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ORIGINAL ARTICLE

Experimental Models of Allergic Disease

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Maternal asthma is associated with persistent changes in allergic offspring antibody glycosylation

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Abstract

Background: Maternal asthma during pregnancy is considered an environmental risk factor for asthma development in children. Immunoglobulin G (IgG) antibodies that are transferred from the mother to the fetus are known to act in a pro- or anti-inflammatory manner depending on their glycosylation status.

Objective: Using a mouse model, we examined how maternal allergic airway inflammation during pregnancy influenced offspring experimental asthma severity, as well as maternal and offspring serum IgG antibody glycosylation patterns. Additionally, the effects of maternal and offspring exposure to the same or different allergens were investigated.

Methods: Female mice were either sham sensitized or sensitized to casein (CAS) or ovalbumin (OVA) before mating. Subsequently, allergic lung inflammation was induced in pregnant dams via aerosol allergen challenge (sham, CAS or OVA). After weaning, pups were subjected to an experimental asthma protocol using OVA. Asn-297 IgG glycosylation was analysed in maternal and offspring serum.

Results: When mothers and offspring were sensitized to the same allergen (OVA-OVA), offspring had more severe experimental asthma. This was evidenced by altered antibody concentrations, increased bronchoalveolar lavage inflammatory cell influx and decreased lung tissue and lung draining lymph node regulatory T cell percentages. When mothers and offspring were sensitized to different allergens (CAS-OVA), this phenotype was no longer observed. Additionally, maternal serum from allergic mothers had significantly higher levels of pro-inflammatory IgG1, shown by decreased galactosylation and sialylation at the Asn-297 glycosylation site. Similar glycosylation patterns were observed in the serum of adult allergic offspring from allergic mothers.

Conclusions and Clinical Relevance: We observed a strong association between maternal experimental asthma during pregnancy, increased offspring airway inflammation and pro-inflammatory IgG glycosylation patterns in mothers and offspring. IgG

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glycosylation is not a standard measurement in the clinical setting, and we argue that it may be an important parameter to include in future clinical studies.

KEYWORDS

allergic airway inflammation, asthma risk, IgG glycosylation, maternal asthma, pregnancy, sialylation

1 | INTRODUCTION

Asthma is a chronic obstructive airway disease characterized by airway inflammation and hyperreactivity leading to recurring episodes of respiratory symptoms including cough and shortness of breath. More than 300 million people around the world suffer from asthma, and its incidence is increasing in many countries, especially among children.[1,2] Though parental history plays a major role in asthma development in children, genetics alone cannot explain why asthma frequency has been increasing over the past decades.[3] In fact, exogenous risk factors during pregnancy and early life are described to play a crucial role in asthma pathogenesis.[4] Supporting this, retrospective human studies indicate that maternal asthma represents a stronger risk factor than paternal asthma.[5-7] Moreover, mouse models demonstrate that maternal asthma exacerbation during pregnancy results in increased asthma severity in the offspring.[8-10] However, the underlying mechanisms that contribute to this phenomenon remain incompletely understood.

During pregnancy and breastfeeding, transfer of maternal immunoglobulin G (IgG) antibodies protects the offspring from pathogens, while the humoral immune response is still inefficient. Importantly, IgG antibodies can act either pro- or anti-inflammatory, depending on complex N-glycans attached to the asparagine (Asn)-297 residue on the fragment crystallizable (Fc) region.[11] Structurally, this N-glycan consists of a core containing four N-acetylglucosamine residues and three mannose molecules, shown in Figure 1A. Integration of galactose and sialic acid residues on these branched carbohydrate chains alters the structure of the antibody, changing antibody receptor binding properties and increasing the anti-inflammatory properties of the molecule,[12,13] shown in Figure 1B.

IgG glycosylation patterns have been correlated with disease severity in humans and serve as a therapeutic target. For instance, in autoimmune diseases, exacerbations are characterized by an increased frequency of agalactosylated IgG (G0) and a decreased frequency of sialic acid.[14-16] This imbalance of IgG glycosylation can be therapeutically compensated by administration of high dose intravenous immunoglobulin (IVIG), which leads to an increase in anti-inflammatory sialylated antibodies.[17] Importantly, in allergen-specific

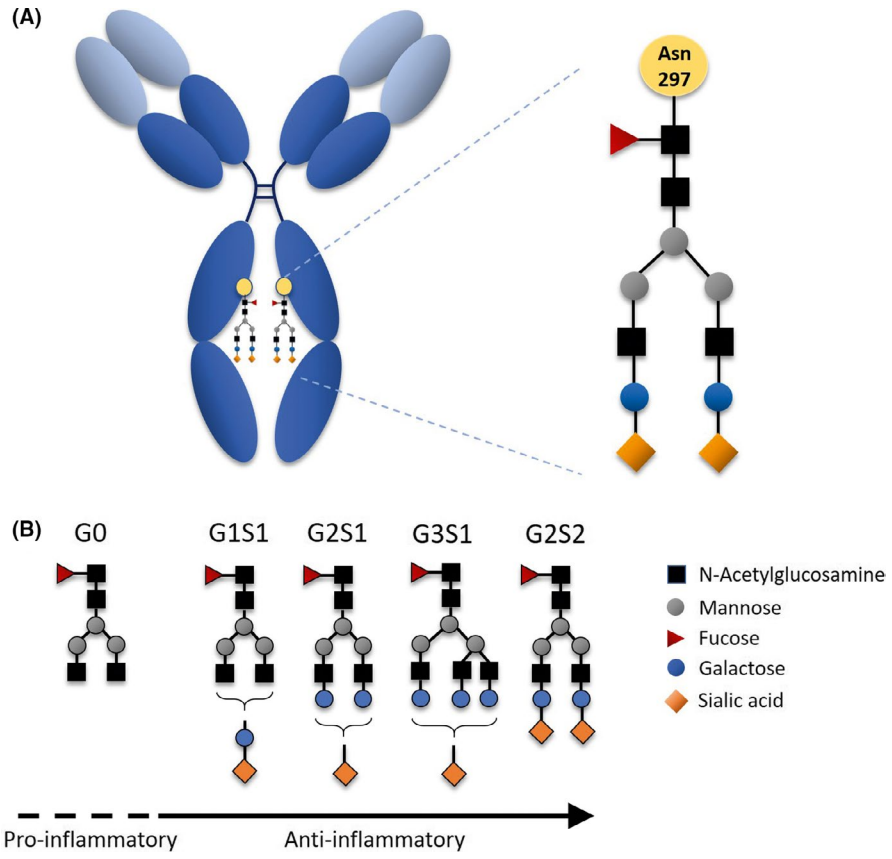


FIGURE 1 Schematic of IgG1 glycosylation. A, Illustration of an IgG molecule showing the two Asn-297 N-liked glycosylation sites in the Fc region of the antibody. The core component of the glycan molecule consists of four N-acetylglucosamine residues (black) and three branched mannose molecules (grey). This core structure can be modified by the addition of a fucose (red), or galactose (blue) and sialic acid (orange). The inset shows a fucosylated, galactosylated and sialylated N-glycan (G2S2). B, Representation of the different Asn-297 glycosylation structures reported in this study. All structures contain a core fucose. Pro-inflammatory agalactosylated glycan: G0. Anti-inflammatory galactose and sialic acid containing glycans: G1S1, G2S1, G3S1, G2S2

immunotherapy, successful tolerance induction is associated with generation of sialylated antigen-specific IgG.[18,19]

IgG glycosylation is known to be influenced by the environment[11] and thus directly reflects the physiological status of an individual. In the case of maternal asthma during pregnancy, since the developing fetus is exposed to transferred IgG from the mother, it is important to consider both IgG concentrations and glycosylation status. Upon transfer to the fetus or neonate, these pro- or anti-inflammatory signals could strongly influence immune system development and predispose the offspring to inflammation or allergy susceptibility. Studies have shown that the levels of galactosylated and sialylated serum IgG increase during pregnancy and decrease postpartum.[20] However, to our knowledge, this is the first study examining IgG glycosylation in relation to maternal asthma during pregnancy and offspring asthma susceptibility.

