HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2024 October 15.

Published in final edited form as:

J Immunol. 2023 October 15; 211(8): 1216–1223. doi:10.4049/jimmunol.2300080.

The eotaxin-1/CCR3 axis and matrix metalloproteinase-9 are critical in anti-NC16A IgE-induced bullous pemphigoid

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Abstract

Bullous pemphigoid (BP) is the most common autoimmune bullous skin disease of humans and is characterized by eosinophilic inflammation and circulating and tissue-bound IgG and IgE autoantibodies directed against two hemidesmosomal proteins: BP180 and BP230. The NC16A domain of BP180 has been found to contain major epitopes recognized by autoantibodies in BP. We recently established the pathogenicity of anti-NC16A IgE through passive transfer of patient-derived autoantibodies to double humanized mice that express the human high affinity IgE receptor, FcεRI, and human NC16A domain (FcεRI/NC16A). In this model, anti-NC16A IgE recruit eosinophils to mediate tissue injury and clinical disease in FcεRI/NC16A mice. The objective of this study was to characterize the molecular and cellular events that underly eosinophil recruitment and eosinophil-dependent tissue injury in anti-NC16A IgE-induced BP. We show that anti-NC16A IgE significantly increase levels of key eosinophil chemoattractants,

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Credit author statement

T.J.M.J., J.C., N.L., and Z.L. designed the study; T.J.M.J., J.C., N.L., S.B., L.W., and Z.L. conducted experiments; T.J.M.J., J.C., N.L., D.C., S.G., P.G., N.E.T., and Z.L. analyzed data; D.C., L.C., and L.A.D. provided critical reagents; T.J.M.J., N.L., S.B., and Z.L. wrote the manuscript.

Conflict of Interest Statement

The authors have declared that no conflicts of interest exist.

eotaxin-1 and eotaxin-2, as well as the proteolytic enzyme matrix metalloproteinase-9 (MMP-9) in the lesional skin of FcεRI/NC16A mice. Importantly, neutralization of eotaxin-1, but not eotaxin-2, and blockade of the main eotaxin receptor, CCR3, drastically reduces anti-NC16A IgE-induced disease activity. We further show that anti-NC16A IgE/NC16A immune complexes induce the release of MMP-9 from eosinophils and that MMP-9-deficient mice are resistant to anti-NC16A IgE-induced BP. Lastly, we find significantly increased levels of eotaxin-1, eotaxin-2, and MMP-9 in blister fluids of BP patients. Taken together, this study establishes the eotaxin-1/CCR3 axis and MMP-9 as key players in anti-NC16A IgE-induced BP and candidate therapeutic targets for future drug development and testing.

Keywords

IgE; autoimmune disease; bullous pemphigoid; eosinophils; eotaxins; matrix metalloproteinase-9

Introduction

Bullous pemphigoid (BP) is the most common autoimmune bullous skin disease of humans and predominately manifests in elderly patients over the age of 70 [1–3]. Histologically, BP[1] is defined by dermal-epidermal separation accompanied by an inflammatory infiltrate predominated by eosinophils and variable numbers of neutrophils and lymphocytes [1, 3]. The presence of circulating and tissue-bound autoantibodies directed against key structural proteins within the hemidesmosome, including BP180 and BP230, is a diagnostic hallmark of the disease [1–3]. BP180 is a 180-kDa transmembrane glycoprotein with an ectodomain composed of 15 collagenous domains interrupted by 16 non-collagenous domains that plays a key role in anchoring basal keratinocytes to the underlying dermis through the basement membrane zone (BMZ) [4]. The 16th non-collagenous domain (NC16A) of BP180's ectodomain has been widely accepted as an immunodominant region containing major pathogenic epitopes recognized by autoantibodies in the majority of BP patients [5–8].

Circulating anti-NC16A[2] IgG autoantibodies have been identified in over 90% of BP patients and correlate with clinical features and disease activity [9–18]. We have previously shown that patient-derived anti-NC16A IgG do not recognize the murine homologue, BP180 NC14A [19]. For this reason, we developed a humanized mouse model where the murine BP180 NC14A domain was replaced with the human BP180 NC16A domain to directly test the pathogenicity of patient-derived anti-NC16A IgG *in vivo* [20]. Passive transfer of anti-NC16A IgG to humanized NC16A mice results in deposition of IgG along the BMZ[3] and subepidermal blister formation that is dependent on complement activation, mast cell degranulation, and neutrophil infiltration [20]. However, anti-NC16A IgG-induced BP in NC16A mice occurs in the notable absence of infiltrating eosinophils which sharply contrasts the lesional skin of BP patients and suggests the presence of anti-NC16A IgG-dependent and -independent mechanisms of disease [20].

1. BP = bullous pemphigoid
2. NC16A = human BP180 non-collagenous 16A domain
3. BMZ = basement membrane zone

Circulating anti-NC16A IgE have also been detected in up to 79% of BP patients and have been shown to correlate with clinical features and disease activity as well [9–13, 21–24]. Early experimental animal models, as well as *in vitro* and *ex vivo* studies, have suggested a pathogenic role for anti-BP180 IgE in BP [24–28]. Additional support for the pathogenicity of IgE autoantibodies in BP has been garnered from individual case reports and case series describing clinical improvement in BP patients following the administration of omalizumab, a humanized monoclonal antibody that prevents IgE from engaging with its cognate receptors [29–36]. We recently demonstrated the pathogenicity of anti-NC16A IgE in BP through passive transfer of patient-derived autoantibodies to double humanized mice that express the human high affinity IgE receptor, FcεRI, and the human BP180 NC16A domain (FcεRI/NC16A) [37]. Passive transfer of anti-NC16A IgE to FcεRI/NC16A mice leads to IgE deposition along the BMZ, eosinophil infiltration, and subepidermal blister formation [37]. In this model, the pathogenicity of anti-NC16A IgE is critically dependent on human FcεRI-expressing eosinophils, but not neutrophils, thereby establishing eosinophils as key pathogenic mediators in BP that are directly involved in tissue injury [37].

Eosinophil recruitment is predominately mediated by the eotaxin family of chemokines including eotaxin-1 (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26) [38, 39]. Interestingly, mice have been shown to express functional eotaxin-1 and eotaxin-2, but not eotaxin-3, thereby limiting the utility of wild-type mice to investigate the role of eotaxin-3 in translational animal models of disease [40, 41]. The local production and release of eotaxins by diverse cell types establish concentration gradients that direct recruitment and migration of eosinophils from the peripheral vasculature into inflamed tissues through binding the main eotaxin receptor, CCR3, on the eosinophil cell surface [38, 39]. While eotaxins and CCR3 have been shown to be upregulated in the lesional skin, blister fluids, and sera of BP patients, their specific roles and contributions to BP disease physiology have not yet been directly established [42–46].

Once recruited to inflamed tissues, eosinophils can act as potent effector cells capable of mediating tissue injury through the release of toxic granule proteins, extracellular traps, and proteolytic enzymes including matrix metalloproteinase-9 (MMP-9) [47, 48]. MMP-9[4], also known as “gelatinase B”, is a 92-kD collagenase secreted as an inactive zymogen by keratinocytes and leukocytes [49, 50]. MMP-9 is proteolytically activated in tissues where it is involved in the degradation of extracellular matrix proteins during physiological remodeling and pathological conditions [49, 50]. MMP-9 has been shown to be upregulated in the lesional skin and blister fluids of BP patients where it has been found to be strongly expressed by eosinophils accumulating at the floor of subepidermal blisters [51–55]. These findings suggest eosinophil-derived MMP-9 may play a role in subepidermal blister formation in BP. However, no experimental evidence has been published to directly support this theory. While neutrophil-derived MMP-9 has been demonstrated to play a direct role in mediating tissue injury in a passive transfer model of anti-murine BP180 (mBP180) IgG-induced BP, the role and relative contribution of eosinophil-derived MMP-9 to BP disease physiology has not yet been characterized [19, 56, 57].

