2012. IgG opsonization of bacteria promotes Th17 responses via synergy between TLRs and FcγRIIa in human dendritic cells. *Blood* 120: 112–121.
13. Vogelpoel, L. T. C., D. L. P. Baeten, E. C. de Jong, and J. den Dunnen. 2015. Control of cytokine production by human fc gamma receptors: implications for pathogen defense and autoimmunity. *Front. Immunol.* 6: 79.
14. Vogelpoel, L. T. C., I. S. Hansen, T. Rispens, F. J. M. Muller, T. M. M. van Capel, M. C. Turina, J. B. Vos, D. L. P. Baeten, M. L. Kapsenberg, E. C. de Jong, and J. den Dunnen. 2014. Fc gamma receptor-TLR cross-talk elicits pro-inflammatory cytokine production by human M2 macrophages. *Nat. Commun.* 5: 5444.
15. Bakema, J. E., C. W. Tuk, S. J. van Vliet, S. C. Bruijns, J. B. Vos, S. Letsiou, C. D. Dijkstra, Y. van Kooyk, A. B. Brenkman, and M. van Egmond. 2015. Antibody-opsonized bacteria evoke an inflammatory dendritic cell phenotype and polyfunctional Th cells by cross-talk between TLRs and FcRs. *J. Immunol.* 194: 1856–1866.
16. Newling, M., W. Hoepel, L. T. C. Vogelpoel, M. H. Heineke, J. A. van Burgsteden, E. W. M. Taanman-Kueter, D. Eggink, T. W. Kuijpers, T. Beaumont, M. van Egmond, et al. 2018. Fc gamma receptor IIa suppresses type I and III interferon production by human myeloid immune cells. *Eur. J. Immunol.* 48: 1796–1809.
17. Polak, M. E., L. Newell, V. Y. Taraban, C. Pickard, E. Healy, P. S. Friedmann, A. Al-Shamkhani, and M. R. Ardern-Jones. 2012. CD70-CD27 interaction augments CD8+ T-cell activation by human epidermal Langerhans cells. *J. Invest. Dermatol.* 132: 1636–1644.
18. Båve, U., M. Magnusson, M.-L. Eloranta, A. Perers, G. V. Alm, and L. Rönnblom. 2003. Fc gamma RIIa is expressed on natural IFN-alpha-producing cells (plasmacytoid dendritic cells) and is required for the IFN-alpha production induced by apoptotic cells combined with lupus IgG. *J. Immunol.* 171: 3296–3302.
19. Means, T. K., E. Latz, F. Hayashi, M. R. Murali, D. T. Golenbock, and A. D. Luster. 2005. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. *J. Clin. Invest.* 115: 407–417.
20. Newling, M., R. H. Fiechter, L. Sritharan, W. Hoepel, J. A. van Burgsteden, A. E. Hak, R. F. van Vollenhoven, M. G. H. van de Sande, D. L. P. Baeten, and J. den Dunnen. 2020. Dysregulated Fcγ receptor IIa-induced cytokine production in dendritic cells of lupus nephritis patients. *Clin. Exp. Immunol.* 199: 39–49.
21. Golebski, K., W. Hoepel, D. van Egmond, E. J. de Groot, G. D. Amatngalim, J. M. Beekman, W. J. Fokkens, C. M. van Drunen, and J. den Dunnen. 2019. FcγRIII stimulation breaks the tolerance of human nasal epithelial cells to bacteria through cross-talk with TLR4. *Mucosal Immunol.* 12: 425–433.
22. Bruhns, P., B. Iannascoli, P. England, D. A. Mancardi, N. Fernandez, S. Jorieux, and M. Daëron. 2009. Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. *Blood* 113: 3716–3725.
23. Vogelpoel, L. T., I. S. Hansen, M. W. Visser, S. Q. Nagelkerke, T. W. Kuijpers, M. L. Kapsenberg, E. C. de Jong, and J. den Dunnen. 2015. FcγRIIa cross-talk with TLRs, IL-1R, and IFNγR selectively modulates cytokine production in human myeloid cells. *Immunobiology* 220: 193–199.
24. Vidarsson, G., A. M. Stemerding, N. M. Stapleton, S. E. Spliethoff, H. Janssen, F. E. Rebers, M. de Haas, and J. G. van de Winkel. 2006. FcRn: an IgG receptor on phagocytes with a novel role in phagocytosis. *Blood* 108: 3573–3579.
25. Saeland, E., G. Vidarsson, J. H. W. Leusen, E. Van Garderen, M. H. Nahm, H. Vile-Weekhout, V. Walraven, A. M. Stemerding, J. S. Verbeek, G. T. Rijkers, et al. 2003. Central role of complement in passive protection by human IgG1 and IgG2 anti-pneumococcal antibodies in mice. *J. Immunol.* 170: 6158–6164.
26. de Taeye, S. W., A. E. H. Bentlage, M. M. Mebius, J. I. Meesters, S. Lissenberg-Thunnissen, D. Falck, T. Sénard, N. Salehi, M. Wuhrer, J. Schuurman, et al. 2020. FcγR binding and ADCC activity of human IgG allotypes. *Front. Immunol.* 11: 740.
27. Lux, A., X. Yu, C. N. Scanlan, and F. Nimmerjahn. 2013. Impact of immune complex size and glycosylation on IgG binding to human FcγRs. *J. Immunol.* 190: 4315–4323.
28. Zhong, Q., F. Y. Gong, Z. Gong, S. H. Hua, K. Q. Zeng, and X. M. Gao. 2018. IgG immunocomplexes sensitize human monocytes for inflammatory hyperactivity via transcriptomic and epigenetic reprogramming in rheumatoid arthritis. *J. Immunol.* 200: 3913–3925.
29. Hansen, I. S., L. Krabbendam, J. H. Bernink, F. Loayza-Puch, W. Hoepel, J. A. van Burgsteden, E. C. Kuijper, C. J. Buskens, W. A. Bemelman, S. A. J. Zaat, et al. 2018. FcαRI co-stimulation converts human intestinal $CD103^{+}$ dendritic cells into pro-inflammatory cells through glycolytic reprogramming. *Nat. Commun.* 9: 863.
30. Hoepel, W., M. Newling, L. T. C. Vogelpoel, L. Sritharan, I. S. Hansen, M. L. Kapsenberg, D. L. P. Baeten, B. Everts, and J. den Dunnen. 2019. FcγR-TLR cross-talk enhances TNF production by human monocyte-derived DCs via IRF5-dependent gene transcription and glycolytic reprogramming. *Front. Immunol.* 10: 739.
31. Hedl, M., J. Yan, and C. Abraham. 2016. IRF5 and IRF5 disease-risk variants increase glycolysis and human M1 macrophage polarization by regulating proximal signaling and Akt2 activation. *Cell Rep.* 16: 2442–2455.
32. Wick, A. N., D. R. Drury, H. I. Nakada, and J. B. Wolfe. 1957. Localization of the primary metabolic block produced by 2-deoxyglucose. *J. Biol. Chem.* 224: 963–969.
33. van Egmond, M., G. Vidarsson, and J. E. Bakema. 2015. Cross-talk between pathogen recognizing Toll-like receptors and immunoglobulin Fc receptors in immunity. *Immunol. Rev.* 268: 311–327.
34. Moseman, E. A., T. Wu, J. C. de la Torre, P. L. Schwartzberg, and D. B. McGavern. 2016. Type I interferon suppresses virus-specific B cell responses by modulating $CD8^{+}$ T cell differentiation. *Sci. Immunol.* 1: eaah3565.
35. Crouse, J., U. Kalinke, and A. Oxenius. 2015. Regulation of antiviral T cell responses by type I interferons. *Nat. Rev. Immunol.* 15: 231–242.
36. Nishimura, Y., R. Gautam, T.-W. Chun, R. Sadjadpour, K. E. Foulds, M. Shingai, F. Klein, A. Gazumyan, J. Golijanin, M. Donaldson, et al. 2017. Early antibody therapy can induce long-lasting immunity to SHIV. *Nature* 543: 559–563.
37. Everts, B., E. Amiel, S. C.-C. Huang, A. M. Smith, C. H. Chang, W. Y. Lam, V. Redmann, T. C. Freitas, J. Blagih, G. J. W. van der Windt, et al. 2014. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKK supports the anabolic demands of dendritic cell activation. *Nat. Immunol.* 15: 323–332.