Here, we demonstrate that asthma exacerbation during pregnancy alters maternal serum IgG1 and IgG3 glycosylation patterns towards a pro-inflammatory phenotype. We further show that these maternal IgG antibody glycosylation patterns persisted in adult allergic offspring and were associated with increased inflammatory responses in offspring experimental asthma.

2 | METHODS

2.1 | Animals

Eight-week-old female BALB/c mice were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and housed four animals per cage in a 12/12-h light/dark cycle. Food and water were available ad libitum. All animal experiments were approved by local authorities and were performed in accordance with German and international guidelines.

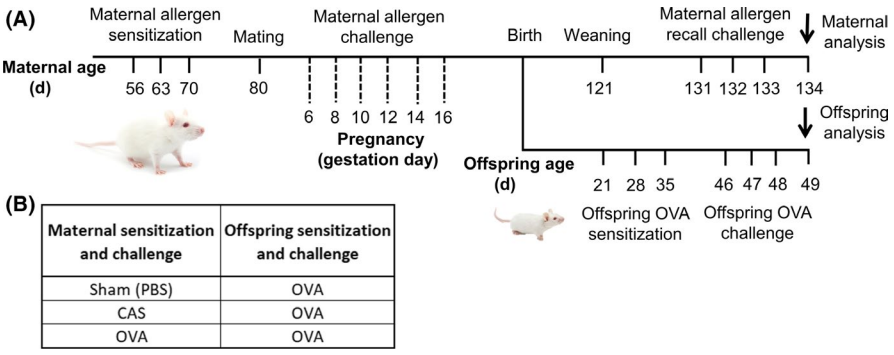
2.2 | Experimental design

2.2.1 | Allergen sensitization and challenge—Dams

Using an adjuvant-free, experimental asthma protocol,[21] 8-week-old female BALB/c mice were subcutaneously sensitized once weekly for three weeks (maternal age 8, 9 and 10 weeks) with either 10 µg casein (CAS) (Merck) or 10 µg ovalbumin (OVA) (grade VI, Merck) in 200 µL phosphate-buffered saline (PBS). Controls were sham sensitized with PBS (Figure 2A,B). After a two-week rest period, 12-week-old sensitized females were mated with non-sensitized, male BALB/c mice, and visualization of a mucous plug was considered gestation day (G)0. On G6, G8, G10, G12, G14 and G16 pregnant mice were exposed for 20 minutes to an aerosol of either 1% CAS or 1% OVA to elicit the asthma phenotype during pregnancy. Control pregnant mice were sham exposed to PBS aerosol. Pups were born and weaned at 21 days of age. Since taking blood can have a substantial impact on mouse physiology,[22] and we did not want to interfere with the pregnant mice or the possible offspring outcome, we chose maternal recall challenge as a method to evaluate maternal allergic airway inflammation. Ten days after offspring weaning, the maternal asthma phenotype was assessed by exposing dams to an aerosol recall challenge of PBS, CAS or OVA, respectively (20 minutes per day for 3 days) (Figure 2A,B).

2.2.2 | Allergen sensitization and challenge—Offspring

At 21 days of age, offspring were weaned from their mothers and immediately sensitized using an adjuvant-free, experimental asthma protocol. Specifically, at 21, 28 and 35 days of age, all female offspring were subcutaneously sensitized with 10 µg OVA (grade VI, Merck) in 200 µL PBS. At 46, 47 and 48 days of age,



Maternal sensitization and challenge	Offspring sensitization and challenge
Sham (PBS)	OVA
CAS	OVA
OVA	OVA

FIGURE 2 Protocol for testing the effect of maternal asthma during pregnancy on offspring asthma risk. This model allows comparison of mothers and offspring that are allergic to different allergens (CAS/OVA) or the same allergen (OVA/OVA). A, *Maternal treatment:* Before mating, female mice were subcutaneously sensitized with either casein (CAS) or ovalbumin (OVA) at 56, 63 and 70 d of age. Control female mice were sham-sensitized with phosphate-buffered saline (PBS). After mating, pregnant mice were aerosol challenged with the respective allergen (CAS or OVA) or control (PBS) every second day from gestation day (G) 6 to G16. Pups were born, and 10 d after weaning, the maternal asthma phenotype was assessed by exposing dams to an aerosol recall challenge of PBS, CAS or OVA, respectively. *Offspring treatment:* At 21, 28 and 35 d of age, offspring were subcutaneously sensitized to OVA. Offspring were aerosol challenged with OVA at 46, 47 and 48 d of age, and analysis was performed 24 h after the last challenge on offspring day 49. B, Summary of the allergens used for maternal and offspring experimental asthma sensitization and challenge

the asthma phenotype was induced by exposing the offspring for 20 minutes to a 1% OVA aerosol (Figure 2A,B). In each group, 2 or more offspring from a minimum of 6 mothers were used.

2.3 | Airway reactivity

Twenty four hours after the last challenge, offspring airway reactivity was measured in 10 mice from each group. Mice were anesthetized by intraperitoneal injection with a mixture of 10 mg/mL ketamine (Merial) and 1 mg/mL xylazine (VMD Livestock). A 20-gauge polyethylene catheter was inserted into the trachea, and mice were ventilated with a flexiVent small animal ventilator® (SCIREQ) as previously described.[23] Lung function was assessed at baseline and following increasing doses of aerosolized methacholine (0, 1.25, 2.5, 5, 10 and 20 g/L) using a 3-sec broadband signal to measure input impedance from 1 to 20.5 Hz and to calculate constant-phase model parameters. Newtonian resistance (Rn) was the main parameter measured during the challenge.

2.4 | Bronchoalveolar lavage (BAL) and differential cell counts

Blood samples were taken from the orbital sinus of terminally anaesthetized mice, and serum was stored at −80°C. Using a tracheal cannula on euthanized mice, BAL was performed once using 1 mL PBS containing 1 × cOmplete protease inhibitor cocktail (Roche). BAL supernatant was stored at −80°C, and total leucocytes were counted with a Neubauer chamber. Cytospin preparations were stained with Diff-Quick (Merz & Dade), and standard morphological criteria were used to identify cell types. Two hundred cells were counted per cytospin by a researcher blinded to the sample names.

2.5 | Lung collection and histology

Immediately after BAL, lungs were fixed with 4% formalin and then embedded in paraffin. Using a method previously described,[24] 4-μm PAS-stained lung sections were microscopically analysed using the new Computer Assisted Stereological Toolbox (newCAST, Visiopharm) by readers blinded to the study groups. For quantification of inflammation, the software randomly selected 100 frames of a coronal lung section (40x objective) and superimposed these frames by grid of points. Point intercepts on leucocytes were considered a point of inflammation (PI). Total PI for each lung was collected and normalized to the surface area measured.