[4]MMP-9 = matrix metalloproteinase-9

The objective of this study was to determine the mechanisms underlying eosinophil recruitment and eosinophil-dependent tissue injury in our passive transfer model of anti-NC16A IgE-induced BP. We show that passive transfer of anti-NC16A IgE to FcεRI/NC16A mice leads to the local production and release of eotaxin-1, eotaxin-2, and MMP-9 in the lesional skin of FcεRI/NC16A mice. Importantly, neutralization of eotaxin-1, but not eotaxin-2, and blockade of the main eotaxin receptor, CCR3, drastically reduces anti-NC16A IgE-induced disease activity We further show that anti-NC16A IgE/NC16A immune complexes induce the release of MMP-9 from eosinophils and mice with genetic deletion of MMP-9 become resistant to anti-NC16A IgE-induced BP. Taken together, this study establishes the eotaxin-1/CCR3 axis and MMP-9 as key players in anti-NC16A IgE-induced BP and candidate therapeutics targets for future drug development and testing.

Material and methods

Patients, sera, and antibody purification

Serum samples were collected from three patients with active BP (BP1, BP2, BP3) and three normal human controls (NC). BP patients presented with generalized tense blisters on dermatologic exam, and dermal-epidermal separation with inflammatory cell infiltration by routine histology. Direct immunofluorescence (IF) showed deposition of IgG along the BMZ in the perilesional skin of each BP patient. Indirect IF[5] showed “roof staining” of salt-split human skin cryosections with an IgG titer of 1:640 (BP1) and 1:320 (BP2 and BP3). Anti-NC16A IgE levels were 292 (BP1), 127 (BP2), and 631 (BP3) index units as determined by ELISA as previously described [9]. Anti-NC16A IgE was purified from BP patient sera using affinity chromatography as previously described [37]. Briefly, IgG was depleted from BP patient sera using a protein G affinity column (Sigma, St. Louis, MO). IgG-deplete BP patient sera was loaded onto an anti-human IgE antibody (ATCC, Manassas, VA)-coupled Affigel-10 affinity column (Bio-Rad, Hercules, CA). Eluted IgE fractions were then loaded onto an NC16A-specific glutathione sepharose column developed in-house [20]. The concentrations of purified IgE were quantified by a human IgE-specific ELISA (Southern Biotechnology, Birmingham, AL). The purity of anti-NC16A IgE was determined by measuring the proportion of IgE in the total protein content of each antibody preparation. Total protein content was determined using the Pierce dye-binding BCA protein assay kit using bovine serum albumin as a standard (Thermofisher). The purity of anti-NC16A IgE was >93%. Purified anti-NC16A IgE and normal control IgE fractions were concentrated by ultrafiltration (Millipore, Billerica, MA) and used for *in vitro* and *in vivo* experiments.

Mice and antibody passive transfer experiments

Double humanized FcεRI/NC16A mice and MMP-9-deficient mice were generated on a C57BL/6J background as previously described and crossed to produce an MMP-9-deficient FcεRI/NC16A mouse line, termed “MMP-9 KO” herein [37, 58]. For antibody passive transfer experiments, adult FcεRI/NC16A and MMP-9 KO[6] mice (8–12 weeks old) were administered either anti-NC16A or control IgE (100 ng/g body weight in 25 μl PBS)

[5]IF = immunofluorescence
[6]MMP-9 KO mouse = MMP-9-deficient FcεRI/NC16A mouse

by intradermal (i.d.) injection to the convex pinnae. In a separate series of experiments, FcεRI/NC16A mice were administered anti-NC16A IgE (100 ng/g body weight in 25 μl PBS) by i.d.[7] injection to the convex pinnae in the presence of an eotaxin-1 neutralizing antibody (250 ng/g body weight, R&D Systems, Minneapolis, MN), eotaxin-2 neutralizing antibody (250 ng/g body weight, R&D Systems, Minneapolis, MN), isotype control antibody (250 ng/g body weight, R&D Systems, Minneapolis, MN), or the selective CCR3 antagonist SB-328437 (10 μg/g body weight, R&D Systems, Minneapolis, MN). Mice were evaluated from 0–48 hours thereafter. Clinical disease activity was measured using the following scoring system. First, the degree of erythema and crusting/scaling of each antibody-injected pinna was subjectively scored as either 0 = a normal, naïve ear, 1= mild, 2= moderate, 3= severe, or 4=very severe. The thickness of each pinna was then measured in millimeters using digital calipers (Fowler, Henderson, NV). Clinical disease scores were calculated by combining the scores for erythema and crusting/scaling with the pinnal thickness measurements. This scoring system shows a direct correlation between the dose of pathogenic antibodies administered to FcεRI/NC16A mice and subsequent clinical disease severity. Mice were then humanely euthanized and pinnae were collected and were either formalin-fixed and processed by routine histology, or snap frozen in liquid nitrogen and stored at −80°C until used in the assays described below.

Quantification of eotaxin-1, −2, and MMP-9 in antibody-injected mouse skin

Levels of eotaxin-1 and −2 were determined in tissue lysates of antibody-injected skin using commercially-available ELISA kits (R&D Systems, Minneapolis, MN) performed following the manufacturers' instructions. Gelatin zymography was used to detect the presence of pro- and active MMP-9 in tissue lysates of antibody-injected skin, as previously described [56]. Briefly, tissue lysates from antibody-injected skin were subjected to SDS-PAGE on 10% acrylamide gels containing 1% gelatin under non-reducing conditions. Following electrophoresis, gels were washed twice with 2.5% Triton X-100 for 30 minutes. Gels were rinsed with water and incubated overnight in reaction buffer (50mM Tris, pH 7.4, 150 mM NaCl, 5mM $CaCl_2$). Gels were then stained with 0.125% Coomassie Brilliant blue. Areas of gelatinolytic activity appeared as clear zones against a dark blue background.

Detection and quantification of eosinophils infiltrating antibody-injected mouse skin.

Eosinophils were detected in mouse skin cryosections by indirect IF using a rat anti-mouse major basic protein monoclonal antibody (Mayo Clinic, Scottsdale, Arizona), followed by an Alexa Fluor 488-conjugated goat anti-rat antibody (Life Technologies). BP180 was identified in mouse skin cryosections by indirect IF using anti-NC16A IgG derived from BP patient sera, followed by an AlexaFluor 488-conjugated goat anti-human IgG antibody (ThermoFisher). Collagen VII was also identified in mouse skin cryosections by indirect IF using a home-made rabbit anti-mouse collagen VII NC1 domain antibody, followed by an AlexaFluor 405-conjugated goat anti-rabbit IgG antibody (ThermoFisher). The number of eosinophils infiltrating antibody-injected skin was quantified by measuring eosinophil peroxidase (EPO) activity using purified eosinophils as a standard, as previously described [37, 59]. Briefly, serial dilutions of skin protein extracts were incubated with the substrate

[7] i.d. = intradermal

o-phenylenediamine at room temperature. Reactions were stopped with the addition of 4N H_2SO_4 and read using a microplate reader (BioTek) at 490 nm. EPO[8] content of skin protein extracts were expressed as relative EPO activity using the following calculation: (the optical density reading $_{490\ nm}$ / mg of protein of each mouse injected with pathogenic antibodies) minus (the mean optical density reading $_{490\ nm}$ / mg of protein of mice injected with isotype control antibodies). Protein concentrations were determined using the Pierce dye-binding BCA protein assay kit using bovine serum albumin as a standard (ThermoFisher).

