

ORIGINAL ARTICLE

Food Allergy and Gastrointestinal Disease

C5a receptor 1^{-/-} mice are protected from the development of IgE-mediated experimental food allergy

Anna Kordowski¹ | Anna T. Reinicke¹ | David Wu² | Zane Orinska^{3,4} |
Philipp Hagemann^{3,4} | Markus Huber-Lang⁵ | Jee-Boong Lee² | Yui-Hsi Wang² |
Simon P. Hogan² | Jörg Köhl^{1,4,6} 

¹Institute for Systemic Inflammation Research, University of Lübeck, Lübeck, Germany

²Division of Allergy and Immunology, Cincinnati Children's Hospital and University of Cincinnati, College of Medicine, Cincinnati, Ohio, USA

³Division of Experimental Pneumology, Research Center Borstel, Leibniz Lung Center, Borstel, Germany

⁴Airway Research Center North (ARCN), German Center for Lung Research (DZL), Borstel, Germany

⁵Institute of Clinical and Experimental Trauma-Immunology, University Hospital of Ulm, Ulm, Germany

⁶Division of Immunobiology, Cincinnati Children's Hospital and University of Cincinnati, College of Medicine, Cincinnati, Ohio, USA

Correspondence

Simon P. Hogan, Division of Allergy and Immunology Cincinnati Children's Hospital and University of Cincinnati, College of Medicine, Cincinnati, OH, USA.

Email: Simon.Hogan@cchmc.org and

Jörg Köhl, Institute for Systemic Inflammation Research, University of Lübeck, Lübeck, Germany.

Email: joerg.koehl@uksh.de

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Abstract

Background: Food-induced anaphylaxis is a serious allergic reaction caused by Fcε-receptor activation on mast cells (MCs). The exact mechanisms breaking oral tolerance and the effector pathways driving food allergy remain elusive. As complement is activated in food-induced anaphylaxis, we aimed to assess the role of C5a in disease pathogenesis.

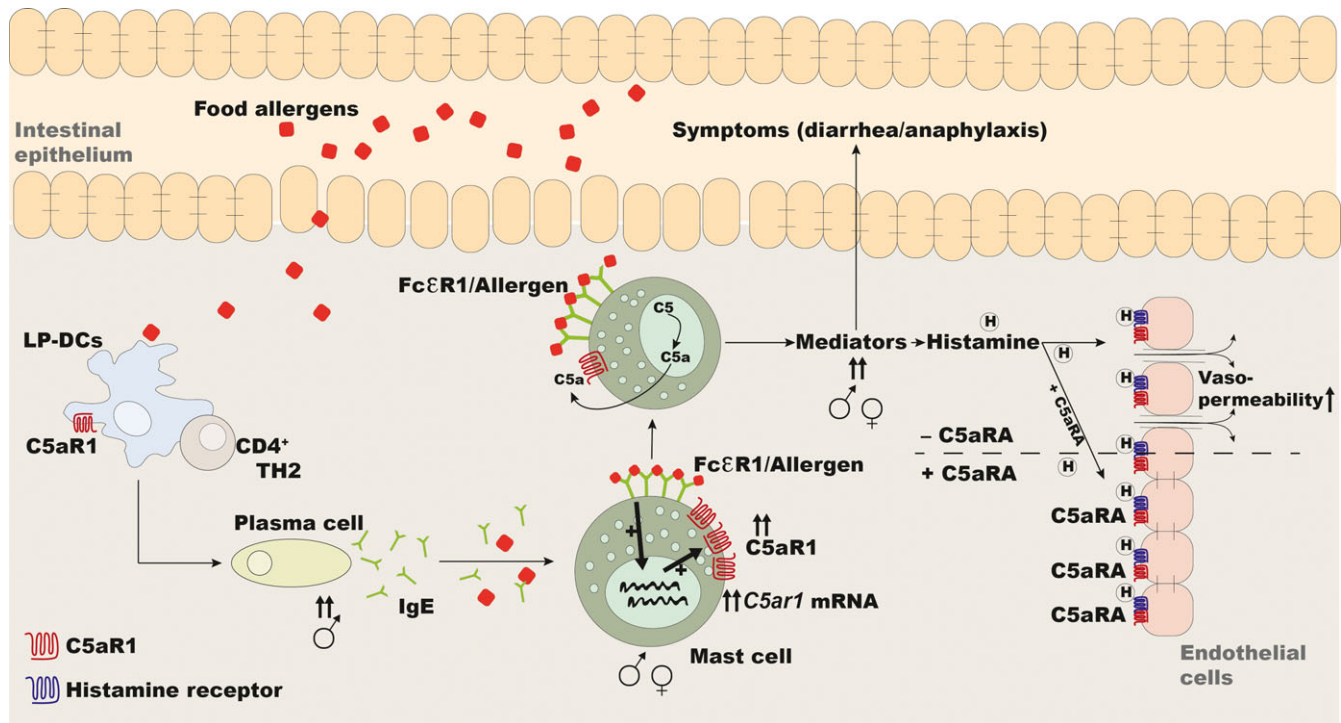
Methods: Oral antigen-induced food-induced anaphylaxis was induced in BALB/c wild-type (wt) and *C5ar1*^{-/-} mice. Readouts included diarrhea development, changes in rectal temperature, hematocrit, antigen-specific serum IgE, MCPT-1, and intestinal MC numbers, as well as FcεR1-mediated MC functions including C5a receptor 1 (C5aR1) regulation. Further, histamine-mediated hypothermia and regulation of endothelial tight junctions were determined.

Results: Repeated oral OVA challenge resulted in diarrhea, hypothermia, increased hematocrit, high OVA-specific serum IgE, and MCPT-1 levels in wt mice. Male *C5ar1*^{-/-} mice were completely whereas female *C5ar1*^{-/-} mice were partially protected from anaphylaxis development. Serum MCPT-1 levels were reduced gender-independent, whereas IgE levels were reduced in male but not in female *C5ar1*^{-/-} mice. Mechanistically, IgE-mediated degranulation and IL-6 production from *C5ar1*^{-/-} BMMCs of both sexes were significantly reduced. Importantly, FcεR1 cross-linking strongly upregulated C5aR1 MC expression in vitro and in vivo. Finally, *C5ar1*^{-/-} male mice were largely protected from histamine-induced hypovolemic shock, which was associated with protection from histamine-induced barrier dysfunction in vitro following C5aR targeting.

Conclusions: Our findings identify C5aR1 activation as an important driver of IgE-mediated food allergy through regulation of allergen-specific IgE production, FcεR1-mediated MC degranulation, and histamine-driven effector functions preferentially in male mice.

KEYWORDS

complement, food allergy, histamine, IgE, mast cell



GRAPHICAL ABSTRACT

The C5a/C5aR1 axis controls the production of allergen-specific IgE in male mice. FcεR1 aggregation upregulates C5aR1 expression in mast cells which amplifies IgE-driven mediator release. C5aR1 signaling controls histamine-mediated effector functions in vivo and in vitro.

1 | INTRODUCTION

Food allergy is an immune-mediated adverse reaction to ingested food and is a substantial and evolving public health issue, emerging over the past decades. Clinical symptoms of food-triggered anaphylaxis range from mild skin reactions to life-threatening systemic anaphylaxis. The onset of symptoms is variable, occurring within seconds to a few hours after exposure to the food allergen. The disease affects not only the gastrointestinal tract but also the cutaneous, respiratory, and cardiovascular systems.¹ Antigen-specific IgE is critical in the pathogenesis of food allergy as it mediates specific degranulation and activation of intestinal tissue-resident MCs and circulating basophils through FcεR1 aggregation.¹ Preformed mediators such as histamine, PAF, and cysteinyl leukotrienes confer many of the symptoms including diarrhea and hypovolemic shock that can lead to the severe anaphylaxis phenotype. However, the mechanisms underlying the break of oral tolerance, induction of maladaptive Th2 immunity, and production of allergen-specific IgE are incompletely understood. Further, our knowledge about the pathways regulating FcεR1 expression on MCs, downstream signaling, and the effects of MC mediators on target cells such as the vascular endothelium is incompletely understood.

Complement activation results in the generation of the anaphylatoxins C3a and C5a. Elevated levels of C3a have been observed in patients and mice with anaphylaxis in response to food

allergens.^{2,3} Previously, it was shown that rodent cutaneous⁴ and synovial⁵ as well as human MCs express receptors for C3a and/or C5a. Further, anaphylatoxins not only induce immediate release of MC mediators independent of IgE immune complexes,⁶ but also act as potent MC chemoattractants.⁷ In experimental peanut-induced shock, peanut extract can directly activate complement leading to C3a formation, which stimulates macrophages, basophils, and MCs to produce PAF and histamine.³ The role of complement in experimental IgE-mediated food allergy has not been explored. Here, we demonstrate that C5a drives the development of food allergy by promoting allergen-specific IgE antibody production, enhancing FcεR1-mediated MC degranulation and histamine-driven effector functions.

2 | METHODS

2.1 | Mice

BALB/c wild-type (wt) mice were originally purchased from Charles River Laboratories (Wilmington, Mass). *C5ar1*^{-/-} mice on the BALB/c background and floxed GFP-C5aR1 knock-in mice on the C57BL/6 background⁸ were housed under specific pathogen-free conditions with a 12-h light/dark cycle with food and water ad libitum. Age-, sex-, and weight-matched littermates were used as control mice in all experiments. Mice were bred and maintained in accordance with guidelines of the National Institutes of Health

and the legal requirements in Germany and the United States. Experiments were approved by the Schleswig-Holstein authorities (Nr. V242-7224.122-39(11-1/15)) and by Cincinnati Children's Hospital (CCHMC IAUCAC #2016-2010) and performed by certified personnel.