2.6 | Immunohistochemistry

After deparaffinization and rehydration, 4-μm paraffin-embedded lung tissue sections were washed in Tris-buffered saline, followed by blocking of endogenous peroxidase via incubation with 3% H_2O_2 in methanol for 30 minutes at room temperature. After incubation with 5% normal serum, the samples were incubated with anti-FoxP3 primary antibody (Clone: FJK-16 – eBioscience) overnight at 4°C. The slides were washed and then incubated for 1 hour at room temperature with biotinylated goat anti-rat secondary antibody (Vector Laboratories). Signal was detected using a liquid DAB + Substrate-Chromogen System (Agilent) at room temperature.

2.7 | Antibody quantification

Serum levels of OVA-specific IgE and IgG1 were measured in both dams and pups by enzyme-linked immunosorbent assay (ELISA). Nunc MaxiSorp flat-bottom 96-well plates (Sigma-Aldrich) were coated with 20 μg/mL OVA, diluted in $NaHCO_3$ buffer and incubated overnight at 4°C. Plates were washed and blocked with PBS-1%BSA buffer for 2 hours at room temperature. After washing, serum samples were added and incubated overnight at 4°C. Plates were then washed and incubated with either biotinylated rat anti-mouse IgE (Pharmingen, USA) or biotinylated goat anti-mouse IgG1 (Sigma-Aldrich) at room temperature for 2 hours. Plates were incubated with streptavidin–peroxidase for 30 minutes, developed with POD substrate (Sigma-Aldrich), and the reaction stopped with 2M sulphuric acid. Colour development was read with a microplate reader (Bio-Rad) at 450 nm. OVA antibody titres of the sample were calculated by comparison with standards. For total IgG1 measurement, a similar protocol was used with the exception that the plates were coated with 0.5 mg/mL rat anti-mouse IgG1. CAS-specific IgE and IgG1 were measured in mothers by the same procedure as the OVA-specific ELISAs, with the exception that plates were coated with 20 mg/mL CAS, and OD 450 nm was reported.

2.8 | Antibody glycosylation analysis

Subclass-specific mouse IgG N-glycosylation analysis was performed as described previously.[25] Briefly, IgG was captured from 15 μL of murine serum using protein G affinity beads in PBS. Proteins interacted with the beads while being shaken for 1.5 hours, after which the beads were washed seven times with 1.5 mL PBS. IgG was eluted with 100 μL of 100 mmol/L formic acid, and eluates were dried for 2 hours in a vacuum concentrator at 60°C. Lyophilized IgG was dissolved in 40 μL 25 mmol/L ammonium bicarbonate, and 0.2 μg of sequencing grade trypsin (Promega) was added. Samples were incubated for 18 h at 37°C. Obtained glycopeptides were desalted on C_{18} beads, dried in a vacuum centrifuge and redissolved in 40 μL of ultrapure water. For nano-LC-ESI-Qq-TOF analysis, five microlitres of sample was injected and separation was achieved on a C_{18} nano-LC column. For data analysis, files were first converted to.mzXML format using ProteoWizard msConvert and processed with LaCy Tools software. Glycopeptide peak areas of doubly and triply charged ions were summed and normalized by total area for each subclass detected.

2.8.1 | Glycan abbreviations

The IgG Asn-297 N-linked glycosylation structures discussed in this study are shown in Figure 1B.

2.9 | Statistical analysis

All samples were tested for normal distribution using a Shapiro-Wilk normality test. Parametric data were analysed using one-way ANOVA and non-parametric data with a Kruskal-Wallis test, followed by Dunnett's multiple comparisons test (all groups compared with each other). For bronchial responsiveness, airway resistances measured for each concentration of methacholine were analysed using two-way ANOVA. Data are presented as mean ± SEM. For correlation analysis, Pearson's *r* was calculated and reported with the *P* value. Calculations were performed with GraphPad Prism 8 software.

3 | RESULTS

3.1 | Dams previously challenged during pregnancy showed distinct allergic airway inflammation upon recall allergen challenge

As expected, dams that were recall challenged with CAS or OVA after their offspring were weaned, displayed a clear allergic phenotype compared to control dams (sham recall challenged with PBS). This was demonstrated by allergen-specific antibody titres in the CAS- and OVA-challenged groups (Figure 3A). Regarding allergic airway inflammation, both CAS and OVA allergic dams displayed increased numbers of leucocytes in the bronchoalveolar lavage (BAL) fluid when compared to controls (Figure 3B). The BAL contained significantly increased numbers of eosinophils and lymphocytes in the allergic groups; OVA allergic mothers additionally exhibited increased numbers of macrophages. Histological quantification of lung tissue inflammation revealed similar profiles for both CAS and OVA allergic groups, both of which had a significantly increased number of lung tissue leucocytes compared to control dams (Figure 3C,D).

3.2 | Maternal allergen type influences the asthma phenotype in allergic offspring

To test the influence of maternal allergic airway inflammation during pregnancy on experimental asthma in the offspring, pups from allergic (CAS or OVA) or sham-treated dams were weaned at 21 days of age and all offspring were subjected to an adjuvant-free OVA asthma protocol (Figure 2A,B). Employing groups in which mothers and offspring were allergic to either the same allergen (OVA-OVA) or the different allergens (CAS-OVA) additionally allowed us to test how the maternal allergen type influenced offspring asthma susceptibility. Indeed, similarities or differences in allergen between mother and offspring were associated with differential antibody concentrations in allergic pups. Considering maternal CAS allergy, OVA allergic offspring from these dams (CAS-OVA) showed significantly increased levels of total IgG1 and OVA-specific IgG1 in the serum when compared with OVA allergic offspring from sham-treated mothers (sham-OVA). OVA allergic offspring from OVA allergic mothers (OVA-OVA) had increased OVA-specific IgG1 and decreased OVA-specific IgE compared to sham-OVA offspring (Figure 4A).

Next, we assessed the effect of maternal allergy during pregnancy on airway inflammation in the allergic pups. When mothers and offspring were sensitized to the same allergen (OVA-OVA), an increase in inflammatory cell influx into the offspring BAL was observed. This effect was shown to be compartment-specific, as leucocyte numbers in lung tissue were comparable between groups (Figure S1A,B). OVA-OVA offspring BAL contained an increased number of leucocytes compared to sham-OVA offspring, which was comprised predominantly of eosinophils, and to a lesser extent lymphocytes and macrophages (Figure 4B). Interestingly, when mothers and offspring were sensitized to different allergens (CAS-OVA), there were no differences in the offspring BAL when compared with the sham-OVA group.

FoxP3 is a marker of T regulatory (Treg) cells, and allergic asthma patients are known to have decreased sputum and blood Treg cell numbers.[26,27] In our study, immunohistochemical staining revealed that when mothers and offspring were both sensitized to the same allergen, OVA-OVA offspring showed significantly less Treg cells in the lung tissue and lung draining lymph nodes compared to the sham-OVA group (Figure 4C,D). This is in accordance with the increased eosinophilic cell influx in the BAL of OVA-OVA offspring. In contrast, when mothers and offspring were sensitized to different allergens (CAS-OVA), no differences in FoxP3 frequency were observed compared to sham-OVA offspring. Although we observed clear differences in allergic airway inflammation parameters in OVA-OVA compared with sham-OVA offspring, these were not correlated with changes in airway reactivity (Figure S1C).

In summary, maternal allergic airway inflammation during pregnancy influenced the offspring experimental asthma phenotype in an allergen-dependent manner. When mothers and offspring were sensitized to the same allergen, airway inflammation in OVA-OVA offspring was more severe. This was evidenced by altered antibody concentrations, increased BAL inflammatory cell influx and decreased lung tissue and lung draining lymph node FoxP3 cell frequency. When mothers and offspring were sensitized to different allergens (CAS-OVA), these differences were no longer apparent compared to the sham-OVA offspring group.