Purification, culture, and activation of mouse eosinophils

Eosinophils were purified from the peripheral blood of FcεRI/NC16A mice using a MACS cell separation system (Miltenyi Biotec, Auburn, CA) as previously described [37, 60]. Purity of eosinophils was greater than 96% as determined by flow cytometry. Freshly isolated eosinophils were resuspended in HBSS with 0.03% gelatin, 10mM HEPES and 200mM cytochrome *c* (Sigma-Aldrich, St. Louis, MO) to a concentration of 5×10^5 cells/ml; 100 μl of the cell suspension was then dispensed into each well of a 96-well flat-bottom microplate. Cells were incubated with either anti-NC16A or control IgE (50 ng/ml), in the presence or absence of recombinant NC16A (rNC16A; 10 ng/ml), for 1 hour at 37°C in 5% CO_2. Gelatin zymography was used to detect the presence of pro-MMP-9 in cell culture supernatants as described above.

Quantification of eotaxin-1, −2, −3 and MMP-9 in human blister fluids

Blister fluids were collected from 12 BP patients with typical clinical, histologic, and immunofluorescent features of BP in the active phase of disease. Suction blister fluids were also collected from 16 NC's[9] using a BFY-IV skin separator (Satellite Medical Equipment CO., LTD, Shaoxing, China). Suction blisters were harvested from the left thigh by applying 40–60 kPa negative pressure for 30–50 minutes. After centrifugation, cell free supernatants of blister fluids were stored at −80°C until used. Levels of eotaxin-1, −2, and −3 in blister fluids were determined using commercially-available ELISA kits (R&D Systems, MN, USA) performed following the manufacturers' instructions. In a separate series of experiments, blister fluids were collected from an additional three BP patients and three patients with pemphigus vulgaris (PV) with typical clinical, histologic, and immunofluorescent features in the active phases of disease. After centrifugation, cell free supernatants of blister fluids were stored at −80°C until used. Levels of MMP-9 in blister fluids were quantified using a commercially-available MMP-9 colorimetric assay kit (BIOMOL Research Laboratories, Plymouth Meeting, PA) performed following the manufacturer's instructions [61].

Statistics

The data are expressed as mean ± SEM and were analyzed using pairwise t-tests. A *p*-value of less than 0.05 was considered statistically significant.

8. EPO = eosinophil peroxidase
9. NC = normal human control

Study approval

Animal care and experiments were performed in accordance with the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill. Written informed consent was received from patients and healthy individuals prior to inclusion in this study. Collection of human samples for this study was approved by our respective institutional ethics committees.

Results

Eotaxin-1 and −2 are increased in the lesional skin of anti-NC16A IgE-injected FcεRI/NC16A mice

We have previously shown that anti-NC16A IgE plays a critical role in recruiting eosinophils to mediate tissue injury in an antibody passive transfer model of experimental BP [37]. However, the specific mechanisms responsible for eosinophil recruitment in anti-NC16A IgE-induced BP remains unknown. We first sought to evaluate the role of two well-characterized eosinophil chemoattractants, eotaxin-1 and −2, in the lesional skin of antibody-injected FcεRI/NC16A mice. The levels of both eotaxin-1 and −2 were significantly higher in the skin of mice injected with anti-NC16A IgE as compared to mice injected with control IgE (Figure 1A). Significant increases in eotaxin-1 and −2 were first noted at 12- and 24-hours post-injection, respectively. These results suggest that both eotaxin-1 and −2 may play a role in mediating eosinophil recruitment in anti-NC16A IgE-induced BP.

The eotaxin-1/CCR3 axis plays a critical role in anti-NC16A IgE-induced experimental BP

We next sought to determine the relative contributions of eotaxin-1 and −2 to eosinophil recruitment in anti-NC16A IgE-induced BP using neutralizing antibodies specific to each chemokine. Mice that received neutralizing antibodies directed against eotaxin-1, but not eotaxin-2, had significantly decreased disease severity scores relative to mice that received an isotype control antibody (Figures 1B, 1C). Similarly, EPO assays showed that significantly lower numbers of eosinophils infiltrated the skin of mice that received neutralizing antibodies directed against eotaxin-1, but not eotaxin-2, relative to mice that received an isotype control antibody (Figure 1D). In a separate series of experiments, adult FcεRI/NC16A mice were passively transferred anti-NC16A IgE in the presence or absence of the selective CCR3 antagonist SB-328437. Mice that received the selective CCR3 antagonist had significantly decreased disease severity, both clinically (Figures 2A, 2B) and histologically (Figure 2C), and had reduced numbers of eosinophils infiltrating the skin (Figure 2D). Taken together, these results clearly show that anti-NC16A IgE-mediated eosinophil recruitment, tissue injury, and disease activity are critically dependent on the eotaxin-1/CCR3 axis.

Anti-NC16A IgE/NC16A immune complexes induce the release of MMP-9 from eosinophils

We next sought to determine the specific mechanism by which eosinophils mediate tissue injury in anti-NC16A IgE-induced BP. We have previously established a critical role for neutrophil-derived MMP-9 in mediating tissue injury in anti-mBP180[10] IgG-induced BP

[56]. As eosinophils in the lesional skin of BP patients are known to strongly express MMP-9, we used gelatin zymography to determine if pro- and activated-MMP-9 are expressed in the lesional skin of anti-NC16A IgE-injected FcεRI/NC16A mice as well. Both pro- and activated-MMP-9 were detected in the skin of FcεRI/NC16A mice that received anti-NC16A IgE, but not control IgE (Figure 3A). To confirm eosinophils as a cellular source for MMP-9 in anti-NC16A IgE-induced BP, purified eosinophils from adult FcεRI/NC16A mice were incubated with either anti-NC16A IgE or control IgE, in the presence or absence of rNC16A[11]. Gelatin zymography was then used to detect the presence of pro-MMP-9 in cell culture supernatants. Pro-MMP-9 was only detected in supernatants of eosinophils that were incubated with anti-NC16A IgE in the presence of rNC16A (Figure 3B). These results clearly show that anti-NC16A IgE/NC16A IgE immune complexes induce the release of MMP-9 from eosinophils and identify the eosinophil as a cellular source of MMP-9 in anti-NC16A IgE-induced BP.