2.2 | IgE-mediated experimental food allergy

Mice were sensitized with 50 µg of OVA (grade V; A-5503; Sigma-Aldrich, Munich, Germany) and 1 mg of alum (Imject Alum Adjuvant; Thermo Scientific, Waltham, MA, USA) in sterile saline by intraperitoneal (i.p.) injection. From day 14 onward, mice were challenged by oral gavage with 50 mg of OVA in saline using i.g. feeding needles (01-290-2B; Fisher Scientific Co., Waltham, MA, USA) three times per week for 3 weeks as described.⁹ Rectal temperature was measured before, 30, and 60 min after oral OVA administration at challenges six and seven. Mice showing profuse liquid stool within 60 min after oral OVA challenge were recorded as diarrhea-positive. Blood samples and intestinal tissues were collected from mice killed immediately after rectal temperature measurements.

2.3 | Histamine-induced hypothermia

Mice were injected i.v. into the lateral tail vein with 5 mg histamine (Sigma-Aldrich, Munich, Germany) in 200 µL saline as described before,¹⁰ and temperature change was monitored every 15 min for 2 h.

2.4 | Hematocrit

Blood was drawn from anesthetized mice from orbital veins into heparinized capillary tubes and centrifuged for 10 min at 16 000 g. The hematocrit was calculated as described.¹¹

2.5 | Flow cytometry

Unless stated otherwise, all fluorochrome-labeled antibodies were from BioLegend (San Diego, CA, USA). Cells were analyzed on LSR II, FACSCalibur, or BD LSRFortessa (BD Immunocytometry Systems; San Jose, CA, USA), and data were analyzed using the FlowJo 10 software (Tree Star; Ashland, OR, USA) or Flowlogic Software 7.2.1. (Invai Technologies, Mentone, Australia).

2.6 | BMMC generation

Bone marrow cells were isolated and cultured as described previously.^{12,13} After 4–6 weeks of culture, bone marrow-derived MCs (BMMCs) were examined for FcεR1α and c-Kit (CD117) expressions by flow cytometry using APC anti-mouse-FcεR1α (MAR1) and PE-Cy7 anti-c-kit antibodies. Double-positive cells were considered BMMCs. BMMC degranulation was measured by hexosaminidase release as described.¹⁴

2.7 | Functional analyses of BMMCs

Degranulation was expressed as the percentage of total β-hexosaminidase activity. The BMMC lysate was used to estimate total β-hexosaminidase activity. To assess the activation after degranulation, BMMCs (5×10^6 /mL) were sensitized with IgE anti-TNP mAb (IgE-3; 0.5 µg/mL; BD, San Jose, CA, USA) for 16 h and challenged with BSA-TNP (10 ng/mL) for 3 h. The supernatant was analyzed for IL-6 production. For kinetic assessment of C5aR1 expression, cells were transferred into fresh BMMC culture medium 30 min after challenge. Three hours, 6, 10, 24, and 48 h after challenge, expression of C5aR1 (PE anti-CD88 [20/70]) was measured by flow cytometry and by RT-qPCR. For intracellular C5 expression, cells were fixed and permeabilized using the BD Cytofix/Cytoperm Kit (BD San Jose, CA, USA) and then stained with anti-C5 (BB5.1; Hycult Biotech, Uden, Netherlands) conjugated with AF647 (Molecular Probes, Invitrogen, CA, USA). In some experiments (Figure 4E–H), BMMCs were stimulated with DNP-HSA, 20 ng/mL LPS, polyinosinic-polycytidylic acid (pIC), or compound 48/80 (all from Sigma-Aldrich, Munich, Germany) for 20 min by flow cytometric analysis of CD107a (Lamp1) surface translocation. Cells were stained with AF647 anti-CD107a (1D4B), FITC anti-CD117 (2B8), and PE anti-C5aR1 (20/70). PI (1 µg/mL) or LIVE/DEAD™ Fixable Blue Stain (Thermo Fisher Scientific, Waltham, MA, USA) was used to exclude dead cells from analysis.

2.8 | Peritoneal MC analyses

Murine peritoneal cells were isolated as described.¹⁵ Cells were resuspended in PBS/1% BSA, incubated in Fc block (CD16/CD32) (eBioscience, San Diego, CA, USA) for 30 min followed by incubation with APC anti-mouse-FcεR1α (MAR1), PE-Cy7 anti-mouse CD117 (2B8), PerCP-Cy5.5 anti-mouse IL33R (DIH9), and PE anti-mouse C5aR1 (20/70) (all from BioLegend, San Diego, CA, USA).

2.9 | Intestinal MC quantification

Tissue from the jejunum tissue was collected 5 cm distal to the stomach, fixed in 10% formalin, and processed by standard histological techniques. Five-µm-thick paraffin-embedded tissue sections were stained for mucosal MCs with chloroacetate esterase (CAE) activity as described¹⁶ and counterstained with hematoxylin. Quantification of mucosal MCs was performed by counting CAE-positive cells per villus and crypt of 40 villi and crypts in 2–3 random sections per mouse.

2.10 | Lamina propria mononuclear cell isolation and identification

The lamina propria (LP) mononuclear cells were isolated using a Percoll gradient as described.¹⁷ LP cells from small intestines were first stained with PerCP-Cy5.5-conjugated mAbs against lineage (Lin) markers (CD11b [M1/70], CD11c [HL3], CD8 [53-6.7], B220 [RA3-6B2], Gr-1 [RB6-8C5], and CD335 [NKP46, 29A.4]) FITC anti-β7 integrin (M293), APC anti-IL-25R, APC-C7 anti-c-Kit (2B8), PE-Cy7

anti-CD3e /145-2C11), Horizon V500 anti-CD4 (RPA-T4), PE anti-C5aR1 (20/70), and biotinylated anti-T1/ST2 (DJ8) (MyBioSource, San Diego, CA, USA) antibodies. Subsequently, cells were counter-stained with BV421-labeled streptavidin before analysis.

2.11 | RNA isolation and PCR

Total RNA from BMMCs was isolated using TRIzol reagent (Thermo Fisher Scientific). The cells from the small intestine were isolated using the LP dissociation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) as described.⁸ Cell suspension of the abdominal aorta was obtained using liberase as described.⁸ RNA from the cell suspensions of the small intestine and abdominal aorta was obtained using the RNeasy Mini Kit (Qiagen, Hilden, Germany). After DNase I (Thermo Fisher Scientific) treatment of the RNA, cDNA was synthesized using a first-strand cDNA synthesis kit for RT-PCR (Roche, Indianapolis, IN, USA). qPCR for *C5ar1* (F:5'-CAGCGGTGTAGAGGAGAAG-3'; R:5'-GAAGGAAGGAAGGAGGAGAGG-3') was performed as described previously.^{18,19} The expression of *Fcer1g* was examined using: F:5'-ATCTTGTCTTGCTCCTTTTGG-3'; R:5'-CATCTGCTTTCTACGGCTG-3' primers. The expression of histamine receptors 1 and 2 was measured using primers: F:5'-CAAGATGTGTGAGGGGAACAG-3'; R:CTACCGA CAGGCTGACAATGT-3' for HR1 and F:5'-CCCAATGGCACGGTT CATTC-3' and R:5'-GCCGACGATTCAAGCTGACA-3' for HR2. All qPCR data were analyzed using the $2^{-\Delta\Delta C_t}$ method with β -actin (F:5'-GCACCACACCTTCTACAATGAG-3'; R:5'-AAATAGCACAGCCTGGA TAGCAAC-3') as reference housekeeping gene. The mean ΔC_t value of wt cells was used as $\Delta C_{t\text{control}}$ to calculate $\Delta\Delta C_t$ of each sample. The temperature profiles were 95°C for 3 min, followed by 34 cycles at 95°C for 30 s, 59°C for 30 s, and 72°C for 30 s. Amplification products were separated on 2% agarose gel and detected by GelRed staining (Biotrend, Cologne, Germany).

2.12 | ELISA

Mouse mast cell protease-1 (MCPT-1) serum levels were measured by ELISA (eBioscience Inc., San Diego, CA, USA). OVA-specific IgE antibodies were measured in serum of OVA-challenged mice as described before.²⁰ C5a serum levels were determined as described.²¹ IL-6 in the supernatant of degranulated BMMCs was

determined using a Mouse IL-6 DuoSet ELISA (R&D Systems Inc., Minneapolis, MN, USA).

2.13 | In vitro permeability

The murine high endothelial venule cell line (mHEVC) was cultured in humidified incubator (5% CO₂, 37°C). Some cells were cultured in the presence of the C5aR antagonist A8^{Δ71-73} (C5aRA²²) at a final concentration of 2 μM. After several passages, 10⁵ cells were seeded on snap wells (3.3 mm diameter, 0.4 μm pore; Corning, Corning, NY, USA) and cultured for 3–4 days. Transendothelial resistance (TER) was monitored with an EVOM/Endohm (WPI Inc, Sarasota, FL, USA). Endothelial monolayers with a TER ≥ 150 Ω were used for all experiments. Some cells were treated with C5aRA (5 μM) one hour before histamine challenge. All cells were placed into the Ussing chamber and allowed to equilibrate for 15 min. Changes in TER were measured in response to the addition of 100 μM of histamine (Sigma-Aldrich, Munich, Germany) on both sides of the transwell as described.²³

