Tolerance induction with T cell–dependent protein antigens induces regulatory sialylated IgGs

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Background: Under inflammatory conditions, T cell–dependent (TD) protein antigens induce proinflammatory T- and B-cell responses. In contrast, tolerance induction by TD antigens without costimulation triggers the development of regulatory T cells. Under both conditions, IgG antibodies are generated, but whether they have different immunoregulatory functions remains elusive.
Objective: It was shown recently that proinflammatory or anti-inflammatory effector functions of IgG molecules are determined by different Fc N-linked glycosylation patterns. We sought to examine the Fc glycosylation and anti-inflammatory quality of IgG molecules formed on TD tolerance induction.
Methods: We administered chicken ovalbumin (OVA) with or without costimulus to mice and analyzed OVA-reactive IgG Fc glycosylation. The anti-inflammatory function of differentially glycosylated anti-OVA IgGs was further investigated in studies with dendritic cell cultures and in an *in vivo* model of allergic airway disease. Additionally, we analyzed the Fc glycosylation pattern of birch pollen–reactive serum IgGs after successful allergen-specific immunotherapy in patients.
Results: Stimulation with TD antigens under inflammatory conditions induces plasma cells expressing low levels of α2,6-sialyltransferase and producing desialylated IgGs. In contrast, plasma cells induced on tolerance induction did not downregulate α2,6-sialyltransferase expression and secreted immunosuppressive sialylated IgGs that were sufficient to block antigen-specific T- and B-cell responses, dendritic cell maturation, and allergic airway inflammation. Importantly, successful specific immunotherapy in allergic patients also induced sialylated allergen-specific IgGs.
Conclusions: Our data show a novel antigen-specific immunoregulatory mechanism mediated by anti-inflammatory sialylated IgGs that are formed on TD tolerance induction. These findings might help to develop novel antigen-specific therapies for the treatment of allergy and autoimmunity.
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Key words: *Tolerance, IgG antibodies, IgG sialylation, dendritic cells, allergy, asthma, antigen-specific immunotherapy, antigen-specific tolerance, antibody therapy*

The initiation and termination of immune responses require tight regulation to prevent inflammatory immune responses to nonpathogenic organisms or antigens and to minimize the risk of immune diseases, such as allergy, autoimmunity, or inflammatory bowel disease. T cell–dependent (TD) protein antigens are known

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Abbreviations used
CFA: Complete Freund adjuvant
DC: Dendritic cell
DTH: Delayed-type hypersensitivity
IVIG: Intravenous immunoglobulin
OVA: Ovalbumin
PC: Plasma cell
SIT: Specific immunotherapy
TD: T cell dependent
TNP: 2,4,6-Trinitrophenyl

to elicit strong inflammatory immune responses if recognized in the presence of inflammatory costimulatory signals, whereas lack of inflammatory costimulation leads to the induction of regulatory T cells and peripheral tolerance.[1-3] IgG antibodies are generated under both conditions, but whether the IgGs themselves provide different immunoregulatory effector functions is yet unknown.

The proinflammatory and anti-inflammatory effector functions of IgG subclass antibodies are mediated by their different affinities to activating Fcγ receptors (FcγRs), and the expression ratio between activating FcγRs and the inhibitory FcγRIIB on immune cells.[4-6] However, it was recently shown that the proinflammatory and anti-inflammatory effector functions of IgG antibodies are also regulated by Fc N-linked glycosylation.[7-9] Desialylation of serum IgG correlates with proinflammatory responses and disease activity in patients with rheumatoid arthritis.[10,11] In contrast, sialylated IgGs mediate the immunosuppressive effects of intravenous immunoglobulin (IVIG), which is purified from pooled human plasma and administered at high doses (2 g/kg) for the treatment of autoimmunity and allergy.[7-9,12,13] However, the inductive mechanisms for the formation of sialylated IgG antibodies are still unknown.

Here we investigated the influence of TD B-cell activation without or in the presence of an inflammatory costimulus on IgG Fc glycosylation. Our data show that tolerance induction with TD antigens induces plasma cells (PCs) expressing high levels of α2,6-sialyltransferase and producing regulatory sialylated IgG_1 in mice. Antigen-specific sialylated murine IgG_1 antibodies thereby protect against dendritic cell (DC) maturation and pathologic TD immune reactions in an antigen-specific manner. Additionally, formation of sialylated IgG antibodies was observed in human allergic patients after successful tolerance induction by using allergen-specific immunotherapy (SIT).