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38. Balic, J. J., H. Albargy, K. Luu, F. J. Kirby, W. S. N. Jayasekara, F. Mansell, D. J. Garama, D. De Nardo, N. Baschuk, C. Louis, et al. 2020. STAT3 serine phosphorylation is required for TLR4 metabolic reprogramming and IL-1β expression. *Nat. Commun.* 11: 3816.
39. Schott, J., S. Reitter, J. Philipp, K. Haneke, H. Schäfer, and G. Stoecklin. 2014. Translational regulation of specific mRNAs controls feedback inhibition and survival during macrophage activation. *PLoS Genet.* 10: e1004368.
40. Wu, D., D. E. Sanin, B. Everts, Q. Chen, J. Qiu, M. D. Buck, A. Patterson, A. M. Smith, C. H. Chang, Z. Liu, et al. 2016. Type 1 interferons induce changes in core metabolism that are critical for immune function. *Immunity* 44: 1325–1336.
41. Gringhuis, S. I., J. den Dunnen, M. Litjens, M. van der Vlist, and T. B. H. Geijtenbeek. 2009. Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to *Mycobacterium tuberculosis*, HIV-1 and *Helicobacter pylori*. *Nat. Immunol.* 10: 1081–1088.
42. Steffen, U., C. A. Koeleman, M. V. Sokolova, H. Bang, A. Kleyer, J. Rech, H. Unterweger, M. Schicht, F. Garreis, J. Hahn, et al. 2020. IgA subclasses have different effector functions associated with distinct glycosylation profiles. *Nat. Commun.* 11: 120.
43. Kara, S., L. Amon, J. J. Lühr, F. Nimmerjahn, D. Dudziak, and A. Lux. 2020. Impact of plasma membrane domains on IgG Fc receptor function. *Front. Immunol.* 11: 1320.
44. Gringhuis, S. I., J. den Dunnen, M. Litjens, M. van der Vlist, B. Wevers, S. C. M. Bruijns, and T. B. H. Geijtenbeek. 2009. Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. *Nat. Immunol.* 10: 203–213.
45. del Fresno, C., D. Soulat, S. Roth, K. Blazek, I. Udalova, D. Sancho, J. Ruland, and C. Ardavín. 2013. Interferon-β production via Dectin-1-Syk-IRF5 signaling in dendritic cells is crucial for immunity to C. albicans. *Immunity* 38: 1176–1186.
46. Irani, V., A. J. Guy, D. Andrew, J. G. Beeson, P. A. Ramsland, and J. S. Richards. 2015. Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases. *Mol. Immunol.* 67: 171–182.
47. White, A. L., H. T. C. Chan, R. R. French, J. Willoughby, C. I. Mockridge, A. Roghanian, C. A. Penfold, S. G. Booth, A. Dodhy, M. E. Polak, et al. 2015. Conformation of the human immunoglobulin G2 hinge imparts superagonistic properties to immunostimulatory anticancer antibodies. *Cancer Cell* 27: 138–148.