3.3 | Maternal allergic airway inflammation during pregnancy is associated with changes in maternal serum IgG1 and IgG3 glycosylation that persist in adult allergic offspring

It is established that glycosylation patterns influence the inflammatory properties of IgG antibodies, and altered antibody glycosylation patterns are commonly observed in inflammatory diseases.[28,29] For

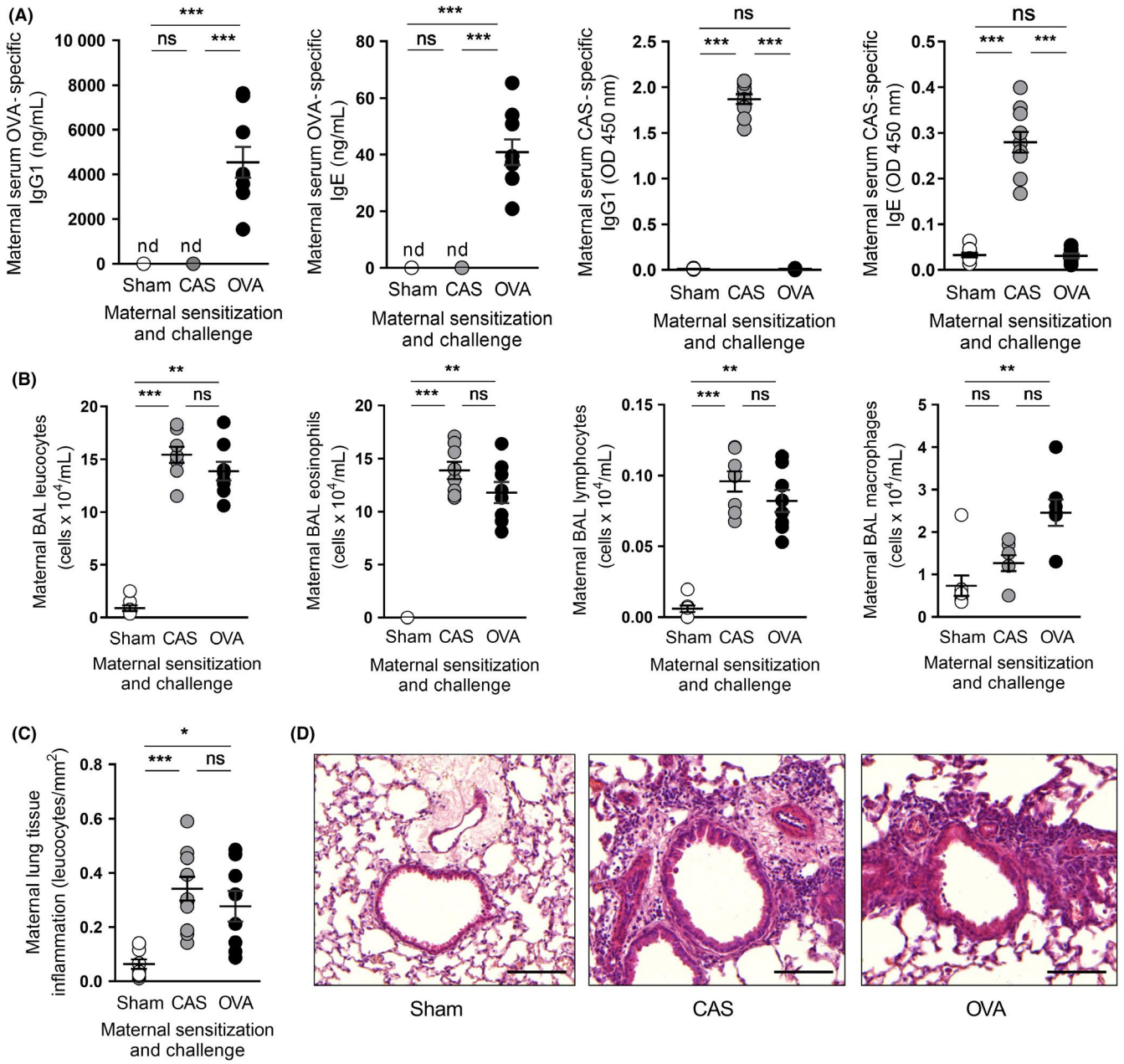
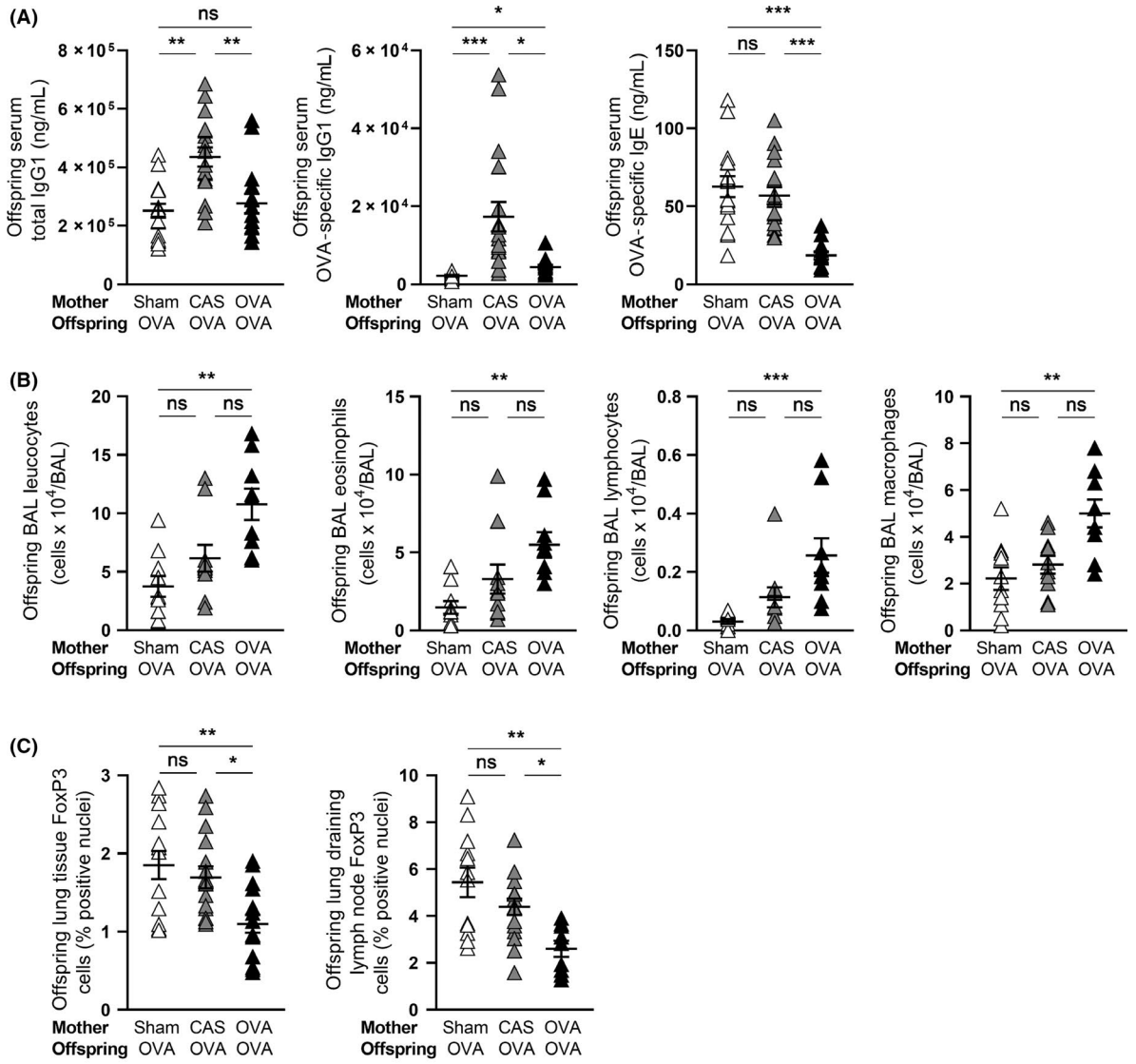
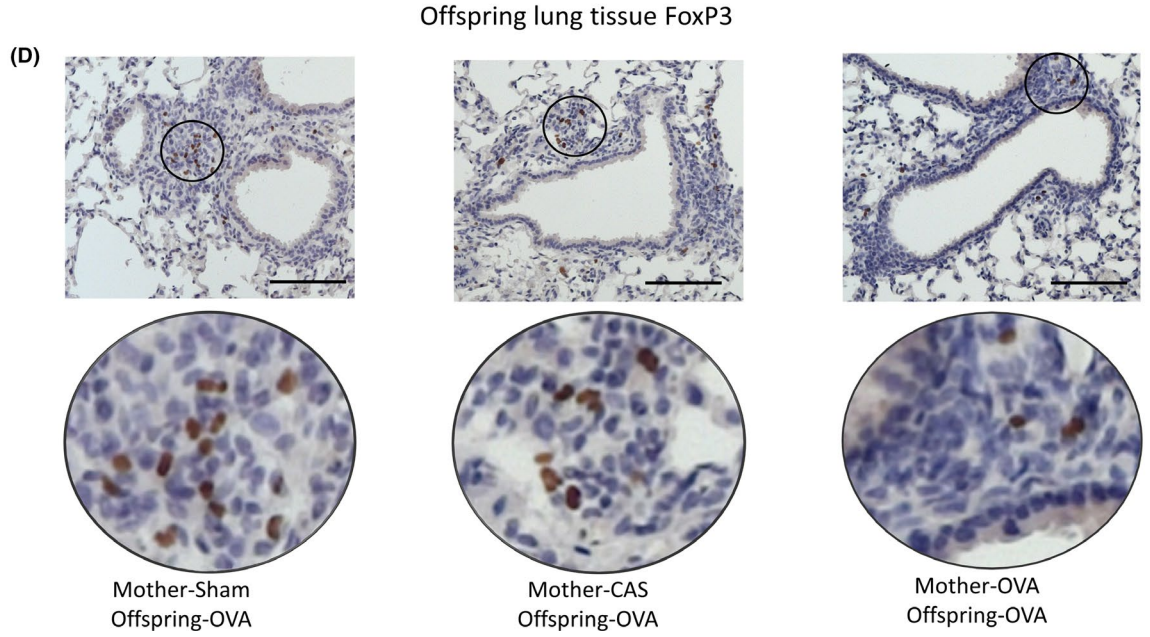