2.14 | Statistical analysis

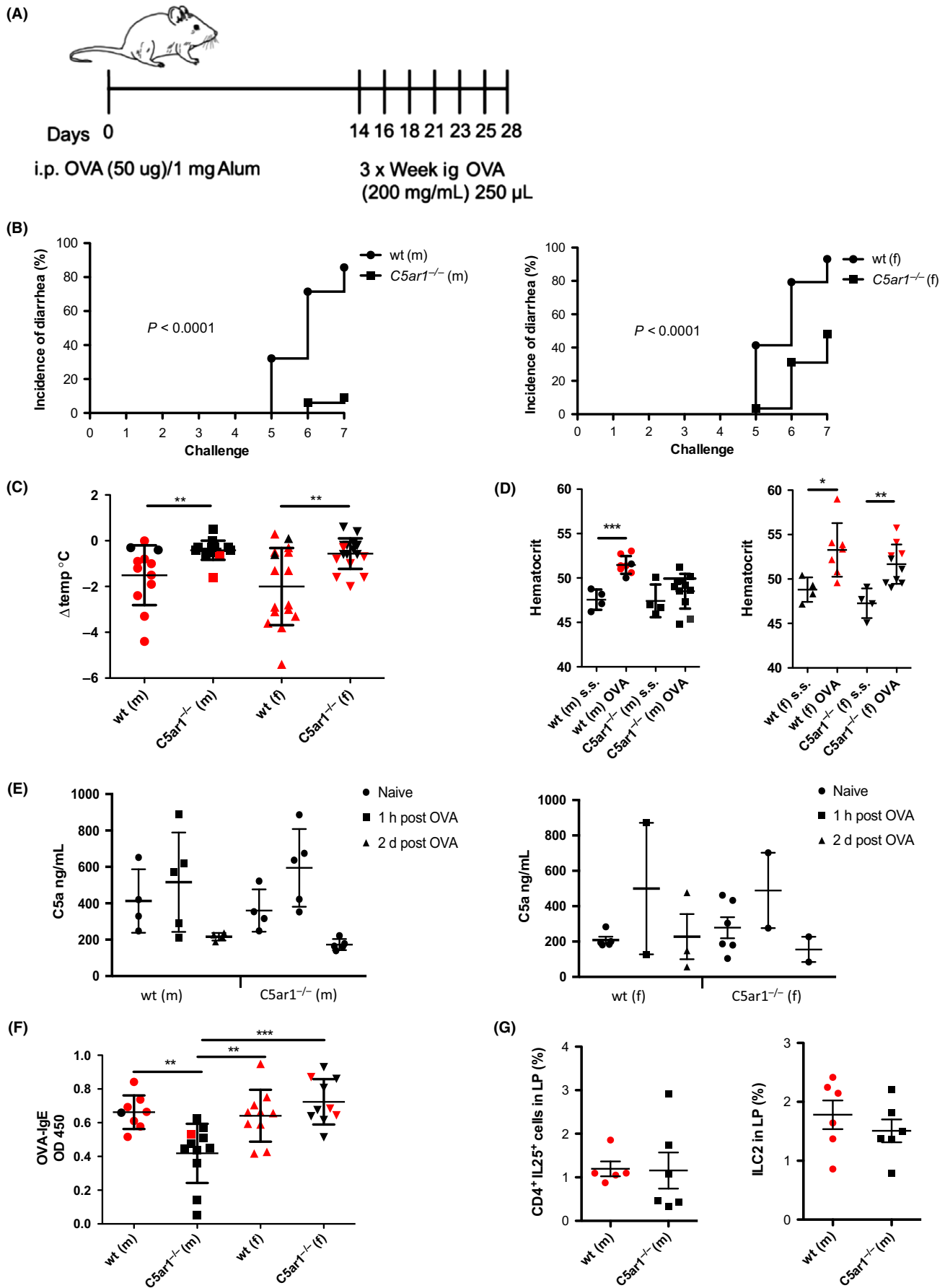
Data are presented as mean ± SEM or as median. Statistically significant differences between different sets of mice or cells were determined by one-way analysis of variance and a Tukey's post hoc test (multiple groups), Kruskal-Wallis one-way analysis of variance and Dunn's test (multiple groups), and nonpaired Student's *t* test or Mann-Whitney *U* test (two groups). *P* < 0.05 was considered significant. The incidence of diarrhea was analyzed using the log-rank (Mantel-Cox) test. All analyses were performed using the Prism 5.0 software (GraphPad Software Inc., San Diego, CA, USA).

3 | RESULTS

3.1 | C5a drives diarrhea and hypothermia in response to oral antigen-induced food allergy

To induce food allergy, BALB/c wt and *C5ar1*^{-/-} mice were sensitized i.p. with OVA/alum. Two weeks later, mice were challenged seven times orally with OVA over three weeks (Figure 1A). Consistent with previous reports,^{20,24} wt mice experienced profound

FIGURE 1 Decreased symptoms of food-induced anaphylaxis in C5aR1-deficient mice. (A) The oral allergen-induced food allergy model. BALB/c mice were sensitized intraperitoneally with 50 μg OVA and 1 mg alum. From day 14 onward, 250 μl of OVA (200 mg/mL of saline) was administered orally 3 times a week. (B) Frequency of diarrhea in *C5ar1*^{-/-} male (m) (n = 33–38/group; left panel) and female (f) (n = 29/group; right panel) mice after repeated oral OVA challenge. (C) Change in body temperature after the 7th OVA challenge in wt and *C5ar1*^{-/-} male and female mice (n = 13–18/group). (D) Hematocrit before oral OVA challenges (s.s.—steady state) and after the 7th OVA challenge in wt and *C5ar1*^{-/-} male and female mice (n = 4–10/group). (E) C5a serum levels in naïve wt and *C5ar1*^{-/-} mice as well as 1 h and 2 days after the final oral OVA challenge in male (left panel) and female mice (right panel) (n = 2–6/group). (F) OVA-specific IgE levels in sera of wt and *C5ar1*^{-/-} male and female mice after the 7th oral OVA challenge (n = 8–11/group). (G) Frequency of IL-25⁺ T cells and ILC2 in LP of wt and *C5ar1*^{-/-} male mice after the 7th oral OVA challenge (n = 6/group). Data in B were analyzed by the log-rank (Mantel-Cox) test. Data in C, D, and F represent the mean ± SEM. Data in C, D, and F were analyzed by one-way ANOVA with Tukey's multiple comparison post hoc test. Data in E represent the median and were analyzed by Kruskal-Wallis test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (C and F: wt vs *C5ar1*^{-/-}; D: steady state (s.s.) vs 7th OVA challenge). Data in G were analyzed by unpaired Student's *t* test. Red symbols in C, D, F, and G show mice suffering from diarrhea after the 7th OVA challenge; black symbols reflect mice that did not develop diarrhea



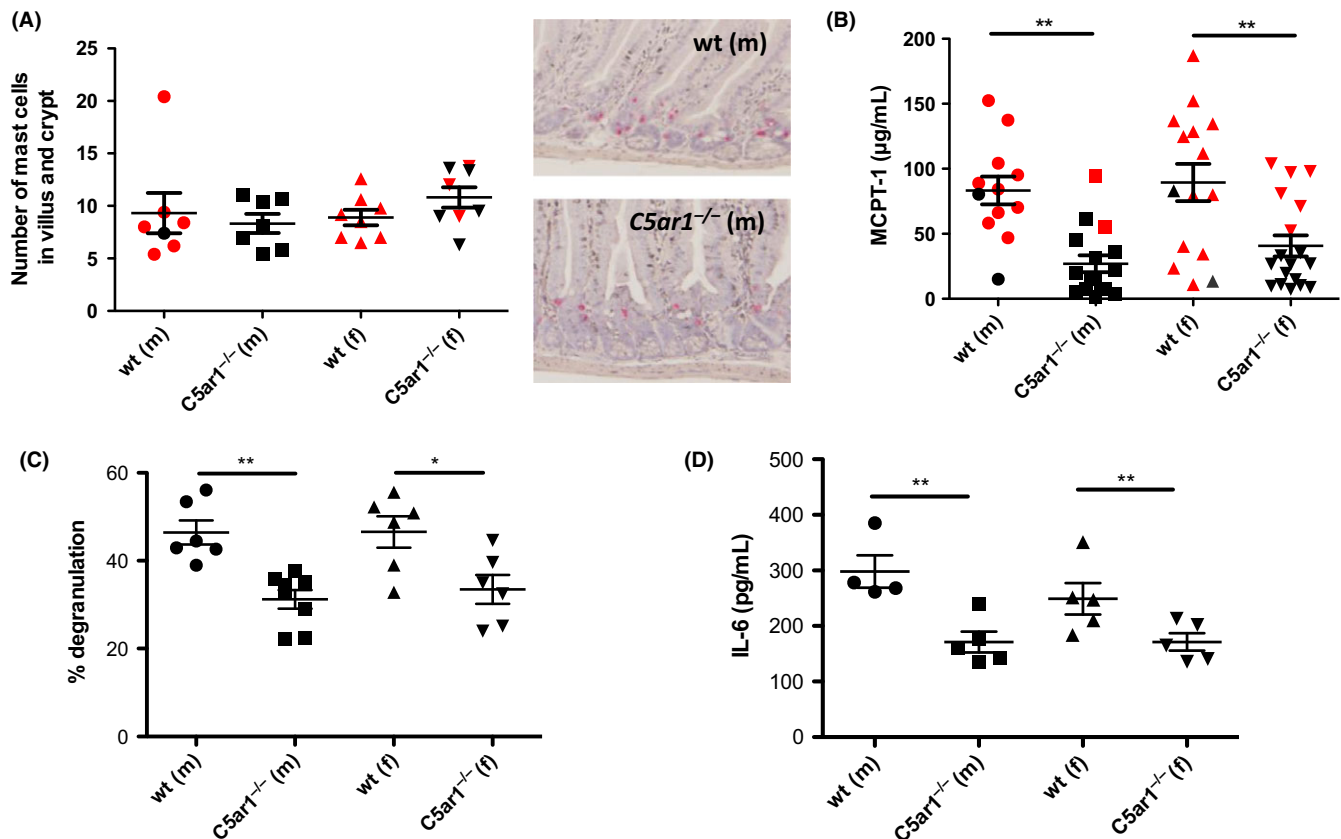


FIGURE 2 Impact of the C5a/C5aR1 axis on mast cell numbers and function. (A) Intestinal MC numbers in wt and of *C5ar1*^{-/-} male and female mice after the 7th OVA challenge (n = 7-8/group). MCs were quantified from CAE-stained paraffin sections of the small intestine. (B) MCPT-1 levels in sera of wt and *C5ar1*^{-/-} male and female mice after the 7th OVA challenge (n = 12-18/group). Red symbols in A and B show mice suffering from diarrhea after the 7th OVA challenge; black symbols show mice with no signs of diarrhea. (C, D) IgE-mediated release of β-hexosaminidase (n = 6-8/group) (C) and IL-6 production (n = 4-5/group) (D) from BMMCs of wt and *C5ar1*^{-/-} male (m) and female (f) mice. β-hexosaminidase activity was determined 30 min (C) and IL-6 synthesis 3 h (D) after IgE-mediated degranulation. BMMCs (5 × 10⁶/mL) were sensitized with IgE anti-TNP (2 μg/mL) and challenged with BSA-TNP (10 ng/mL) (n = 5-6/group). Data in A-E represent the mean ± SEM and were analyzed by one-way ANOVA with Tukey's multiple comparison post hoc test *P < 0.05, **P < 0.01