MMP-9 plays a critical role in anti-NC16A IgE-induced BP

To determine the role of MMP-9 in our model of anti-NC16A IgE-induced BP, we crossed FcεRI/NC16A and MMP-9-deficient mice to generate an MMP-9 deficient strain of FcεRI/NC16A mice, termed “MMP-9 KO” herein. Adult FcεRI/NC16A and MMP-9 KO mice were passively transferred anti-NC16A IgE and were clinically evaluated and humanely euthanized 48h thereafter. MMP-9 KO mice had significantly lower disease severity scores relative to FcεRI/NC16A mice at 48h (Figure 4A). Similarly, routine histologic staining revealed typical dermal-epidermal separation in FcεRI/NC16A mice, but not in MMP-9 KO mice, at 48h (Figure 4B). While eosinophil infiltration was detected in the skin of both FcεRI/NC16A and MMP-9 KO mice at 48h by routine histology, the level of eosinophil infiltration was reduced in MMP-9 KO mice as determined by indirect IF (Figure 4C). Taken together, these results demonstrate that anti-NC16A IgE-induced eosinophil recruitment, tissue injury, and disease activity are critically dependent on MMP-9 as well.

Eotaxin-1, eotaxin-2, and MMP-9 are increased in the blister fluids of BP patients

To support the clinical relevance of our animal model findings, we next sought to determine if the eotaxins and MMP-9 are increased in the blister fluids of BP patients. The concentrations of eotaxin-1, −2, and −3 were quantified in the blister fluids of BP patients and suction blister fluids of NC’s by ELISA. Consistent with our animal model findings, significantly higher levels of eotaxin-1 and −2, but not eotaxin-3, were detected in BP blister fluids as compared to NC suction blister fluids (Figure 5A). Levels of MMP-9 were also quantified in the blister fluids of patients with BP and PV[12] (control) using a MMP-9 colorimetric assay [61]. Consistent with our animal model findings, significantly higher levels of MMP-9 were detected in BP blister fluids relative to PV blister fluids (Figure 5B). These results further support eotaxin-1 and MMP-9 as critical players in BP disease physiology.

[10] mBP180 = murine BP180
[11] rNC16A = recombinant NC16A protein
[12] PV = pemphigus vulgaris

Discussion

Eosinophils have long been suspected to play a key role in mediating tissue injury in BP through releasing proteolytic enzymes, granule proteins, and eosinophil extracellular traps [48]. Indeed, we recently established a direct role for eosinophils in mediating tissue injury in the passive transfer model of anti-NC16A IgE-induced BP used in this study [37]. However, the specific molecular and cellular events that mediate eosinophil recruitment and eosinophil-dependent tissue injury in BP have not yet been unraveled. Here, we clearly establish that the eotaxin-1/CCR3 axis and MMP-9 play key roles in eosinophil recruitment and eosinophil-dependent tissue injury, respectively, in our *in vivo* model of anti-NC16A IgE-induced BP. In doing so, we identify the eotaxin-1/CCR3 axis and MMP-9 as candidate therapeutic targets for future drug development and testing in BP.

Eotaxin-1 is a well-characterized eosinophil chemoattractant that has been found to be significantly upregulated in the lesional skin, blister fluids, and sera of BP patients [42–46, 62]. Eotaxin-1 levels are significantly higher in the blister fluids of BP patients relative to matched patient sera, consistent with its local production and release in inflamed skin [43, 46]. Eotaxin-1 levels in BP patient blister fluids and sera have been found to positively correlate with the number of eosinophils infiltrating the skin and clinical disease severity, respectively, suggesting eotaxin-1 plays a role in mediating eosinophil recruitment in BP [43, 45, 46, 62]. However, there has been no experimental evidence to confirm this theory until now. Here, we clearly establish a direct role for eotaxin-1 in mediating eosinophil recruitment in anti-NC16A IgE-induced BP. We show that eotaxin-1 levels are significantly increased in the lesional skin of FcεRI/NC16A mice following passive transfer of anti-NC16A IgE. Moreover, eosinophil recruitment and clinical disease activity are significantly decreased in anti-NC16A IgE-injected FcεRI/NC16A mice with the administration of eotaxin-1 neutralizing antibodies. These results are consistent with a previous study where eotaxin-1 neutralizing antibodies were shown to significantly decrease the *in vitro* migratory behavior of human eosinophils in response to BP patient blister fluids [42]. Our results establish eotaxin-1 as a promising candidate therapeutic target for future drug development and testing in BP. In fact, a fully humanized anti-eotaxin-1 monoclonal antibody (i.e., bertilimumab) has already undergone an open-label, proof-of-concept, single-arm, phase 2 clinical trial (NCT02226146) evaluating its safety and efficacy in nine BP patients. In this study, bertilimumab decreased mean disease severity scores by 81% and provided a significant steroid-sparing effect to BP patients without any serious drug-associated adverse events [63, 64].

To our knowledge, levels of eotaxin-2 and −3 have been evaluated in BP in only one previous study. In this study, there were no significant differences in serum levels of eotaxin-2 between BP patients, PV patients, and NC's [43]. While the levels of eotaxin-2 in the blister fluids of three BP patients were not found to be significantly different than in matched patient sera, BP patient blister fluids were not directly compared with blister fluids from a control group in this study [43]. In contrast, we found significantly increased levels of eotaxin-2 in the skin of FcεRI/NC16A mice injected with anti-NC16A IgE, but not control IgE, as well as in the blister fluids of 12 BP patients compared to suction blister fluids of 16 NC's. However, administration of eotaxin-2 neutralizing antibodies did

not significantly impact eosinophil recruitment or clinical disease activity in FcεRI/NC16A mice following passive transfer of anti-NC16A IgE. Interestingly, we found relatively higher levels of eotaxin-1 than eotaxin-2 in diseased FcεRI/NC16A mice, but relatively higher levels of eotaxin-2 than eotaxin-1 in BP patient blister fluids. The discrepancy in relative levels of eotaxin-1 and eotaxin-2 between experimentally-induced BP in FcεRI/NC16A mice and naturally-occurring BP in humans could be due to species-specific differences in disease physiology, or differences in the stage of disease evaluated between these two populations.

In the same previous study, eotaxin-3 was shown to be significantly increased in the lesional skin and sera of BP patients [43]. Moreover, levels of eotaxin-3 were found to be significantly higher in the sera of BP patients with severe disease, but not mild to moderate disease, suggesting a role for eotaxin-3 in specific BP clinical phenotypes [43].While levels of eotaxin-3 were also found to be significantly higher in the blister fluids of three BP patients relative to matched patient sera, BP patient blister fluids were not directly compared with blister fluids from a control group in this study either [43]. In contrast, we did not find a significant increase in eotaxin-3 levels in the blister fluids of 12 BP patients when compared to suction blister fluids of 16 NC's. While a homologous gene for eotaxin-3 has been identified in the mouse genome, functional expression of eotaxin-3 has not yet been detected in murine tissues suggesting the sequence encoding eotaxin-3 is a pseudogene [40, 41]. For these reasons, the role of eotaxin-3 in our model of anti-NC16A IgE-induced BP was not investigated. While our results demonstrate a minor role for eotaxin-2 in our model of anti-NC16A IgE-induced BP, the clinical relevance of eotaxin-2 and −3 in naturally occurring BP is less clear. It is possible that levels of eotaxin-2 and −3 in BP patient blister fluids may vary depending on undefined patient-specific factors.