FIGURE 3 Recall allergen challenge of sensitized dams generates robust allergic airway inflammation that is similar in OVA and CAS allergic groups. Pregnant mice were sensitized with either PBS (sham), CAS or OVA before mating, then challenged during pregnancy with PBS, CAS or OVA, respectively. After the offspring were weaned, dams were recall challenged with PBS or the respective allergen. A, Maternal serum OVA-specific IgG1, OVA-specific IgE, CAS-specific IgG1, CAS-specific IgE. B, Maternal BAL numbers of leucocytes, eosinophils, lymphocytes and macrophages. C, Quantification of maternal lung inflammation. D, Lung histology images from control, CAS and OVA allergic mothers. Scale bar = 100μm. nd = not detectable, ns = not significant. Means ± SEM are shown, sham (n ≥ 8), CAS (n ≥ 8), OVA (n ≥ 8). Results represent two independently performed experiments. Significance is represented by **P* < .05, ***P* < .01, ****P* < .001, ANOVA or Kruskal-Wallis with Dunnett's multiple comparisons test

FIGURE 4 Increased lung inflammation is observed in OVA allergic offspring from OVA allergic dams. Graphs show OVA allergic offspring from sham-treated dams or dams with allergic airway inflammation to either CAS or OVA during pregnancy. A, Total serum IgG1, OVA-specific IgG1 and OVA-specific IgE. B, Differential cell counts in the BAL: leucocytes, eosinophils, lymphocytes and macrophages. C, Quantification of lung tissue and lung draining lymph node FoxP3 staining from OVA allergic offspring. D, Representative images of offspring lung tissue immunohistochemistry—OVA allergic offspring from sham-treated and allergic mothers. Scale bar = 100 μm. Means ± SEM are shown, sham-OVA (n = 16), CAS-OVA (n = 17), OVA-OVA (n = 15). In each group, a minimum of 2 offspring from at least 6 different mothers were included. Results represent two independently performed experiments. Significance is represented by **P* < .05, ***P* < .01, ****P* < .001, ANOVA or Kruskal-Wallis with Dunnett's multiple comparisons test


(A)
Offspring serum total IgG1 (ng/mL)
Offspring serum OVA-specific IgG1 (ng/mL)
Offspring serum OVA-specific IgE (ng/mL)
Mother Sham CAS OVA
Offspring OVA OVA OVA
(B)
Offspring BAL leucocytes (cells x 10^4/BAL)
Offspring BAL eosinophils (cells x 10^4/BAL)
Offspring BAL lymphocytes (cells x 10^4/BAL)
Offspring BAL macrophages (cells x 10^4/BAL)
(C)
Offspring lung tissue FoxP3 cells (% positive nuclei)
Offspring lung draining lymph node FoxP3 cells (% positive nuclei)


Offspring lung tissue FoxP3
(D)
Mother-Sham
Offspring-OVA
Mother-CAS
Offspring-OVA
Mother-OVA
Offspring-OVA

this reason, we next examined Asn-297 IgG antibody glycosylation in both maternal and offspring sera as a possible mediator for the increased airway inflammation observed in our OVA-OVA offspring. In OVA allergic mothers, analysis of IgG1 glycosylation patterns revealed significantly increased levels of pro-inflammatory G0 compared to CAS allergic dams (Figure 5A). Analysis of the offspring of these mothers revealed an amplification of this effect, with OVA-OVA offspring having significantly increased levels of G0 compared to both sham-OVA and CAS-OVA offspring (Figure 5B).

The addition of sialic acid to the IgG Asn-297 region is strongly anti-inflammatory, and reduced sialylation is associated with a pro-inflammatory phenotype.[30] In our study, analysis of maternal sialylation revealed that OVA allergic dams had significantly decreased percentages of IgG1 sialic acid residues, including G1S1, G2S1, G3S1 and G2S2 compared to the sham-sensitized group (Figure 5A). Though a similar trend was apparent in IgG1 sialylation in CAS allergic dams, only G3S1 and G2S2 were significant. Examination of the offspring IgG1 revealed that these sialic acid reductions were mirrored and amplified in adult allergic offspring. The strongest decreases were observed when mother and offspring were sensitized to the same allergen (OVA-OVA) with G1S1, G2S1, G3S1 and G2S2 being significantly reduced compared to sham-OVA offspring. When mother and offspring were sensitized to different allergens (CAS-OVA), decreases in offspring IgG1 sialylation were also observed, but to a lesser extent (Figure 5B).