diarrhea starting with the 5th OVA challenge (Figure 1B). Strikingly, the incidence of allergic diarrhea was markedly reduced in *C5ar1*^{-/-} mice as compared with wt mice. Following the 7th oral OVA challenge, the incidence of intestinal diarrhea was 80% in wt male and 95% in wt female mice. In sharp contrast, only 9% of male and 50% of *C5ar1*^{-/-} female mice suffered from diarrhea (Figure 1B). Further, we observed hypothermia in wt but not in *C5ar1*^{-/-} mice after the 7th OVA challenge (Figure 1C). Consistent with hypovolemic shock, the 7th OVA challenge induced an increase in hematocrit in wt male and female mice that remained unchanged in male *C5ar1*^{-/-} mice (Figure 1D). In female *C5ar1*^{-/-} mice, we observed a significant increase in hematocrit; however, when we compared the hematocrit of female *C5ar1*^{-/-} mice that did not develop diarrhea with that of female *C5ar1*^{-/-} control mice, we found no significant differences (data not shown). To assess whether the intestinal inflammation results in systemic complement activation, we compared C5a serum levels in naïve mice with those obtained 1 h and 2 days after the 7th OVA challenge. We found at best a minor increase that was gender-independent (Figure 1E).

3.2 | Activation of the C5a/C5aR1 axis induces allergen-specific IgE production in male wt mice

Antigen-specific IgE antibodies are crucial for the manifestations of food allergy symptoms including allergen-mediated anaphylaxis.¹ We found antigen-specific IgE production in the serum of male and female wt mice, which was significantly decreased in *C5ar1*^{-/-} male but not in female animals (Figure 1F). This finding suggests that the C5a/C5aR1 axis is critical for B-cell-mediated production of allergen-specific IgE and may explain, in part, the reduced incidence of diarrhea in male *C5ar1*^{-/-} mice. In search for mechanisms underlying the C5a-mediated regulation of IgE antibody production in male mice, we assessed the numbers of IL-25R⁺ T cells (lin⁻ CD3⁺ CD4⁺ IL-25⁺) and ILC2 (lin⁻ CD4⁻ CD3⁻ c-kit⁻ IL-25R⁺ ST2⁺) cells in the LP of the small intestine after the 7th challenge.¹⁷ No significant differences were observed in male (Figure 1G) or female mice (data not shown). OVA-specific IgE levels were not decreased in *C5ar1*^{-/-} female mice suggesting that the diminished anaphylaxis phenotype in *C5ar1*^{-/-} females is not attributed to reduced IgE levels.

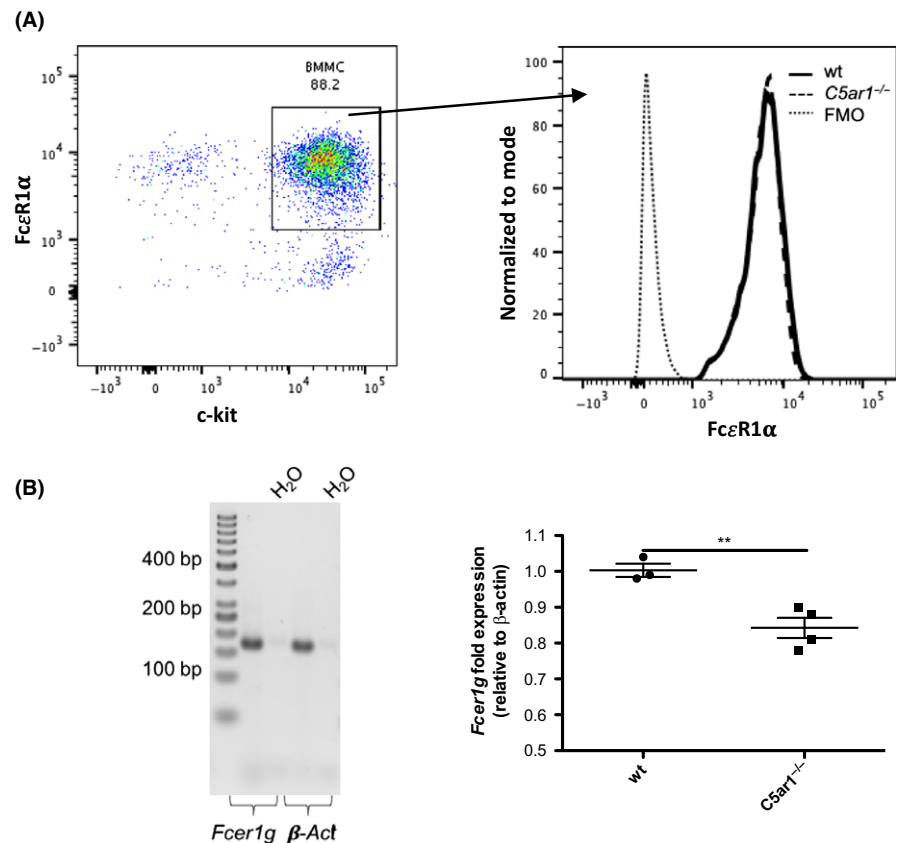


FIGURE 3 FcεR1α and FcεR1γ expression in BMMCs from wt and C5aR1-deficient mice. (A) FcεR1α receptor expression. Left panel: BM cells expressing FcεR1α and c-kit were identified as MCs. Right panel: FcεR1α expression of FcεR1⁺c-kit⁺ BMMCs from wt (solid line) and *C5aR1*^{-/-} (dashed line) mice. The dotted line depicts the FMO control. The histogram is representative of three independent experiments. (B) FcεR1γ expression in BMMCs. Shown is the mRNA (left) and relative mRNA (right) expression in cells from wt and *C5aR1*^{-/-} mice as determined by PCR or qPCR (n = 3–4/group). Data on the right panel represent the mean ± SEM and were analyzed by unpaired Student's *t* test. ***P* < 0.01 [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Mast cell functions are reduced in vivo and in vitro in C5aR1-deficient mice

Intestinal MCs including MC progenitors (iMCP) and IL-9-producing mucosal mast cells (MMC9) drive the clinical phenotype in experimental food allergy models.^{1,17} Surprisingly, we found no difference in MC (Figure 2A), iMCP, and MMC9 (data not shown) numbers between wt and C5aR1-deficient males after the 7th OVA challenge. Next, we assessed whether the activation of MCs was different in wt and *C5aR1*^{-/-} mice. For this purpose, we determined serum levels of MCPT-1, a MC protease that is stored in the granules and released upon IgE-mediated MC degranulation²⁵ and serves as a surrogate marker for in vivo MC activation and food-induced anaphylaxis.² Consistent with the reduced anaphylaxis phenotype, the concentration of serum mMCPT-1 was significantly lower in *C5aR1*^{-/-} than in wt mice (Figure 2B) suggesting decreased activation and degranulation of MC in response to FcεR1 cross-linking in vivo in *C5aR1*^{-/-} mice.

To further test this hypothesis, BMMC from wt and C5aR1-deficient mice were loaded with TNP-specific IgE and subsequently stimulated with TNP-BSA. The supernatants were tested for β-hexosaminidase release and IL-6 production. The spontaneous degranulation after IgE binding was 1%–5% and did not differ between the treatment groups (data not shown). In contrast, β-hexosaminidase release and IL-6 production in response to FcεR1 aggregation were significantly reduced in *C5aR1*^{-/-} BMMCs as compared with wt cells (Figure 2C,D). These data strongly suggest that the activation of the C5a/C5aR1 axis is critical for IgE-mediated degranulation and

activation of MCs. Differences in FcεR1α surface expression did not account for these differences as they were comparable in wt and *C5aR1*^{-/-} mice (Figure 3A). However, we found a significant down-regulation of FcR γ-chain (*FcεR1γ*) mRNA expression in cells from *C5aR1*^{-/-} mice (Figure 3B), suggesting that C5aR1 signaling may affect FcεR1 signaling in BMMCs.