Eotaxins mediate eosinophil chemotaxis through binding the main eotaxin receptor, CCR3, on infiltrating eosinophils. Unsurprisingly, CCR3 has been found to be upregulated in the lesional skin of BP patients where it is primarily expressed by infiltrating eosinophils and T-lymphocytes [46]. In our study, we have demonstrated a direct role for CCR3 in mediating eosinophil recruitment in our model of anti-NC16A IgE-induced BP. We show that administration of a selective antagonist of CCR3 significantly decreases eosinophil recruitment and clinical disease activity in FcεRI/NC16A mice following passive transfer of anti-NC16A IgE. These findings are consistent with a previous study where anti-CCR3 neutralizing antibodies were shown to significantly decrease the *in vitro* migratory behavior of human eosinophils in response to blister fluids from BP patients [42]. Taken together, these findings establish CCR3 as an additional candidate therapeutic target for future drug development and testing in BP. Indeed, a randomized, double-blinded, phase 2 clinical trial (NCT04499235) evaluating the safety and efficacy of an oral CCR3 small molecule inhibitor (i.e., lazucirnon hydrochloride, or "AKST4290") as adjunctive treatment for mild to moderate BP was recently completed. Results from this study have not yet been released.

In our study, we clearly show that targeting eosinophil recruitment through inhibition of the eotaxin-1/CCR3 axis attenuates both eosinophil infiltration and clinical disease activity in anti-NC16A IgE-induced BP. However, the specific mediators of eosinophil-dependent tissue injury in BP remain uncharacterized. While eosinophil-derived granule proteins,

proteolytic enzymes, and eosinophil extracellular traps have been identified in the lesional skin of BP patients, their relative contributions to BP disease physiology have not been experimentally determined [48]. The role of the proteolytic enzyme MMP-9 in BP has been a topic of interest for over two decades as it has been shown to be significantly increased in the lesional skin and blister fluids of BP patients [51–55]. Several lines of evidence have established a critical role for MMP-9 in mediating tissue injury in BP. First, MMP-9 has been shown to degrade the extracellular domain of BP180 *in vitro* [51, 53]. Second, inhibition of MMP-9 prevents granulocyte-mediated tissue injury in a human skin cryosection model of IgG-mediated BP [65]. Third, MMP-9-deficient mice are resistant to developing BP following passive transfer of anti-mBP180 IgG [56, 61, 66]. While we have previously established a critical role for neutrophil-derived MMP-9 in mediating tissue injury in anti-mBP180 IgG-induced BP, neutrophils are not a predominating cell type in the lesional skin of BP patients [19, 56, 57]. Rather, the lesional skin of BP patients is predominately characterized by eosinophilic inflammation. Moreover, eosinophils accumulating along the floor of subepidermal blisters in BP have been shown to strongly express MMP-9 [51]. For these reasons, we sought to determine the role of eosinophil-derived MMP-9 in our model of anti-NC16A IgE-induced BP where tissue injury and disease activity is critically dependent on infiltrating eosinophils [51]. We show that MMP-9 is significantly upregulated in the lesional skin of FcεRI/NC16A mice following passive transfer of anti-NC16A IgE. Furthermore, we show that anti-NC16A IgE/NC16A immune complexes induce the release of MMP-9 from eosinophils *in vitro*. Importantly, MMP-9 KO mice are resistant to tissue injury and clinical disease following passive transfer of anti-NC16A IgE. Interestingly, reduced numbers of eosinophils were found to infiltrate the skin of MMP-9 KO mice compared to FcεRI/NC16A mice following passive transfer of anti-NC16A IgE. This suggests that byproducts of eosinophil-mediated tissue injury are also involved in eosinophil chemotaxis in BP. Taken together, these findings establish eosinophils as a cellular source of MMP-9 in anti-NC16A IgE-induced BP where MMP-9 plays a critical role in mediating tissue injury and eosinophil recruitment. While neutralizing monoclonal antibodies and small molecule inhibitors of MMP-9 have been evaluated in human clinical trials for the treatment of various malignancies, the safety and efficacy of selective MMP-9 inhibition in the treatment of BP has remained entirely unexplored [67].

In conclusion, the results of the study presented here have clearly established critical roles for the eotaxin-1/CCR3 axis and MMP-9 in mediating eosinophil recruitment and eosinophil-dependent tissue injury in our model of anti-NC16A IgE-induced BP. In doing so, we identify the eotaxin-1/CCR3 axis and MMP-9 as candidate therapeutic targets for future drug development and testing in BP. This process is already well underway with the recent development and evaluation of eotaxin-1 neutralizing monoclonal antibodies and CCR3 small molecule inhibitors in human clinical trials. Future studies are needed to determine the clinical relevance and roles of eotaxin-2 and −3 in BP disease physiology, as well as confirm the safety and efficacy of therapeutics targeting the eotaxin-1/CCR3 axis in BP patients.

Acknowledgements

The authors would like to thank Elena Montero Mulligan for her technical support. Results from this study were previously presented in poster format at the 2022 SID annual meeting in Portland, OR and published as an abstract: Jordan TJM, Chen J, Li N, et al. 2022. Eotaxin-1 and matrix metalloproteinase-9 are critical in anti-BP180 IgE-induced experimental bullous pemphigoid. *J Invest Dermatol.* 142:S9

Funding

This study was supported in part by the National Institutes of Health grants AR070276 and AR072694 (ZL), T32OD011130 (TJMJ), and the Milstein Medical Asian American Partnership Foundation (JC).

Data availability

Data will be made available upon request.

Abbreviations used in this article:

BP	bullous pemphigoid
BMZ	basement membrane zone
EPO	eosinophil peroxidase
IF	immunofluorescence
MMP-9	matrix metalloproteinase-9
mBP180	murine BP180
MMP-9 KO mice	MMP-9-deficient FcεRI/NC16A mice
NC	normal human control
NC16A	human BP180 non-collagenous 16A domain
PV	pemphigus vulgaris
rNC16A	recombinant human NC16A

REFERENCES:

[1]. Schmidt E, Schmidt E, and Zillikens D. 2013. Pemphigoid diseases. Lancet. 381: 320–332. [PubMed: 23237497]

[2]. Amber KT, Murrell DF, Schmidt E, Joly P, and Borradori L. 2018. Autoimmune subepidermal bullous diseases of the skin and mucosae: clinical features, diagnosis, and management. Clinic Rev Allerg Immunol. 54: 26–51.

[3]. Egami S, Yamagami J, and Amagai M. 2020. Autoimmune bullous skin diseases, pemphigus and pemphigoid. J Allergy Clinical Immunol. 145: 1031–1047. [PubMed: 32272980]

[4]. Giudice GJ, Emery DJ, and Diaz LA. 1992. Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP180. J Invest Dermatol. 99: 243–250. [PubMed: 1324962]

[5]. Zillikens D, Rose PA, Balding SD, Liu Z, Olague-Marchan M, Diaz LA, and Giudice GJ. 1997. Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. J Invest Dermatol. 109: 573–579. [PubMed: 9326393]

[6]. Fairley JA, Fu CL, and Giudice GJ. 2005. Mapping the binding sites of anti-BP180 immunoglobulin E autoantibodies in bullous pemphigoid. J Invest Dermatol. 125: 467–472. [PubMed: 16117787]

[7]. Giudice GJ, Emery DJ, Zelickson BD, Anhalt GJ, Liu Z, and Diaz LA. 1993. Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. Journal Immunol. 151: 5742–5750. [PubMed: 8228259]

[8]. Matsumura K, Amagai M, Nishikawa T, and Hashimoto T. 1996. The majority of bullous pemphigoid and herpes gestationis serum samples react with the NC16a domain of the 180-kDa bullous pemphigoid antigen. Arch of Dermatol Res. 288: 507–509. [PubMed: 8874743]