In addition to IgG1, IgG3 antibodies also showed altered glycosylation patterns. Though there were no differences in pro-inflammatory G0 between any of the groups, anti-inflammatory sialylation (G1S1 and G2S1) was significantly decreased in OVA mothers and their OVA allergic offspring. It is notable that no differences were observed in CAS mothers or CAS-OVA offspring when compared to sham-exposed mothers or sham-OVA offspring, respectively (Figure 6A,B).

3.4 | Maternal serum IgG1 glycosylation percentages are strongly correlated with IgG1 glycosylation percentages in adult allergic offspring

To better assess the relationship between maternal and offspring IgG glycosylation patterns, we next calculated Pearson correlation coefficients for IgG1 and IgG3 glycosylation subtypes. As shown in

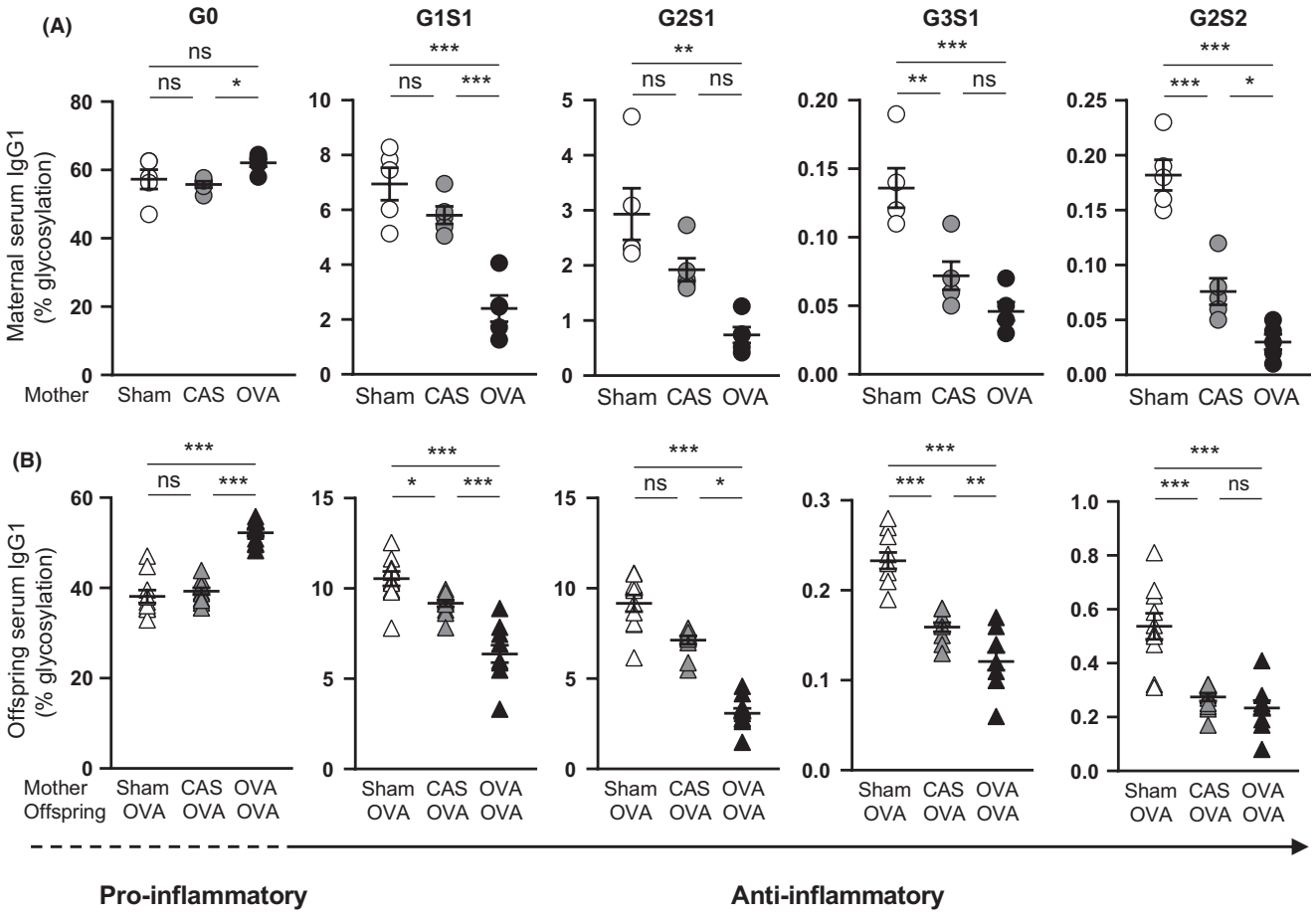


FIGURE 5 Serum IgG1 antibodies from allergic mothers and their adult allergic offspring have significantly increased pro-inflammatory (agalactosylated) and reduced anti-inflammatory (sialylated) glycans. A, Asn-297 glycosylation of maternal serum IgG1 antibodies from sham-treated, as well as CAS and OVA allergic mothers. B, Asn-297 glycosylation of serum IgG1 antibodies from OVA allergic offspring derived from sham-treated, CAS or OVA allergic mothers. Means ± SEM are shown: maternal sham (n = 5), maternal CAS (n = 5), maternal OVA (n = 5), sham-OVA offspring (n = 10), CAS-OVA offspring (n = 11) and OVA-OVA offspring (n = 10). Results represent two independently performed experiments. Significance is represented by *$P < .05$, **$P < .01$, ***$P < .001$, ANOVA or Kruskal-Wallis with Dunnett's multiple comparisons test