3.4 | C5aR1 expression is upregulated on BMMCs and peritoneal MCs after FcεR1 aggregation

Next, we determined C5 and C5aR1 expression in BMMCs. Clearly, we found C5 protein production in naïve BMMCs (Figure 4A). Surprisingly, we found C5aR1 mRNA but only minor cell surface expression on naïve BMMCs (Figure 4B). To assess whether FcεR1 cross-linking regulates C5aR1 expression, we loaded BMMCs with TNP-specific IgE, which was subsequently cross-linked by TNP-OVA. Already after IgE loading, we observed a slight increase in C5aR1 expression. Three hours after FcεR1 aggregation, we found a 100-fold increase in *C5aR1* mRNA expression (Figure 4C) resulting in strong C5aR1 surface expression 6 h after FcεR1 activation, which further increased during the next 18 h and declined after 48 h (Figure 4D). The *C5aR1* mRNA expression was stable between 3 and 6 h after FcεR1 aggregation and steadily declined thereafter (Figure 4C). The observed effect was specific for IgE cross-linking, since TLR activation or degranulation with compound 48/80 had minor or no effects (Figure 4E). Further, we found that the antigen dose required to drive strong

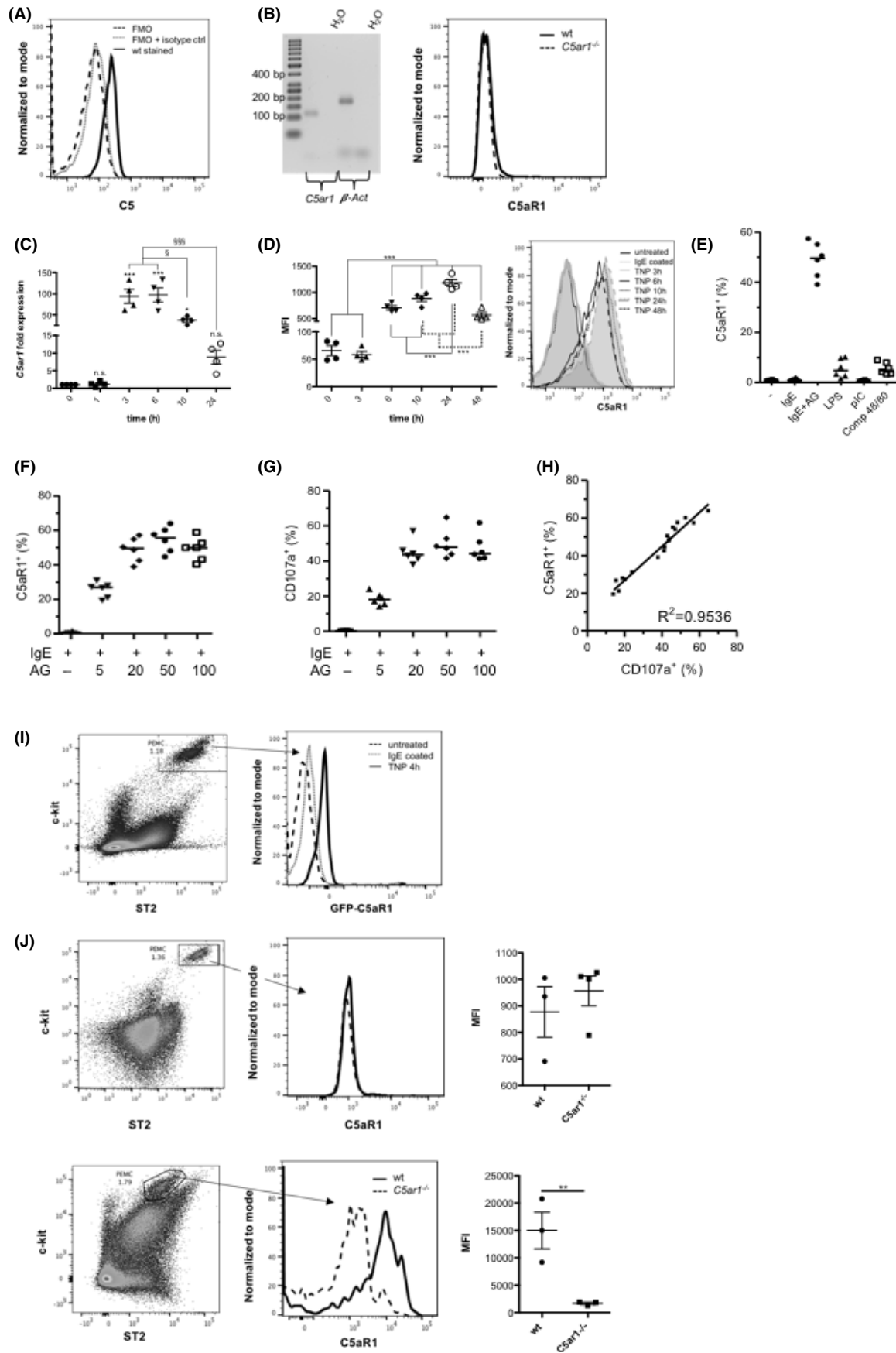


FIGURE 4 Expression of C5 and C5aR1 on BMMCs and peritoneal MCs. (A) C5 protein expression in BMMCs from BALB/c mice. Histogram: FMO—dashed line; FMO + isotype ctrl—gray, dotted line; C5-specific staining—solid line. (B) mRNA (left panel) and surface C5aR1 expression (right panel) in BMMCs. Histogram: signal obtained with cell from *C5ar1*^{-/-} mice—dashed line; signals obtained with cells from wt mice—solid line. (C,D) Time course of *C5ar1* mRNA (C) and C5aR1 surface expression (D) in wt BMMCs in response to FcεR1 receptor aggregation (n = 4/group). The histogram shows the change in C5aR1 surface expression between 3 and 48 h after FcεR1 receptor cross-linking. Data shown in C and D represent the mean ± SEM. Statistical differences between time points were determined by one-way ANOVA with Tukey's multiple comparison post hoc test *⁵P < 0.05, **P < 0.01, ***⁵⁵⁵P < 0.001. (E) Frequency of C5aR1⁺ BMMCs in response to FcεR1 aggregation, LPS, Poly (I:C), or compound 48/80 stimulation (n = 6/group). (F, G) Dose-dependent induction of (F) MC degranulation determined as surface translocation of CD107a or (G) C5aR1 expression in BMMCs (n = 6/group). (H) Regression analysis of C5aR1⁺ and CD107a⁺ BMMCs in response to stimulation with increasing concentrations of antigen. Data shown in E-G represent the median. (I) GFP-C5aR1 expression in naïve PEMCs and 4 h after ex vivo FcεR1 receptor aggregation. Left panel: PEMCs were identified as ST2⁺c-kit⁺FcεR1⁺ cells; right panel: GFP-C5aR1 expression was determined in PEMCs from floxed GFP-C5aR1 knock-in mice. Histograms: signal obtained from PEMCs of naïve floxed GFP-C5aR1 knock-in mice—dashed line; signal obtained from IgE-coated floxed GFP-C5aR1 knock-in mice—dotted line; signal obtained from PEMCs of floxed GFP-C5aR1 knock-in mice 4 h after ex vivo FcεR1 receptor aggregation—solid line. The histogram is representative of three independent experiments. (J) C5aR1 expression in PEMCs isolated from naïve wt BALB/c or *C5ar1*^{-/-} mice (upper panel) or after the 7th oral OVA challenge (lower panel). Peritoneal cells positive for ST2 and c-kit (dot plots; left panels) were identified as MCs. C5aR1 surface expression on PEMCs was determined using C5aR1-specific mAb 20/70 (middle panels; histograms: signal obtained with PEMCs from C5aR1-deficient mice—dashed line; signal obtained with PEMCs from wt mice—solid line). The graphs on the very right depict differences in C5aR1 expression in PEMCs from naïve wt and *C5ar1*^{-/-} mice (upper graph) or mice after the 7th oral OVA challenge (lower graph) (n = 3-4/group). Data shown are the median. Statistical differences between the groups were determined by Mann-Whitney U test, **P < 0.01 [Colour figure can be viewed at wileyonlinelibrary.com]

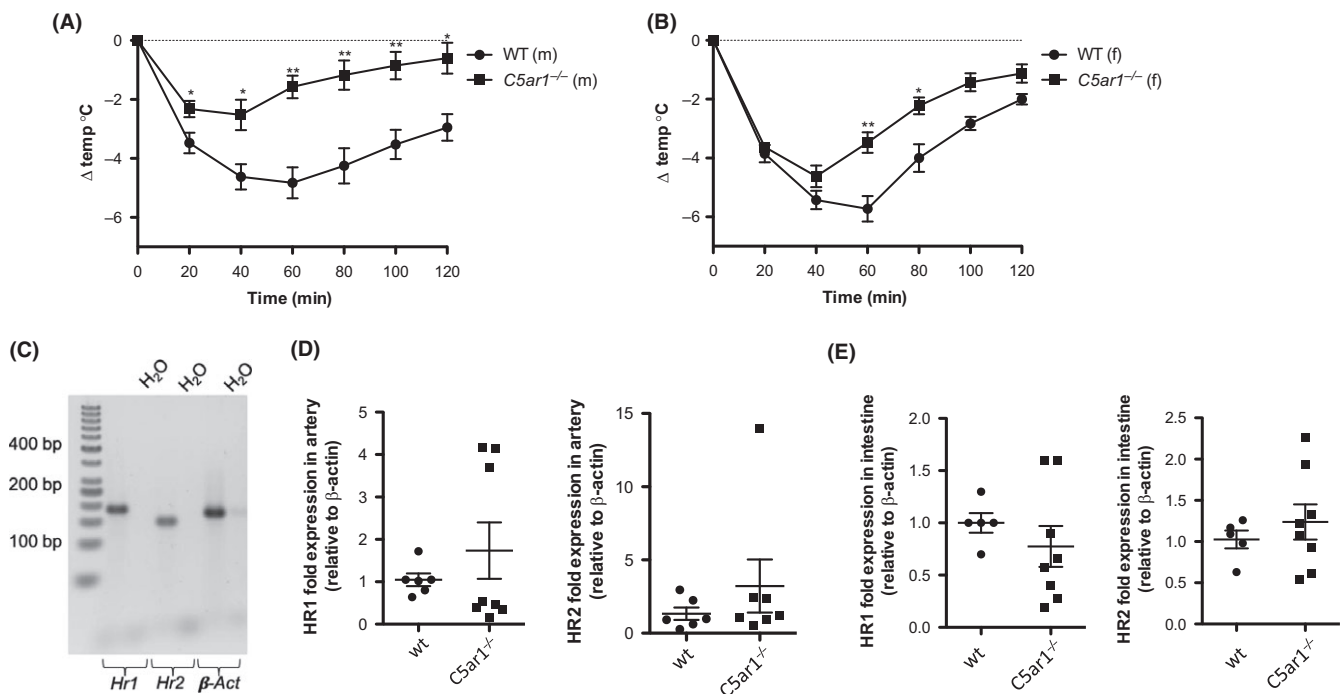


FIGURE 5 Histamine-induced hypothermia in wild-type BALB/c and C5aR1-deficient mice and histamine receptor expression in the abdominal aorta or the small intestine. (A, B) Change of the rectal temperature between 0 and 120 min following the i.v. injection of histamine (5 mg/200 μL of saline) in (A) male (m) and (B) female (f) wt and *C5ar1*^{-/-} mice. Each data point represents the mean ± SEM of 4 mice from one out of 4 independent experiments. The temperature changes at the indicated time points were compared using an unpaired Student's *t* test (wt vs *C5ar1*^{-/-}). *P < 0.05, **P < 0.01. Δtemp °C: rectal temperature at the indicated time point minus rectal temperature before histamine administration. (C-E) Histamine 1 and 2 receptor expression in two target tissues. Shown is the mRNA (C) and relative mRNA expression in the abdominal aorta (D) or the small intestine (E) of BALB/c wt or *C5ar1*^{-/-} mice (n = 5-8/group). Data in D and E represent the mean ± SEM. Differences between groups were analyzed by Student's *t* test

FcεR1-mediated degranulation (Figure 4F; Figure S1A) also resulted in strong and similar C5aR1 expression in BMMCs of male and female origin (Figure 4G; Figure S1B) again linking FcεR1 signaling to upregulation of C5aR1 (Figure 4H).