[9]. Messingham KAN, Noe MH, Chapman MA, Giudice GJ, and Fairley JA. 2009. A novel ELISA reveals high frequencies of BP180-specific IgE production in bullous pemphigoid. J Immunol Methods. 346: 18–25. [PubMed: 19422829]

[10]. Hashimoto T, Ohzono A, Teye K, Numata S, Hiroyasu S, Tsuruta D, Hachiya T, Kuroda K, Hashiguchi M, Kawakami T, and Ishii N. 2017. Detection of IgE autoantibodies to BP180 and BP230 and their relationship to clinical features in bullous pemphigoid. Br J Dermatol. 177: 141–151. [PubMed: 27716903]

[11]. Ishiura N, Fujimoto M, Watanabe R, Nakashima H, Kuwano Y, Yazawa N, Echigo T, Okochi H, and Tamaki K. 2008. Serum levels of IgE anti-BP180 and anti-BP230 autoantibodies in patients with bullous pemphigoid. J Dermatol Sci. 49: 153–161. [PubMed: 17920818]

[12]. van Beek N, Lüttmann N, Huebner F, Recke A, Karl I, Schulze FS, Zillikens D, and Schmidt E. 2016. Correlation of serum levels of IgE autoantibodies against BP180 with bullous pemphigoid disease activity. JAMA Dermatol. 153: 30–38.

[13]. Iwata Y, Komura K, Kodera M, Usuda T, Yokoyama Y, Hara T, Muroi E, Ogawa F, Takenaka M, and Sato S. 2008. Correlation of IgE autoantibody to BP180 with a severe form of bullous pemphigoid. Arch Dermatol. 144: 41–48. [PubMed: 18209167]

[14]. Zillikens D, Mascaro JM, Rose PA, Liu Z, Ewing SM, Caux F, Hoffmann RG, Diaz LA, and Giudice GJ. 1997. A highly sensitive enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. J of Invest Dermatol. 109: 679–683. [PubMed: 9347799]

[15]. Schmidt E, Obe K, Bröcker E, and Zillikens D. 2000. Serum levels of autoantibodies to BP180 correlate with disease activity in patients with bullous pemphigoid. Arch Dermat. 136: 174–178.

[16]. Amo Y, Ohkawa T, Tatsuta M, Hamada Y, Fujimura T, Katsuoka K, and Hashimoto T. 2001. Clinical significance of enzyme-linked immunosorbent assay for the detection of circulating anti-BP180 autoantibodies in patients with bullous pemphigoid. J Dermatol Sci. 26: 14–18. [PubMed: 11323216]

[17]. Tsuji-Abe Y, Akiyama M, Yamanaka Y, Kikuchi T, Sato-Matsumura KC, and Shimizu H. 2005. Correlation of clinical severity and ELISA indices for the NC16A domain of BP180 measured using BP180 ELISA kit in bullous pemphigoid. J Dermatol Sci. 37: 145–149. [PubMed: 15734283]

[18]. Kobayashi M, Amagai M, Kuroda-Kinoshita K, Hashimoto T, Shirakata Y, Hashimoto K, and Nishikawa T. 2002. BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid. J Dermatol Sci. 30: 224–232. [PubMed: 12443845]

[19]. Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, and Giudice GJ. 1993. A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. J Clin Invest. 92: 2480–2488. [PubMed: 7693763]

[20]. Liu Z, Sui W, Zhao M, Li Z, Li N, Thresher R, Giudice GJ, Fairley JA, Sitaru C, Zillikens D, Ning G, Marinkovich MP, and Diaz LA. 2008. Subepidermal blistering induced by human autoantibodies to BP180 requires innate immune players in a humanized bullous pemphigoid mouse model. J Autoimun. 31: 331–338.

[21]. Cho Y, Liao S, Wang L, and Chu C. 2016. High serum anti-BP180 IgE levels correlate to prominent urticarial lesions in patients with bullous pemphigoid. J Dermatol Sci. 83: 78–80. [PubMed: 27021592]

[22]. Döpp R, Schmidt E, Chimanovitch I, Leverkus M, Bröcker E, and Zillikens D. 2000. IgG4 and IgE are the major immunoglobulins targeting the NC16A domain of BP180 in bullous pemphigoid: serum levels of these immunoglobulins reflect disease activity. J Am Acad Dermatol. 42: 577–583. [PubMed: 10727301]

[23]. Kalowska M, Ciepiela O, Kowalewski C, Demkow U, Schwartz RA, and Wozniak K. 2016. Enzyme-linked immunoassay index for anti-NC16a IgG and IgE auto-antibodies correlates with severity and activity of bullous pemphigoid. Acta Derma Venereol. 96: 191–196.

[24]. Dimson OG, Giudice GJ, Fu CL, Van den Bergh F, Warren SJ, Janson MM, and Fairley JA. 2003. Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. J Invest Dermatol. 120: 784–788. [PubMed: 12713582]

[25]. Zone JJ, Taylor T, Hull C, Schmidt L, and Meyer L. 2007. IgE basement membrane zone antibodies induce eosinophil infiltration and histological blisters in engrafted human skin on SCID mice. J Invest Dermatol. 127: 1167–1174. [PubMed: 17235329]

[26]. Freire PC, Muñoz CH, and Stingl G. 2017. IgE autoreactivity in bullous pemphigoid: eosinophils and mast cells as major targets of pathogenic immune reactants. Br J Dermatol. 177: 1644–1653. [PubMed: 28868796]

[27]. Fairley JA, Burnett CT, Fu C, Larson DL, Fleming MG, and Giudice GJ. 2007. A pathogenic role for IgE in autoimmunity: bullous pemphigoid IgE reproduces the early phase of lesion development in human skin grafted to nu/nu mice. J Invest Dermatol. 127: 2605–2611. [PubMed: 17611576]

[28]. Messingham KN, Srikantha R, DeGueme AM, and Fairley JA. 2011. FcR-independent effects of IgE and IgG autoantibodies in bullous pemphigoid. J Immunol. 187: 553–560. [PubMed: 21646296]

[29]. Fairley JA, Baum CL, Brandt DS, and Messingham KAN. 2009. Pathogenicity of IgE in autoimmunity: successful treatment of bullous pemphigoid with omalizumab. J Allergy Clin Immunol. 123: 704–705. [PubMed: 19152970]

[30]. London VA, Kim GH, Fairley JA, and Woodley DT. 2012. Successful treatment of bullous pemphigoid with omalizumab. Arch Dermatol. 148: 1241–1243. [PubMed: 23165827]

[31]. Dufour C, Souillet AL, Chaneliere C, Jouen F, Bodemer C, Jullien D, Cambazard F, Joly P, and Reix P. 2012. Successful management of severe infant bullous pemphigoid with omalizumab. B J Dermatol. 166: 1140–1142.