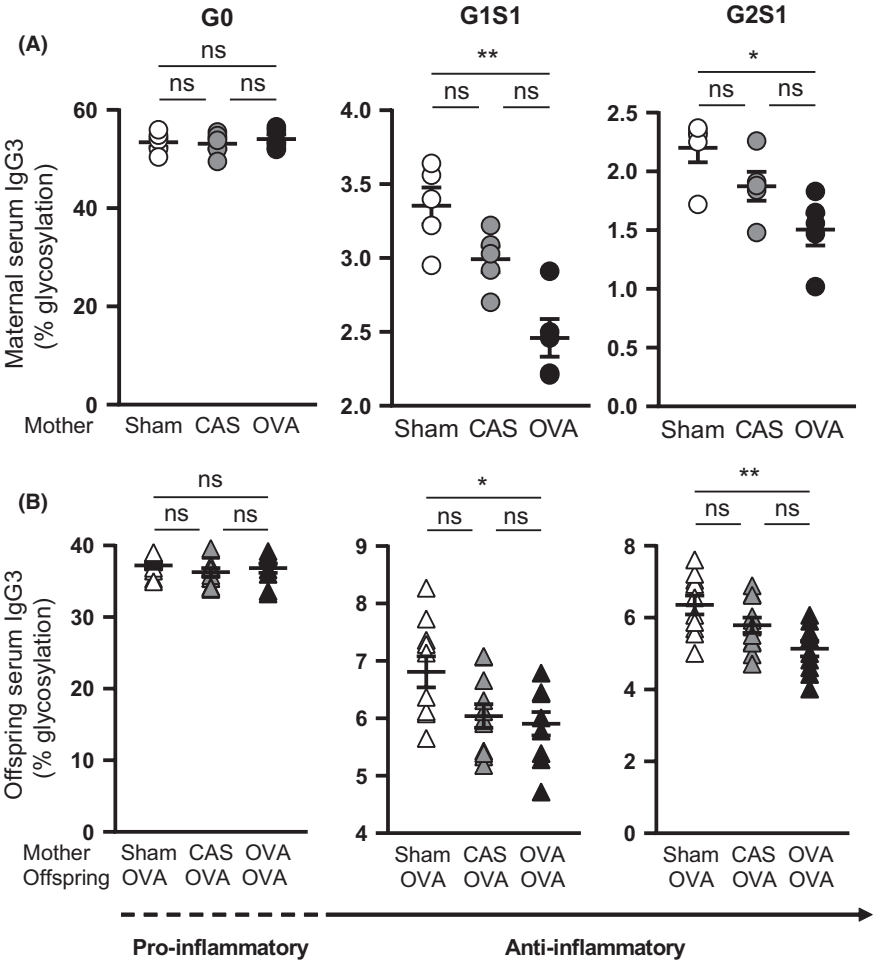


FIGURE 6 Serum IgG3 antibodies from OVA allergic mothers and their adult OVA allergic offspring have significantly reduced anti-inflammatory (sialylated) glycans. A, Asn-297 glycosylation of maternal serum IgG3 antibodies from sham-treated, as well as CAS and OVA allergic mothers. B, Asn-297 glycosylation of serum from OVA allergic offspring derived from sham-treated, CAS or OVA allergic mothers. Means ± SEM are shown: maternal sham (n = 5), maternal CAS (n = 5), maternal OVA (n = 5), sham-OVA offspring (n = 10), CAS-OVA offspring (n = 11) and OVA-OVA offspring (n = 10). Results represent two independently performed experiments. Significance is represented by *$P < .05$, **$P < .01$, ANOVA or Kruskal-Wallis with Dunnett's multiple comparisons test

Figure 7A, maternal and offspring IgG1-G0 percentages were significantly correlated, with a moderate r of 0.43. Supporting our hypothesis further, in all sialylated IgG1 groups, mothers and offspring were significantly and strongly correlated, with r ranging from 0.74 to 0.89. Contrarily, measurement of IgG3 correlation between mothers and offspring revealed that only IgG3-G1S1 was significant with a moderate r of 0.37 (Figure 7B). These data indicate that aglycosylated and sialylated IgG1 percentages are strongly correlated between mothers and their adult allergic offspring.

In summary, analysis of IgG1 Asn-297 glycosylation patterns revealed that 1) allergic dams have increased pro-inflammatory (G0) and decreased anti-inflammatory (sialic acid) glycan structures in the Fc region compared to sham controls, 2) the imbalance of pro- and anti-inflammatory IgG is aggravated in OVA allergic mothers compared with CAS allergic mothers, and 3) the IgG1 glycosylation patterns found in the mothers persisted in adult allergic offspring.

4 | DISCUSSION

Using a mouse model, we demonstrate here that maternal allergic airway inflammation during pregnancy is associated with an increased airway inflammation in the offspring. Further, the extent of this response was clearly associated with mothers and offspring being sensitized to the same allergen (OVA-OVA). In contrast to CAS-OVA offspring, OVA-OVA offspring had significantly increased allergic airway inflammation and decreased numbers of pulmonary Treg cells compared to sham-OVA offspring. Glycosylation patterns in OVA mothers and their OVA allergic offspring were also significantly more pro-inflammatory than in the CAS-OVA or sham-OVA group.

Analysis of the humoral immune response revealed that CAS-OVA offspring had significantly increased levels of total IgG1, and both CAS-OVA and OVA-OVA had significantly increased OVA-specific IgG1 in the serum, compared to sham-OVA allergic offspring. OVA-specific serum IgE, however, was significantly decreased in OVA-OVA offspring. While decreased allergen-specific IgE titres may initially seem counterintuitive for an asthma risk model, this is likely because both mother and offspring were sensitized to the same allergen. Supporting this, Jarrett and Hall showed in rats that maternal sensitization with OVA before pregnancy resulted in suppression of OVA-specific IgE and enhanced production of IgG in OVA-sensitized pups. The effect was allergen-specific as maternal immunization with keyhole limpet haemocyanin did not suppress IgE in the offspring.[31] As the underlying mechanisms of offspring IgE suppression are not fully understood, our model provides an excellent opportunity to further study this phenomenon.

An additional puzzling aspect of our study was the question of how maternal allergy led to differential allergen-specific effects

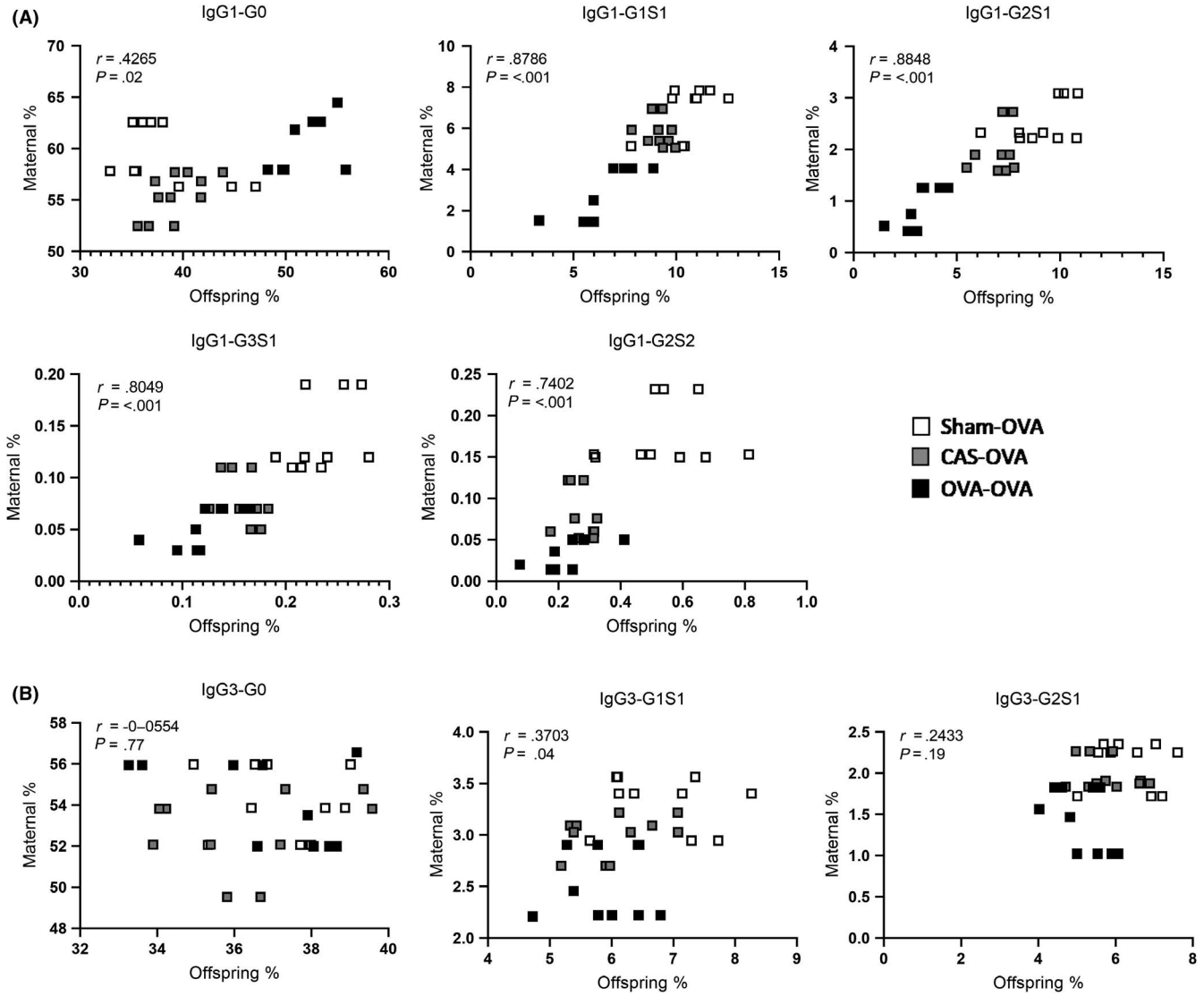


FIGURE 7 Maternal serum Asn-297 IgG1 glycosylation percentages are strongly correlated with adult allergic offspring IgG1 glycosylation percentages. Correlation analysis showing A) correlation between maternal and adult allergic offspring IgG1 glycosylation percentages and B) correlation between maternal and adult allergic offspring IgG3 glycosylation percentages. Sham-OVA offspring (n = 10), CAS-OVA offspring (n = 11), OVA-OVA offspring (n = 10) compared with their respective mothers. Correlation is given in Pearson's *r*