Similar to BMMCs, peritoneal MCs (PEMC) from naïve GFP-C5aR1 reporter mice⁸ stained negative for C5aR1. However, IgE loading induced a slight shift of the GFP signal, which became strong 4 h after FcεR1 activation (Figure 4I). Two days after the 7th OVA

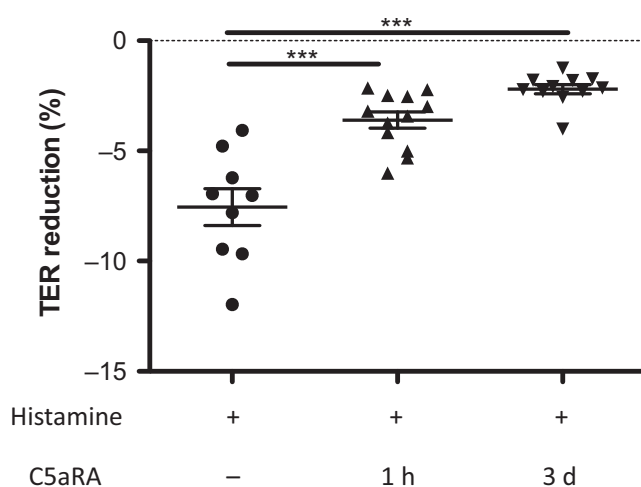


FIGURE 6 Impact of C5aR1 blockade on histamine-induced change in transendothelial resistance in mHEVC. Confluent mHEVC were either treated with the C5aRA A8 Δ 71-73 1 h (5 μ M) or 3 day (2 μ M) before the stimulation with histamine or without C5aRA ($n = 9$ -12/group). Data represent the mean $\pm$ SEM. Differences between groups were determined using one-way ANOVA with Tukey's multiple comparison post hoc test. *** $P < 0.001$

challenge, PEMCs strongly expressed C5aR1 at the cell surface (Figure 4J) demonstrating C5aR1 expression on MCs after cross-linking of Fc ϵ R1 in vivo. Unfortunately, direct detection of C5aR1 expression on MCs from the intestine was not possible as the C5aR1-specific Ab did not work on paraffin sections of the intestine.

3.5 | C5aR1 signaling increases the sensitivity of the vascular system toward the MC mediator histamine

One important mediator released from MC upon Fc ϵ R1 aggregation is histamine,²⁶ which promotes hypovolemic shock observed in experimental food-induced anaphylaxis through activation of the G protein-coupled (GPCR) H1 and H2 receptors.²⁷ We and others have shown that C5aR1 activation regulates the function of other GPCRs including the bradykinin B2 receptor (B2R)²⁸ and CCR5.^{29,30} Thus, we hypothesized that C5aR1 may regulate the shock response through an impact of histamine receptor-mediated dysfunction of the vascular endothelium. To test this hypothesis, wt and C5aR1 $^{-/-}$ mice were subjected to histamine-induced hypothermia. As expected, we found a strong drop in body temperature of approximately 4°C in wt male (Figure 5A) and female (Figure 5B) mice during the first 20 min after histamine administration, which further decreased during the next 20-40 min to -5°C (male) or -6°C (female). Between 60 and 120 min, wt mice recovered slowly although the temperature was still 3°C (male mice) or 2°C below the starting value. In contrast, the initial temperature reduction was less pronounced in male C5aR1 $^{-/-}$ mice. Further, 120 min after histamine administration the rectal temperature was indistinguishable from the starting values in male C5aR1 $^{-/-}$ mice (Figure 5A). In C5aR1 $^{-/-}$ female mice, the initial temperature drop was similar to wt mice but

attenuated between 40 and 60 min. Also, the mice recovered much faster than the wt control animals (Figure 5B). These findings suggest that activation of the C5a/C5aR1 axis regulates the histamine-induced hypovolemic shock and suggest a role for the C5a/C5aR1 axis on vascular endothelial function. In support of this view, we found no differences in H1 and H2 mRNA receptor expression levels in the abdominal aorta (Figure 5C,D) or the small intestine (Figure 5E) from wt and C5aR1 $^{-/-}$ mice.

3.6 | C5aR1 activation controls histamine-mediated opening of tight junctions in human endothelial cells

The mechanisms underlying histamine-mediated increase in vascular endothelial leak are not entirely clear but involve phospholipase C activation and increase in intracellular Ca²⁺, both of which are also important downstream targets of C5aR1 signaling.³¹ To directly assess the impact of C5aR1 activation on histamine-driven regulation of vascular endothelial barrier function, we examined histamine-induced vascular endothelial barrier dysfunction in mHEVC in the presence or absence of C5aR1 blockade. Histamine induced a significant vascular endothelial barrier dysfunction as evidenced by a strong decrease of TER in control-mHEVC cells (Figure 6). Notably, one hour pretreatment with the C5aRA protected mHEVCs from histamine-induced barrier dysfunction. The protective effect was even more pronounced when the C5aRA was present throughout the histamine stimulation (Figure 6) strongly suggesting that C5aR1 signaling amplifies histamine-induced barrier dysfunction of the vascular endothelium.

4 | DISCUSSION

Although the complement system has been intensively studied over last decades, its role in allergy has not been well explored. Previous work revealed a dual role for C5aR1 and a pro-allergic role for C3aR in allergic asthma.³² The available data support the view that C5a protects from the development of T_H2 response during allergen sensitization and promotes pro-allergic functions in the allergic effector phase. In contrast to the results derived from allergic asthma models,³² C5aR1 deficiency resulted in decreased antigen-specific IgE production, at least in male mice. It is well appreciated that that sexual dimorphism exists for mouse C5³³ and that C5 levels are considerably higher in male than in female mice³³ contributing, for example, to TRALI development in male but not in female mice.³⁴ In several studies, male sex has been associated with an increased risk to develop food allergy.^{35,36} While it is unclear at this point whether the results from the mouse model can be transferred to human food allergy, a recent study demonstrated that the C5a/C5aR1 axis regulates adaptive immunity in human T cells.³⁷

MCs are the dominant effector cells in food allergy reactions. Intestinal MCs numbers positively correlate with the severity of food allergy symptoms,¹⁶ and expression of C3aR and C5aR has been reported in human MCs. Functionally, both anaphylatoxins are

potent MC chemoattractants and can induce MC degranulation independent of IgE.^{7,38,39} Further, activation of C5aR1 and C3aR enhances IgE-mediated MC degranulation in a passive cutaneous anaphylaxis model.⁴ Given that the decrease in diarrhea development in female mice was not associated with reduced allergen-specific IgE production, we considered differences in MC functions as another potential mechanism accounting for the reduced incidence in diarrhea and hypovolemic shock in female *C5ar1*^{-/-} mice, in particular, as we found similar numbers of intestinal MCs, MMC9, and iMPC in wt and *C5ar1*^{-/-} male and female mice. MCPT-1 serum levels were markedly reduced in *C5ar1*^{-/-} mice of both sexes suggesting reduced IgE-mediated degranulation of MCs. In line with this and previous findings,⁴ we observed significantly attenuated FcεR1-mediated degranulation and cytokine production in *C5ar1*^{-/-} MCs. These results support the view that C5aR1-deficient MCs are less sensitive to FcεR1-mediated degranulation. While FcεR1α expression on wt and *C5ar1*^{-/-} BMMCs was comparable, we found a significant reduction of *Fcer1g* expression in *C5ar1*^{-/-} MCs, which is required for FcεR1 signaling⁴⁰ and might therefore explain the reduced degranulation and activation of C5aR1-deficient MCs. In support of this view, C5aR1 drives upregulation of *Fcer1g* in pulmonary macrophages.⁴¹ *Fcer1g* gene expression is controlled by several transcription factors including Sp1, GABP, and Elf-1 binding to transcriptional enhancer elements. One of such elements is similar to a binding motif of the C/EBP family of transcription factors,⁴² which can be induced by C5aR1 signaling.⁴³ Thus, C5aR1 may control *Fcer1g* expression through this pathway. The available data support a model, in which C5aR1 activation of neutrophils and macrophages controls IgG Fc receptor expression⁴⁴ and vice versa, and FcγRIIb signaling controls C5aR1 signaling.⁴⁵ Our findings add to this view by showing that low C5aR1 expression in MCs is associated with low *Fcer1g* expression. Alternatively, C5a may regulate the differentiation of BMMCs and thereby affect FcεR1-mediated activation. In support of this view, the C5a/C5aR1 axis was shown to regulate myeloid suppressor cell generation from BM cells,⁴⁶ neuronal progenitor cells,⁴⁷ or osteoclast differentiation from mesenchymal stem cells.⁴⁸

Also, we uncovered a new mechanism, by which FcεR1 cross-linking drives marked enhancement of *C5ar1* mRNA expression in BMMCs and peritoneal MCs eventually resulting in strong C5aR1 surface expression. It is tempting to speculate that the induction of diarrhea that begins only after repeated oral OVA challenge results from the upregulation of C5aR1 expression in intestinal MCs and that it is the combined activation of FcεR1 and C5aR1 of such MCs that finally drives diarrhea development, temperature drop, and anaphylaxis.