[32]. Gönül M, Keseroglu H, Ergin C, Özcan I, and Erdem Ö. 2016. Bullous pemphigoid successfully treated with omalizumab. Indian J Dermatol Venereol Leprol. 82: 577–579. [PubMed: 27297272]

[33]. Yu KK, Crew AB, Messingham KAN, Fairley JA, and Woodley DT. 2014. Omalizumab therapy for bullous pemphigoid. J Am Acad Dermatol. 71: 468–474. [PubMed: 24954907]

[34]. Balakirski G, Alkhateeb A, Merk HF, Leverkus M, and Megahed M. 2016. Successful treatment of bullous pemphigoid with omalizumab as corticosteroid-sparing agent: report of two cases and review of literature. J Eur Acad Dermatol Venereol. 30: 1778–1782. [PubMed: 27357866]

[35]. Yalcin AD, Genc GE, and Gumuslu S. Anti-IgE monoclonal antibody (omalizumab) is effective in treating bullous pemphigoid and its effects on soluble CD200. Clin Lab. 60:523–540. [PubMed: 24697134]

[36]. Lonowski S, Sachsman S, Patel N, Truong A, and Holland V. 2020. Increasing evidence for omalizumab in the treatment of bullous pemphigoid. JAAD Case Rep. 6: 228–233. [PubMed: 32140524]

[37]. Lin L, Hwang B, Culton DA, Li N, Burette S, Koller BH, Messingham KA, Fairley JA, Lee JJ, Hall RP, An L, Diaz LA, and Liu Z. 2018. Eosinophils mediate tissue injury in the autoimmune skin disease bullous pemphigoid. J Invest Dermatol. 138: 1032–1043. [PubMed: 29246800]

[38]. Klion AD, Ackerman SJ, and Bochner BS. 2020. Annual Review of Pathology: Mechanisms of Disease Contributions of Eosinophils to Human Health and Disease. Annu Rev of Pathol. 15: 179–209. [PubMed: 31977298]

[39]. Zajkowska M and Mroczko B. 2021. From allergy to cancer-clinical usefulness of eotaxins. Cancers. 13:128. [PubMed: 33401527]

[40]. Pope SM, Fulkerson PC, Blanchard C, Akei HS, Nikolaidis NM, Zimmermann N, Molkentin JD, and Rothenberg ME. 2005. Identification of a cooperative mechanism involving interleukin-13

and eotaxin-2 in experimental allergic lung inflammation. J Biol Chem. 280: 13952–13961. [PubMed: 15647285]

[41]. Zlotnik A and Yoshie O. 2012. The chemokine superfamily revisited. Immunity. 36: 705–716. [PubMed: 22633458]

[42]. Gounni Abdelilah S, Wellemans V, Agouli M, Guenounou M, Hamid Q, Beck LA, and Lamkhioued B. 2006. Increased expression of Th2-associated chemokines in bullous pemphigoid disease. Role of eosinophils in the production and release of these chemokines. Clinical Immunol. 120: 220–231. [PubMed: 16782408]

[43]. Günther C, Wozel G, Meurer M, and Pfeiffer C. 2011. Up-regulation of CCL11 and CCL26 is associated with activated eosinophils in bullous pemphigoid. Clin Exp Immunol. 166: 145–153. [PubMed: 21985360]

[44]. Shrikhande M, Hunziker T, Braathen LR, Pichler WJ, Dahinden CA, and Yawalkar N. 2000. Increased coexpression of eotaxin and interleukin 5 in bullous pemphigoid. Acta Derm Venereol. 80: 277–280. [PubMed: 11028861]

[45]. Wakugawa M, Nakamura K, Hino H, Toyama K, Hattori N, Okochi H, Yamada H, Hirai K, Tamaki K, and Furue M. 2000. Elevated levels of eotaxin and interleukin-5 in blister fluid of bullous pemphigoid: correlation with tissue eosinophilia. Br J Dermatol. 143: 112–116. [PubMed: 10886144]

[46]. Frezzolini A, Teofoli P, Cianchini G, Barduagni S, Ruffelli M, Ferranti G, Puddu P, and De Pita O. 2002. Increased expression of eotaxin and its specific receptor CCR3 in bullous pemphigoid. Eur J Dermatology. 12: 27–31.

[47]. Simon D, Borradori L, and Simon H. 2017. Eosinophils as putative therapeutic targets in bullous pemphigoid. Exp Dermatol. 26: 1187–1192. [PubMed: 28833620]

[48]. Amber KT, Valdebran M, Kridin K, and Grando SA. 2018. The role of eosinophils in bullous pemphigoid: a developing model of eosinophil pathogenicity in mucocutaneous disease. Front Med. 5:201.

[49]. Cirillo N and Prime SS. 2021. A scoping review of the role of metalloproteinases in the pathogenesis of autoimmune pemphigus and pemphigoid. Biomolecules. 11: 1506. [PubMed: 34680139]

[50]. Amar S, Smith L, and Fields GB. 2017. Matrix metalloproteinase collagenolysis in health and disease. Biochim Biophysica Acta Mol Cell Res. 1864: 1940–1951.

[51]. Ståhle-Bäckdahl M, Inoue M, Giudice GJ, and Parks WC. 1994. 92-kD gelatinase is produced by eosinophils at the site of blister formation in bullous pemphigoid and cleaves the extracellular domain of recombinant 180-kD bullous pemphigoid autoantigen. J Clin Invest. 93: 2022–2030. [PubMed: 8182134]

[52]. Niimi Y, Pawankar R, and Kawana S, 2006. Increased expression of matrix metalloproteinase-2, matrix metalloproteinase-9 and matrix metalloproteinase-13 in lesional skin of bullous pemphigoid. Int Arch Allergy Immunol. 139: 104–113. [PubMed: 16374020]

[53]. Verraes S, Hornebeck W, Bernard P, Polette M, and Borradori L. 2001. Respective contribution of neutrophil elastase and matrix metalloproteinase 9 in the degradation of BP180 (type XVII collagen) in human bullous pemphigoid. J Invest Dermatol. 117: 1091–1096. [PubMed: 11710917]

[54]. Oikarinen A, Kylmäniemi M, Autio-Harmainen H, Autio P, and Salo T. 1993. Demonstration of 72-kDa and 92-kDa forms of type IV collagenase in human skin: variable expression in various blistering diseases, induction during re-epithelialization, and decrease by topical glucocorticoids. J Invest Dermatol. 101: 205–210. [PubMed: 8345222]

[55]. Paquet P, Nusgens BV, Piérard GE, and Lapière CM. 1998. Gelatinases in drug-induced toxic epidermal necrolysis. Eur J Clin Invest. 28: 528–532. [PubMed: 9726031]

[56]. Liu Z, Shipley JM, Vu TH, Zhou X, Diaz LA, Werb Z, and Senior RM. 1998. Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. J Exp Med. 188: 475–482. [PubMed: 9687525]

[57]. Liu Z, Giudice GJ, Zhou X, Swartz SJ, Troy JL, Fairley JA, Till GO, and Diaz LA. 1997. A major role for neutrophils in experimental bullous pemphigoid. J Clin Invest. 100: 1256–1263. [PubMed: 9276744]

[58]. Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, and Werb Z. 1998. MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell. 93: 411–422. [PubMed: 9590175]

[59]. Schneider T, and Issekutz AC. 1996. Quantitation of eosinophil and neutrophil infiltration into rat lung by specific assays for eosinophil peroxidase and myeloperoxidase Application in a Brown Norway rat model of allergic pulmonary inflammation. J Immunol Methods. 198: 1–14. [PubMed: 8914592]

[60]. Li Z, Garantziotis S, Jia W, Potts EN, Lalani S, Liu Z, He Y, Foster WM, and Hollingsworth JW. 2008. The extracellular matrix protein mindin regulates trafficking of murine eosinophils into the airspace. J Leukoc Biol. 85: 124–131. [PubMed: 18818374]

[61]. Liu Z, Li N, Diaz LA, Shipley M, Senior RM, and Werb Z. 2005. Synergy between a plasminogen cascade and MMP-9 in autoimmune disease. J Clin Invest. 115: 879–887. [PubMed: 15841177]

[62]. Nakashima H, Fujimoto M, Asashima N, Watanabe R, Kuwano Y, Yazawa N, Maruyama N, Okochi H, Kumanogoh A, and Tamaki K. 2007. Serum chemokine profile in patients with bullous pemphigoid. Br J Dermatol. 156: 454–459. [PubMed: 17300233]

[63]. Lee J, Werth VP, Hall RP, Eming R, Fairley JA, Fajgenbaum DC, Harman KE, Jonkman MF, Korman NJ, Ludwig RJ, Murrell DF, Musette P, Naik HB, Sadik CD, Yamagami J, Yale ML, and Payne AS. 2018. Perspective from the 5th international pemphigus and pemphigoid foundation scientific conference. Front Med. 5: 306.