in the offspring. We propose for the first time that IgG antibody glycosylation is an important additional factor that could substantially contribute to distinct aspects of the asthma phenotype. Glycosylation is a critical posttranslational protein modification, and it has been described that N-linked glycans in the Fc region influence antibody effector functions. The addition of sialic acid residues onto these glycan chains generates IgG molecules that bind mouse Icam-3 grabbing non–integrin-related 1 (SIGNR1), which actively induces an anti-inflammatory cascade.[30] Illustrating this, successful allergen-specific immunotherapy in allergic patients is correlated with increased IgG sialylation.[19,32] In our model of maternal transmission of asthma risk, the group with the most severe airway inflammation (OVA-OVA offspring) had significantly increased agalactosylated IgG1 levels as well as decreased sialic acid residues on both IgG1 and IgG3 molecules, indicating a strong pro-inflammatory status. The CAS-OVA offspring, which showed a trend towards increased asthma severity, also had decreased IgG1 and IgG3 sialylation, though to a much lesser extent than the OVA-OVA offspring. Thus, maternal allergic airway inflammation during pregnancy was associated with decreased frequencies of protective IgG antibodies in the offspring.

Finally, to explore the relationship between mother and offspring, we also examined serum IgG glycosylation in asthmatic dams which were recall challenged to allergen. Maternal IgG1 serum glycosylation patterns strongly correlated with the patterns observed in allergic offspring. This indicates that maternal IgG antibodies may play a role in offspring allergy development, and highlights the importance of measuring antibody glycosylation in addition to concentration in allergy studies. Our results highlight a key role for IgG glycosylation in the transgenerational transmission

of offspring asthma risk that warrants further investigation. Since the current study measured IgG glycosylation in recall challenged mothers and adult allergic offspring, future studies will include analyses of mothers and offspring at several gestational and neonatal time points. Our newly developed mouse model provides a resource to understand in more detail how maternal IgG glycosylation during pregnancy influences offspring immune system development and subsequent susceptibility to inflammatory diseases such as asthma later in life.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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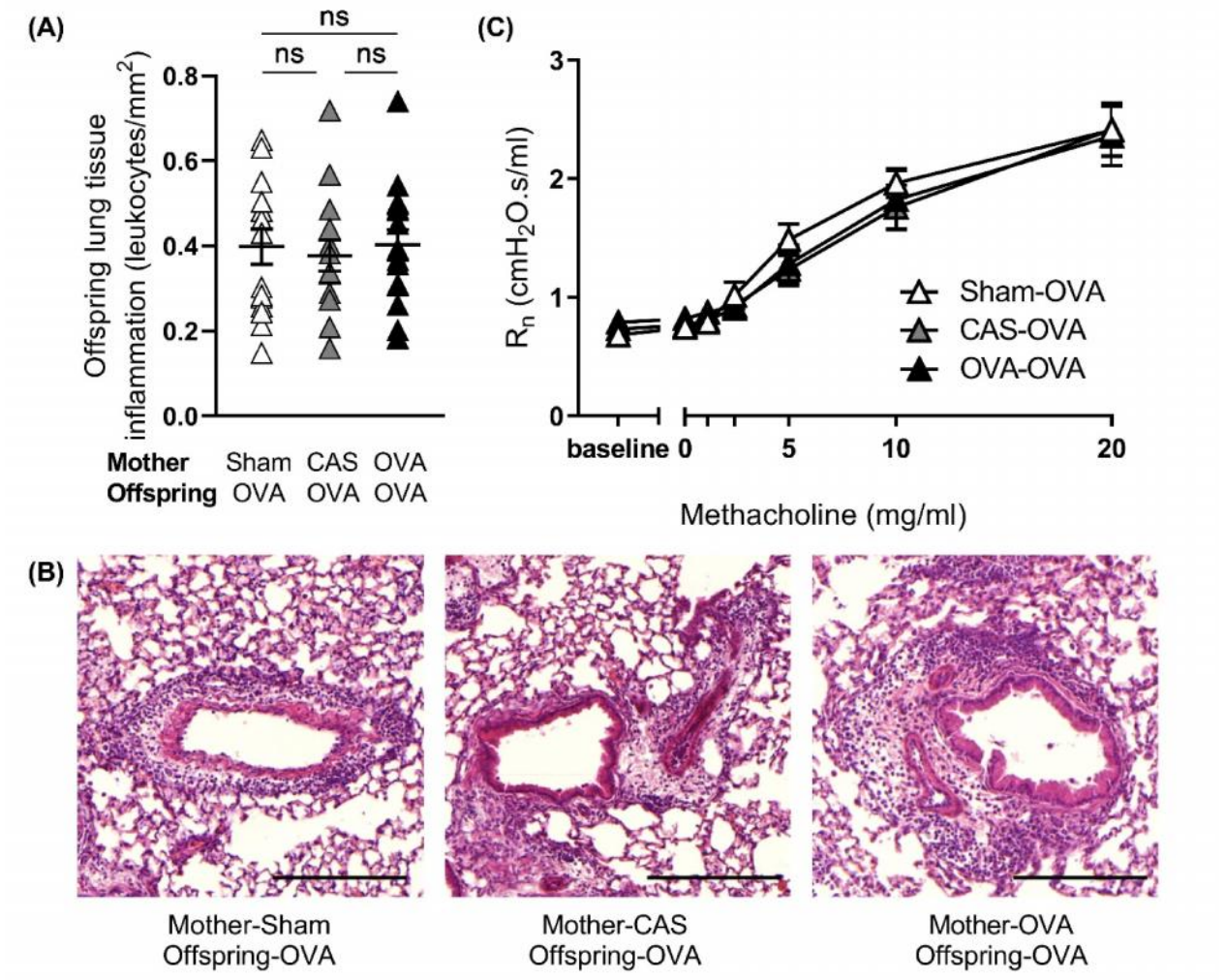

Supplementary Figure 1: Maternal experimental asthma during pregnancy does not influence allergic offspring lung tissue inflammation or airway reactivity.
Graphs show OVA allergic offspring from sham treated dams or dams with allergic airway inflammation to either CAS or OVA during pregnancy. (A) Allergic offspring lung tissue inflammation. Sham-OVA (*n*=16), CAS-OVA (*n*=17), OVA-OVA (*n*=15). (B) Representative images of allergic offspring lung histology. Scale bar = 100µm. (C) Allergic offspring airway reactivity data (*n*=10/group). Results represent two independently performed experiments. Means ± SEM are shown. ns = not significant, lung tissue inflammation: ANOVA or Kruskal-Wallis with Dunnett's multiple comparisons test. Airway reactivity data were compared for each dose of methacholine using a two-way ANOVA.