Finally, we observed that *C5ar1*^{-/-} male mice were less sensitive to the histamine-induced hypovolemic shock during the first 20 min. Also, the recovery was much faster, both in male and female C5aR1-deficient mice. Similar to what we observed in *C5ar1*^{-/-} female (Figure 5B), Hox et al reported that not the initial temperature drop during the first 20 min after histamine administration but the recovery between 20 and 120 min was faster in

estrogen receptor-targeted mice suggesting that estradiol controls the recovery from histamine-mediated anaphylaxis.⁴⁹ It is possible that C5aR1 impacts the recovery from histamine-induced anaphylaxis via a similar mechanism to the female steroid hormone, estradiol.

Histamine-induced hypothermia involves the activation of H1 and H2 receptors, both of which are GPCRs. The exact cell type and site at which histaminergic signaling drives hypothermia remains unknown.⁵⁰ Some studies reported that HR1 and HR2 signaling in the hypothalamus is responsible for the decrease in temperature,^{51,52} although the exact cell type mediating this effect remains elusive. Importantly, C5aR1 is expressed in the brain by microglia, astrocytes, and neurons.^{53,54} Thus, C5aR1 signaling may act in concert with H1 and H2 receptors to control central thermoregulation by histamine. Our previous findings clearly demonstrate that C5aR1 interacts with other GPCRs such as B2R²⁸ on dendritic cells and CCR2^{29,30} on monocyte-derived macrophages and regulates their functions.

On the other hand, it was reported that activation of HR1 and HR2 induces various cellular responses in vascular endothelial cells including changes in vascular permeability⁵⁵ which leads to hypovolemia and finally hypothermia.⁵⁶ The data derived from in vitro experiments on mHEVC show that C5aR1 targeting reduced histamine-induced barrier dysfunction. The potential mechanism could involve the formation of heterodimers comprising H1/H2R and C5aR1. Such heterodimerization of C5aR1 with another GPCR has been already reported. CCR5 forms dimers with C5aR1, which is critical for the entrance of HIV into the macrophages.³⁰ Interestingly, blockade of C5aR signaling decreased the CCR5 expression. HR1 and HR2 are expressed on murine and human vascular smooth muscle and endothelial cells.⁵³ The same cells were reported to express C5aR1.⁵⁷ Thus, C5aR1 signaling may enhance the responsiveness of endothelial cells toward histamine and increase hypothermia in vivo through a direct cross talk with histamine receptors, the expression of which was similar in target cells/tissues of wt and *C5ar1*^{-/-} mice. It will be important to assess the exact mechanisms of such cross talk in future studies.

In summary, our findings demonstrate that complement drives the development of allergic responses in a model of experimental food allergy by several mechanisms that act in concert and operate in a gender-dependent way. C5aR1 signaling promotes allergen-specific IgE antibody production in male mice, enhances IgE-mediated MC degranulation in male and female mice, and controls important effector functions of the MC mediator histamine with some differences between male and female mice. Altogether, the data suggest that C5aR1 may serve as potential new therapeutic target in this emerging disease.

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CONFLICT OF INTEREST

None of the authors has a conflict of interest in relation to this work.

AUTHOR CONTRIBUTIONS

A.K., A.T.R., D.W., J.-B.L., M.H.-L., P.H., Y.-H.W., and Z.O. performed experiments and analyzed the data. A.K. and J.K. wrote the manuscript. S.P.H. and J.K. designed the study, analyzed data, and wrote the manuscript. All authors contributed to critical revision of the manuscript and approved the final version for publication.

ORCID

Jörg Köhl  <http://orcid.org/0000-0003-1121-3178>

REFERENCES

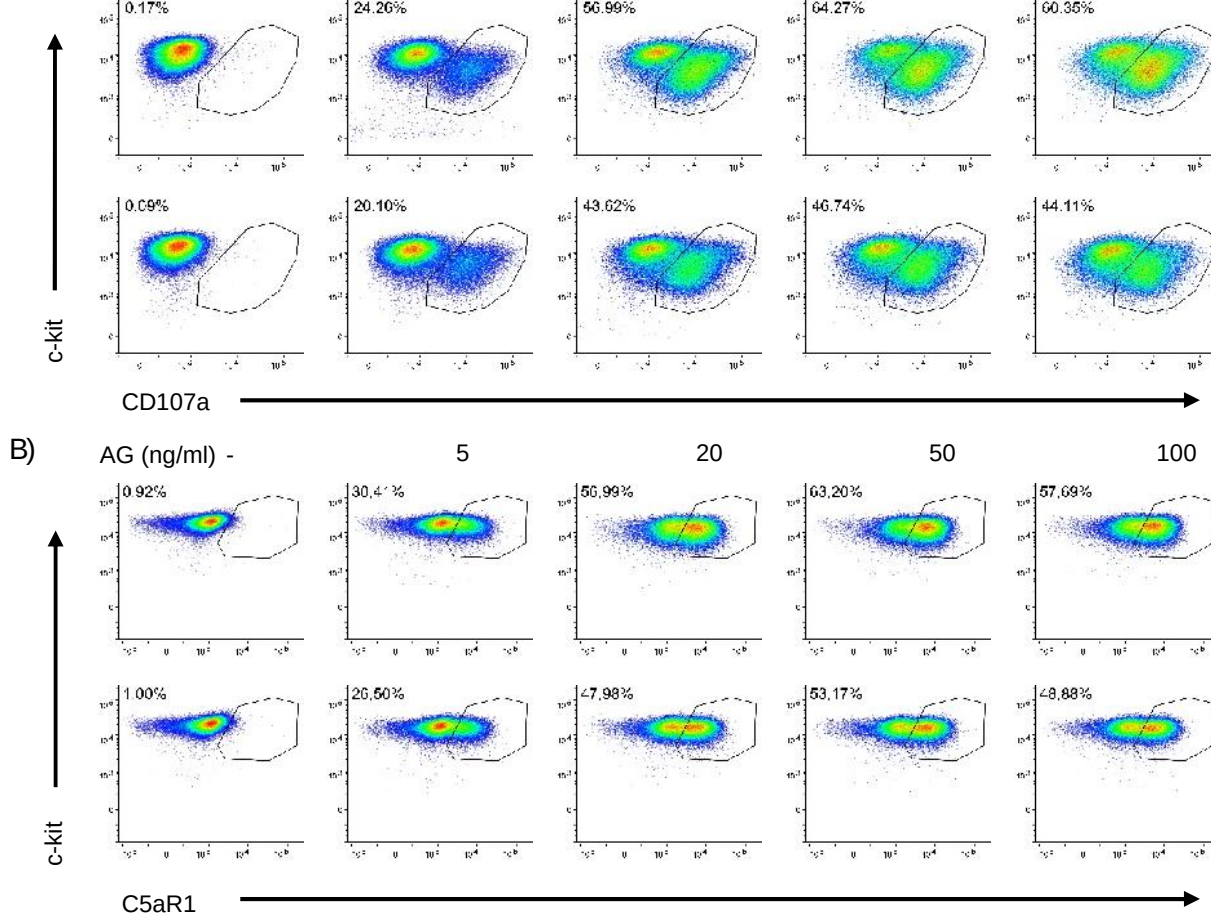
- Tordesillas L, Berin MC, Sampson HA. Immunology of food allergy. *Immunity*. 2017;47:32-50.
- Brown SGA, Stone SF, Fatovich DM, et al. Anaphylaxis: clinical patterns, mediator release, and severity. *J Allergy Clin Immunol*. 2013;132:1141-1149.
- Khodoun M, Strait R, Orekov T, et al. Peanuts can contribute to anaphylactic shock by activating complement. *J Allergy Clin Immunol*. 2009;123:342-351.
- Schäfer B, Piliponsky AM, Oka T, et al. Mast cell anaphylatoxin receptor expression can enhance IgE-dependent skin inflammation in mice. *J Allergy Clin Immunol*. 2013;131:541-548.
- Nigrovic PA, Malbec O, Lu B, et al. C5a receptor enables participation of mast cells in immune complex arthritis independent of Fc γ receptor modulation. *Arthritis Rheum*. 2010;62:3322-3333.
- Gaudenzio N, Sibilano R, Marichal T, et al. Different activation signals induce distinct mast cell degranulation strategies. *J Clin Invest*. 2016;126:3981-3998.
- Hartmann K, Henz BM, Krüger-Krasagakes S, et al. C3a and C5a stimulate chemotaxis of human mast cells. *Blood*. 1997;89:2863-2870.
- Karsten CM, Laumonnier Y, Eurich B, et al. Monitoring and cell-specific deletion of C5aR1 using a novel floxed GFP-C5aR1 reporter knock-in mouse. *J Immunol*. 2015;194:1841-1855.
- Ahrens R, Osterfeld H, Wu D, et al. Intestinal mast cell levels control severity of oral antigen-induced anaphylaxis in mice. *Am J Pathol*. 2012;180:1535-1546.
- Arumugam M, Ahrens R, Osterfeld H, et al. Increased susceptibility of 129SvEvBrd mice to IgE-Mast cell mediated anaphylaxis. *BMC Immunol*. 2011;12:14.
- Strait RT, Morris SC, Smiley K, Urban JF, Finkelman FD. IL-4 exacerbates anaphylaxis. *J Immunol*. 2003;170:3835-3842.
- Groschwitz KR, Ahrens R, Osterfeld H, et al. Mast cells regulate homeostatic intestinal epithelial migration and barrier function by a chymase/Mcpt4-dependent mechanism. *Proc Natl Acad Sci*. 2009;106:22381-22386.
- Reitz M, Hartmann W, Rüdiger N, Orinska Z, Brunn M-L, Breloer M. Interleukin-9 promotes early mast cell-mediated expulsion of *Strongyloides ratti* but is dispensable for generation of protective memory. *Sci Rep*. 2018;8:8636.
- Saitoh S, Arudchandran R, Manetz TS, et al. LAT is essential for Fc ϵ RI-mediated mast cell activation. *Immunity*. 2000;12:525-535.
- Wang N, McKell M, Dang A, et al. Lipopolysaccharide suppresses IgE-mast cell-mediated reactions. *Clin Exp Allergy*. 2017;47:1574-1585.
- Brandt EB, Strait RT, Hershko D, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest*. 2003;112:1666-1677.
- Chen C-Y, Lee J-B, Liu B, et al. Induction of interleukin-9-producing mucosal mast cells promotes susceptibility to IgE-mediated experimental food allergy. *Immunity*. 2015;43:788-802.
- Zhang X, Schmutte I, Laumonnier Y, et al. A critical role for C5L2 in the pathogenesis of experimental allergic asthma. *J Immunol*. 2010;185:6741-6752.
- Zhang X, Lewkowich IP, Köhl G, Clark JR, Wills-Karp M, Köhl J. A protective role for C5a in the development of allergic asthma associated with altered levels of B7-H1 and B7-DC on plasmacytoid dendritic cells. *J Immunol*. 2009;182:5123-5130.
- Forbes EE, Groschwitz K, Abonia JP, et al. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J Exp Med*. 2008;205:897-913.
- Huber-Lang M, Younkin EM, Sarma JV, et al. Generation of C5a by phagocytic cells. *Am J Pathol*. 2002;161:1849-1859.
- Otto M, Hawlisch H, Monk PN, et al. C5a mutants are potent antagonists of the C5a receptor (CD88) and of C5L2 position 69 is the locus that determines agonism or antagonism. *J Biol Chem*. 2004;279:142-151.
- Wu D, Ahrens R, Osterfeld H, et al. Interleukin-13 (IL-13)/IL-13 receptor α 1 (IL-13R α 1) signaling regulates intestinal epithelial cystic fibrosis transmembrane conductance regulator channel-dependent Cl⁻ secretion. *J Biol Chem*. 2011;286:13357-13369.
- Strait RT, Mahler A, Hogan S, Khodoun M, Shibuya A, Finkelman FD. Ingested allergens must be absorbed systemically to induce systemic anaphylaxis. *J Allergy Clin Immunol*. 2011;127:982-989.
- Brown JK, Knight PA, Wright SH, Thornton EM, Miller HRP. Constitutive secretion of the granule chymase mouse mast cell protease-1 and the chemokine, CCL2, by mucosal mast cell homologues. *Clin Exp Allergy J Br Soc Allergy Clin Immunol*. 2003;33:132-146.
- Reber LL, Hernandez JD, Galli SJ. The pathophysiology of anaphylaxis. *J Allergy Clin Immunol*. 2017;140:335-348.
- Makabe-Kobayashi Y, Hori Y, Adachi T, et al. The control effect of histamine on body temperature and respiratory function in IgE-dependent systemic anaphylaxis. *J Allergy Clin Immunol*. 2002;110:298-303.
- Schmitz V, Almeida LN, Svensjö E, Monteiro AC, Köhl J, Scharfstein J. C5a and bradykinin receptor cross-talk regulates innate and adaptive immunity in *Trypanosoma cruzi* infection. *J Immunol*. 2014;193:3613-3623.
- Hüttenrauch F, Pollok-Kopp B, Oppermann M. G protein-coupled receptor kinases promote phosphorylation and β -arrestin-mediated internalization of CCR5 homo- and hetero-oligomers. *J Biol Chem*. 2005;280:37503-37515.
- Moreno-Fernandez ME, Aliberti J, Groeneweg S, Köhl J, Chougnat CA. A novel role for the receptor of the complement cleavage fragment C5a, C5aR1, in CCR5-mediated entry of HIV into macrophages. *AIDS Res Hum Retroviruses*. 2015;32:399-408.