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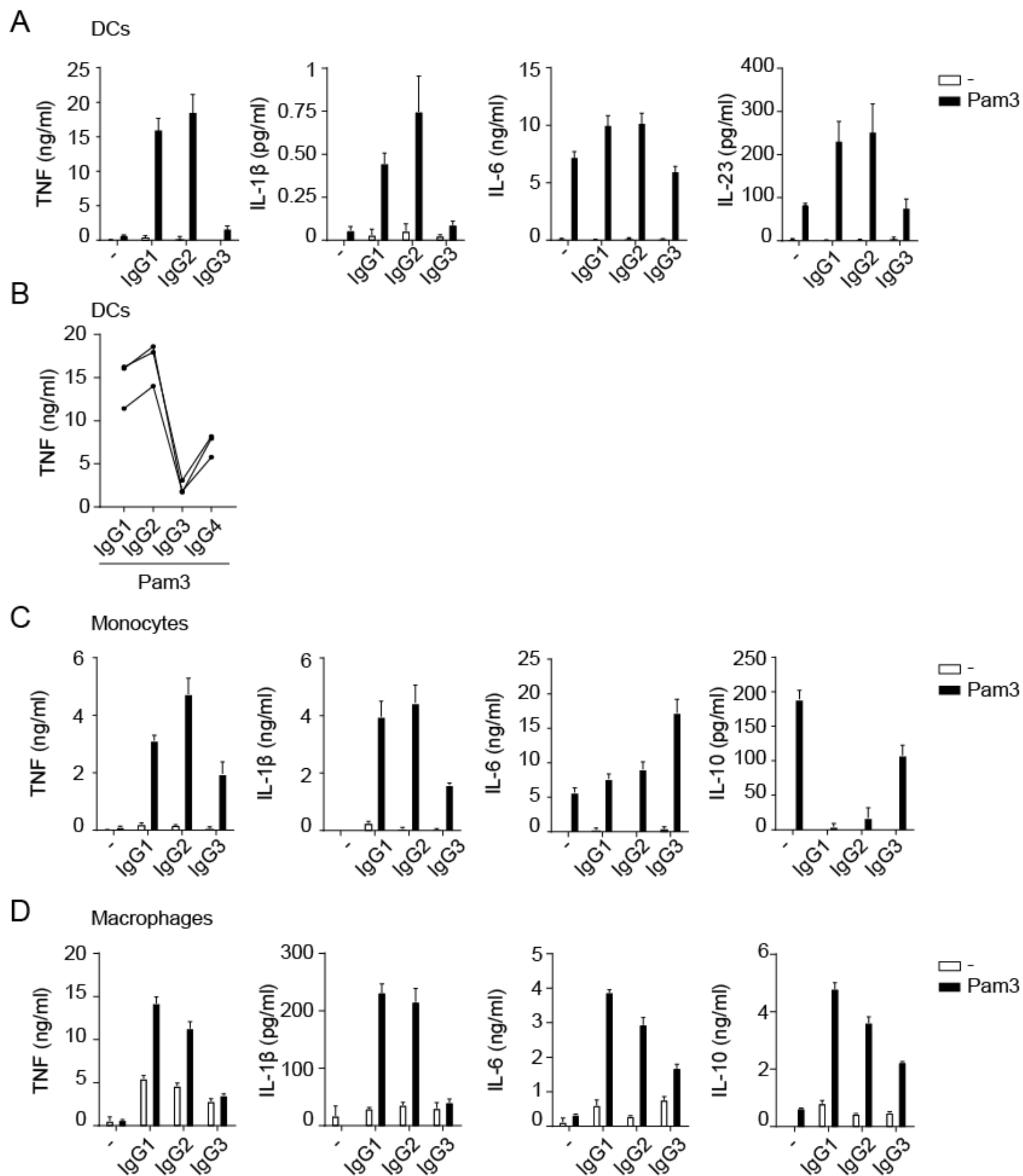
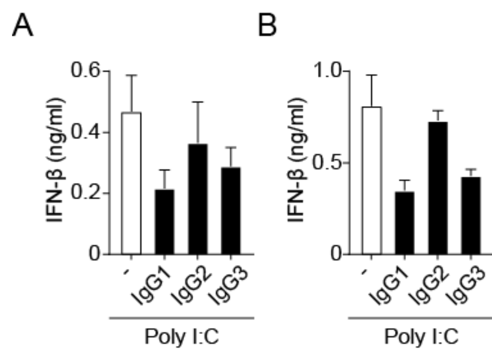
Suppl Figure 1