METHODS

Mice

C57BL/6 and BALB/c mice were purchased from Charles River Laboratories (Bar Harbor, Me). FcγRIIB$^{-/-}$ and IL-4 receptor α$^{-/-}$ mice were on the C57BL/6 background.[14-16] Mice were bred and maintained in accordance with institutional guidelines. Eight- to 12-week-old female mice were analyzed.

Reagents

Ovalbumin (OVA) was purchased from Sigma (St Louis, Mo; no. A5503). 2,4,6-Trinitrophenyl (TNP)(12)-FICOLL and TNP(12)-BSA were purchased from Biosearch Technology (Novato, Calif). TNP(3)-OVA was prepared with TNP-e-aminocaproyl-OSu (Biosearch Technologies, no. T-1030) in house. IVIG (Intracet) was from Biotest (Boca Raton, Fla). LPS from *Escherichia coli* (no. L4524), complete Freund adjuvant (CFA; no. F5881; 1 mg mycobacterium tuberculosis/mL), and incomplete Freund adjuvant (no. F5506)) were purchased from Sigma. Enriched CFA was prepared by adding heat-killed *Mycobacterium tuberculosis* H37 RA (DIFCO Laboratories, Detroit, Mich) to incomplete Freund adjuvant (5 mg mycobacterium tuberculosis/mL).

CD4$^+$ cell depletion and inhibition of the CD40/CD40 ligand interaction

Mice were treated with sequential intraperitoneal injections of 250 μg of anti-mouse CD4 (clone GK1.5) or anti-mouse CD154 (MR-1), respectively, to deplete CD4$^+$ cells or to block the CD40/CD40 ligand interaction.

Synthesis of Dec-OVA in 293T human embryonic kidney cells

Dec-OVA IgG_1[17] fusion antibodies were produced by means of polyethylenimine-mediated cotransfection of human embryonic kidney cells in serum-free medium containing 0.03% Primatone RL/UF (Sheffield Bio-Science, Norwich, NY) with IgH and IgL chain–encoding plasmid DNA.

OVA immunization and anti-OVA IgG antibody purification

Eight-week-old C57BL/6 mice were immunized as indicated. Serum samples were taken at day 14, and pooled serum IgG antibodies were purified with protein G–sepharose. OVA-reactive IgG was purified by using OVA coupled to cyanogen bromide–activated sepharose 4B (GE Healthcare, Fairfield, Conn) columns (in-house preparation). Enrichment of OVA-reactive IgG was verified by using ELISA.

In vitro sialylation and desialylation of IgG antibodies

The *in vitro* sialylation of the murine anti-TNP IgG_1 (clone H5)[18] and anti-OVA IgG_1 hybridoma (clone 4C9, unpublished) antibodies was performed in a 2-step procedure, as described previously.[8] Briefly, antibodies were galactosylated with human β1,4-galactosyltransferase and uridine diphosphate galactose and subsequently sialylated with human α2,6-sialyltransferase and CMP-sialic acid (all from Calbiochem, Nottingham, United Kingdom). Desialylation of the anti-OVA IgG_1 (4C9) was performed with a ProZyme Sialidase kit (no. GK80040; ProZyme, Hayward, Calif).

Human samples

Human blood was provided by healthy volunteers (n = 5), volunteers with untreated birch pollen allergy in or after the pollen season (n = 7), and 2 patients with birch pollen allergy after the pollen season (n = 2, SIT P1 *1998 and SIT P2 *1959) who had received complete and successful subcutaneous SIT (Allergovit; Allergopharma, Zurich, Switzerland) over 3 years (autumn 2008 to summer 2011). Serum analyses from patients were approved by local authorities, and written informed consent was obtained from the patients.

Total and anti–Bet v 1 human serum IgG purification

Human serum IgG was purified with protein G–sepharose. Bet v 1–reactive IgG was enriched by using rBet v 1 coupled to cyanogen bromide–activated sepharose 4B columns (in-house preparation). Enrichment of Bet v 1–reactive IgG was verified by means of ELISA. rBet v 1 was expressed in *E coli* BL21 by cloning the gene for Bet v 1 (accession no. X15877)[19] into the pET23b vector and adding a C-terminal His-tag sequence (Novagen). Bet v 1 purification was performed by using metal affinity chromatography (Talon Superflow; Clontech, Mountain View, Calif).

Glycan analysis

IgG samples were digested with recombinantly expressed endoglycosidase S from *Streptococcus pyogenes* purified as previously described.[20] The