31. Jiang H, Kuang Y, Wu Y, Smrcka A, Simon MI, Wu D. Pertussis toxin-sensitive activation of phospholipase C by the C5a and fMet-Leu-Phe receptors. *J Biol Chem*. 1996;271:13430-13434.
32. Schmutte I, Laumonnier Y, Köhl J. Anaphylatoxins coordinate innate and adaptive immune responses in allergic asthma. *Semin Immunol*. 2013;25:2-11.
33. Baba A, Fujita T, Tamura N. Sexual dimorphism of the fifth component of mouse complement. *J Exp Med*. 1984;160:411-419.
34. Strait RT, Hicks W, Barasa N, et al. MHC class I-specific antibody binding to nonhematopoietic cells drives complement activation to induce transfusion-related acute lung injury in mice. *J Exp Med*. 2011;208:2525-2544.
35. Kotz D, Simpson CR, Sheikh A. Incidence, prevalence, and trends of general practitioner-recorded diagnosis of peanut allergy in England, 2001 to 2005. *J Allergy Clin Immunol*. 2011;127:623-630.
36. Pyrhönen K, Näyhä S, Kaila M, Hiltunen L, Läärä E. Occurrence of parent-reported food hypersensitivities and food allergies among children aged 1–4 yr. *Pediatr Allergy Immunol*. 2009;20:328-338.
37. Arbore G, West EE, Spolski R, et al. T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4⁺ T cells. *Science*. 2016;352:aad1210.
38. Nilsson G, Johnell M, Hammer CH, et al. C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. *J Immunol*. 1996;157:1693-1698.
39. Halova I, Draberova L, Draber P. Mast cell chemotaxis – chemoattractants and signaling pathways. *Front Mol Innate Immun*. 2012;3:119.
40. Oliver JM, Burg DL, Wilson BS, McLaughlin JL, Geahlen RL. Inhibition of mast cell Fc epsilon R1-mediated signaling and effector function by the Syk-selective inhibitor, piceatannol. *J Biol Chem*. 1994;269:29697-29703.
41. Shushakova N, Skokowa J, Schulman J, et al. C5a anaphylatoxin is a major regulator of activating versus inhibitory Fc gammaRs in immune complex-induced lung disease. *J Clin Invest*. 2002;110:1823-1830.
42. Takahashi K, Hayashi N, Shimokawa T, Umehara N, Kaminogawa S, Ra C. Cooperative regulation of Fc receptor gamma-chain gene expression by multiple transcription factors, including Sp1, GABP, and Elf-1. *J Biol Chem*. 2008;283:15134-15141.
43. Gong B, Pan Y, Zhao W, et al. IVIG immunotherapy protects against synaptic dysfunction in Alzheimer's disease through complement anaphylatoxin C5a-mediated AMPA-CREB-C/EBP signaling pathway. *Mol Immunol*. 2013;56:619-629.
44. Kumar V, Ali SR, Konrad S, et al. Cell-derived anaphylatoxins as key mediators of antibody-dependent type II autoimmunity in mice. *J Clin Invest*. 2006;116:512-520.
45. Karsten CM, Pandey MK, Figge J, et al. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIB and dectin-1. *Nat Med*. 2012;18:1401-1406.
46. Schmutte I, Ströver HA, Vollbrandt T, et al. C5a receptor signalling in dendritic cells controls the development of maladaptive Th2 and Th17 immunity in experimental allergic asthma. *Mucosal Immunol*. 2013;6:807-825.
47. Coulthard LG, Hawksworth OA, Li R, et al. Complement C5aR1 signaling promotes polarization and proliferation of embryonic neural progenitor cells through PKCζ. *J Neurosci*. 2017;37:5395-5407.
48. Kalbasi Anaraki P, Patecki M, Larmann J, et al. Urokinase receptor mediates osteogenic differentiation of mesenchymal stem cells and vascular calcification via the complement C5a receptor. *Stem Cells Dev*. 2014;23:352-362.
49. Hox V, Desai A, Bandara G, Gilfillan AM, Metcalfe DD, Olivera A. Estrogen increases the severity of anaphylaxis in female mice through enhanced endothelial nitric oxide synthase expression and nitric oxide production. *J Allergy Clin Immunol*. 2015;135:729-736.
50. Tabarean IV. Histamine receptor signaling in energy homeostasis. *Neuropharmacology*. 2016;106:13-19.
51. Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. *Prog Neurobiol*. 2001;63:637-672.
52. Green MD, Cox B, Lomax P. Histamine H1- and H2-receptors in the central thermoregulatory pathways of the rat. *J Neurosci Res*. 1975;1:353-359.
53. Osaka H, McGinty A, Höpken UE, Lu B, Gerard C, Pasinetti GM. Expression of C5a receptor in mouse brain: role in signal transduction and neurodegeneration. *Neuroscience*. 1999;88:1073-1082.
54. Stahel PF, Kariya K, Shohami E, et al. Intracerebral complement C5a receptor (CD88) expression is regulated by TNF and lymphotoxin-α following closed head injury in mice. *J Neuroimmunol*. 2000;109:164-172.
55. Jutel M, Akdis M, Akdis CA. Histamine, histamine receptors and their role in immune pathology. *Clin Exp Allergy*. 2009;39:1786-1800.
56. Hogan SP, Wang YH, Strait R, Finkelman FD. Food-induced anaphylaxis: mast cells as modulators of anaphylactic severity. *Semin Immunopathol*. 2012;34:643-653.
57. Haviland DL, McCoy RL, Whitehead WT, et al. Cellular expression of the C5a anaphylatoxin receptor (C5aR): demonstration of C5aR on nonmyeloid cells of the liver and lung. *J Immunol*. 1995;154:1861-1869.

SUPPORTING INFORMATION

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

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Suppl. Fig. 1 FcεRI-mediated stimulation induces dose-dependent C5aR1 upregulation in BMMCs. BMMCs of male (upper rows in A and B) or female (lower rows in A and B) origin were loaded with IgE (200ng/ml, clone SP-7) for 18h and stimulated with indicated concentrations of DNP-HSA. **(A,)** FcεRI-mediated degranulation was measured after 20 min by CD107a translocation to the cell surface. CD107a⁺ cells are indicated. **(B)** C5aR1 expression was measured 24h later. C5aR1⁺ cells are indicated. Data shown are from one representative BMMC culture of male or female mice out of six.