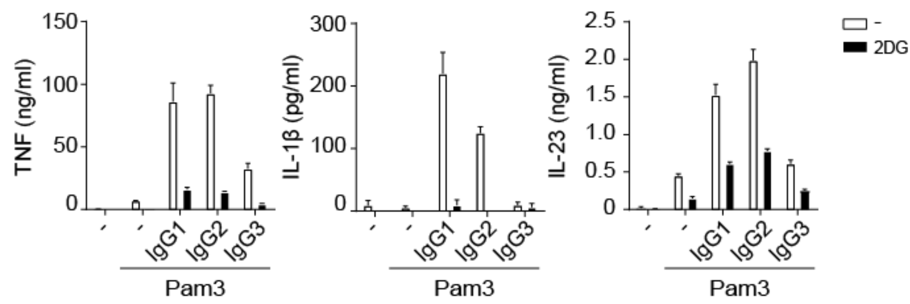
Supplementary figure 1. IgG-induced pro-inflammatory cytokine production is dependent on IgG1 and IgG2**. (a,b)** Human monocyte-derived dendritic cells (DCs), **(c)** human monocytes, and **(d)** human monocyte-derived macrophages were stimulated for 24h with 10µg/ml Pam3CSK4 (Pam3), IgG-IC or a combination and cytokine production in supernatant was measured by ELISA. **(a, c, d)** representative example performed in triplicate of **(a)** 9, **(c)** 6, or **(d)** 4 independent donors (Mean ± SEM). **(b)** Data shown of 3 independent experiments with different donors performed in triplicate. Each line represents one donor.

Suppl Figure 2



Supplementary figure 2. Suppression of type I IFNs is dependent on IgG1 and IgG3. **(a-c)** DCs were stimulated for 24h with 20µg/ml Poly I:C or in combination with IgG-IC. **(a,b)** IFN-β production in supernatant was determined by ELISA. **(a)** representative example performed in triplicate of **(b)** four independent experiment with different donors (Mean ± SEM).

Suppl Figure 3



Supplementary figure 3. Pro-inflammatory cytokine production by all IgG subclasses is dependent on glycolysis. Human monocyte-derived dendritic cells (DCs) were stimulated for 24h with 10µg/ml Pam3CSK4 (Pam3) in combination with IgG-IC made from the different IgG subclasses together with 10mM of the glycolytic inhibitor 2-deoxglucose (2DG) and cytokine production in supernatant was measured by ELISA. Representative example of 3 independent experiments with different donors performed in triplicate (Mean ± SEM).