[64]. Izumi K, Bieber K, and Ludwig RJ, 2019. Current clinical trials in pemphigus and pemphigoid. Front. Immunol 10:978. [PubMed: 31130959]

[65]. Shimanovich I, Mihai S, Oostingh GJ, Ilenchuk TT, Bröcker E, Opdenakker G, Zillikens D, and Sitaru C. 2004. Granulocyte-derived elastase and gelatinase B are required for dermal-epidermal separation induced by autoantibodies from patients with epidermolysis bullosa acquisita and bullous pemphigoid. J Pathol. 204: 519–527. [PubMed: 15538734]

[66]. Liu Z, Zhou X, Shapiro SD, Shipley JM, Twining SS, Diaz LA, and Senior RM. 2000. The serpin α1-proteinase inhibitor is a critical substrate for gelatinase B/MMP-9 in vivo. Cell. 102: 647–655. [PubMed: 11007483]

[67]. Augoff K, Hryniewicz-Jankowska A, Tabola R, and Stach K. 2022. MMP9: a tough target for targeted therapy for cancer. Cancers. 14:1847. [PubMed: 35406619]

Key Points

- Anti-NC16A IgE recruit eosinophils in experimental BP through the eotaxin-1/CCR3 axis
- Eosinophil-derived MMP-9 mediates tissue injury in experimental BP
- The eotaxin-1/CCR3 axis and MMP-9 are candidate therapeutic targets in BP

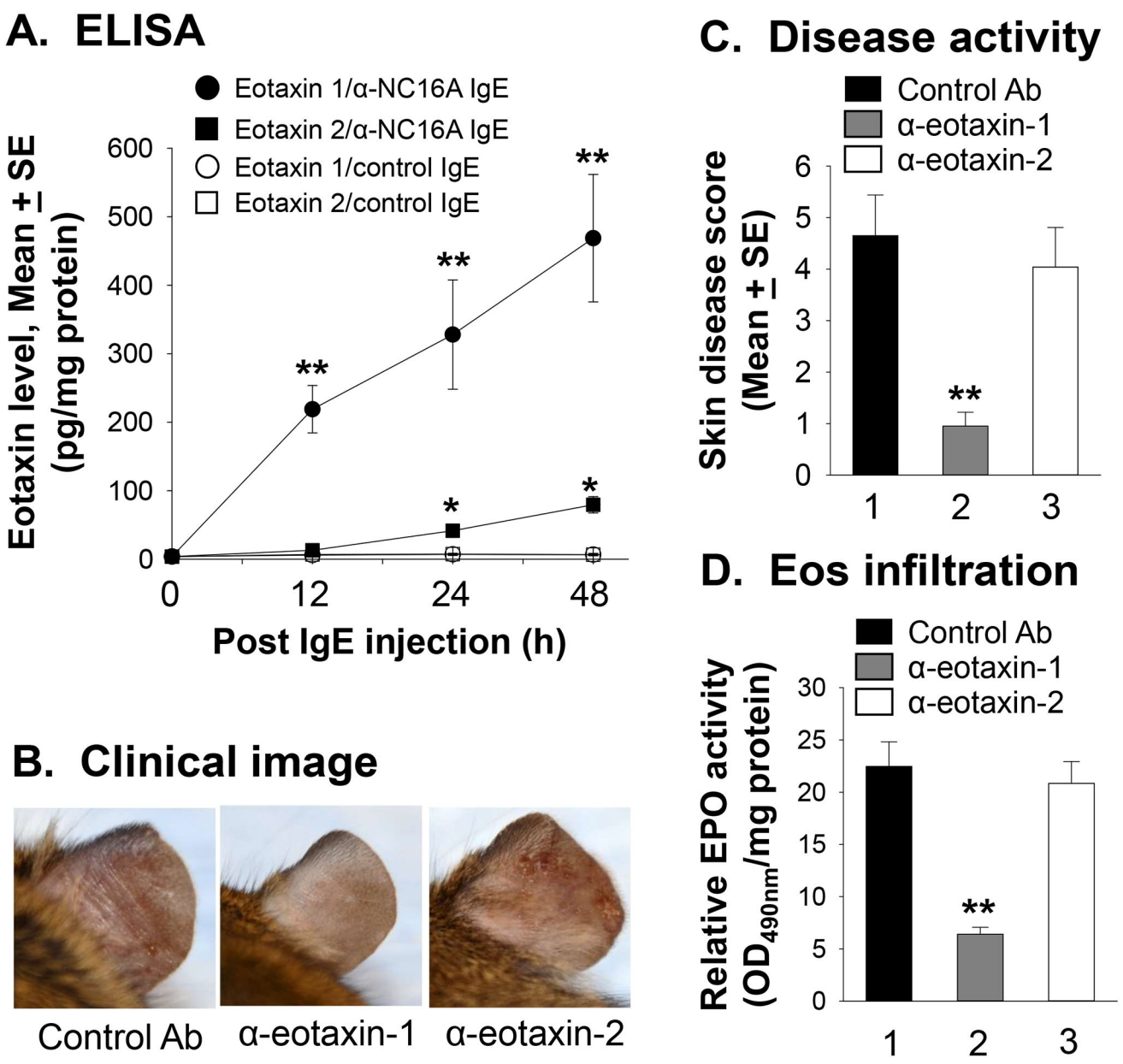

Figure 1: Eotaxin-1 is critical in anti-NC16A IgE-induced BP in FcεRI/NC16A mice.

Adult FcεRI/NC16A mice were injected in the convex pinnae with either anti-NC16A IgE or normal human control IgE in the presence or absence of eotaxin-1 neutralizing antibodies, eotaxin-2 neutralizing antibodies, or isotype control antibodies. Mice were then evaluated for 0–48h post-injection. (A) The lesional skin of anti-NC16A IgE-injected mice exhibited significantly higher levels of eotaxin-1 and eotaxin-2 compared to control IgE-injected mice. At each timepoint, eotaxin-1/α-NC16A IgE was compared to eotaxin-1/control IgE, and eotaxin-2/α-NC16A IgE was compared to eotaxin-2/control IgE. (B, C) Neutralizing antibodies directed against eotaxin-1, but not eotaxin-2, significantly attenuated the severity of skin disease induced by anti-NC16A IgE in FcεRI/NC16A mice; bar 1 vs. bar 2. (D) Significantly reduced numbers of eosinophils infiltrated the skin of mice treated with

neutralizing antibodies directed against eotaxin-1, but not eotaxin-2; bar 1 vs. bar 2. n=6, *p<0.05, **p<0.01, paired t-test.

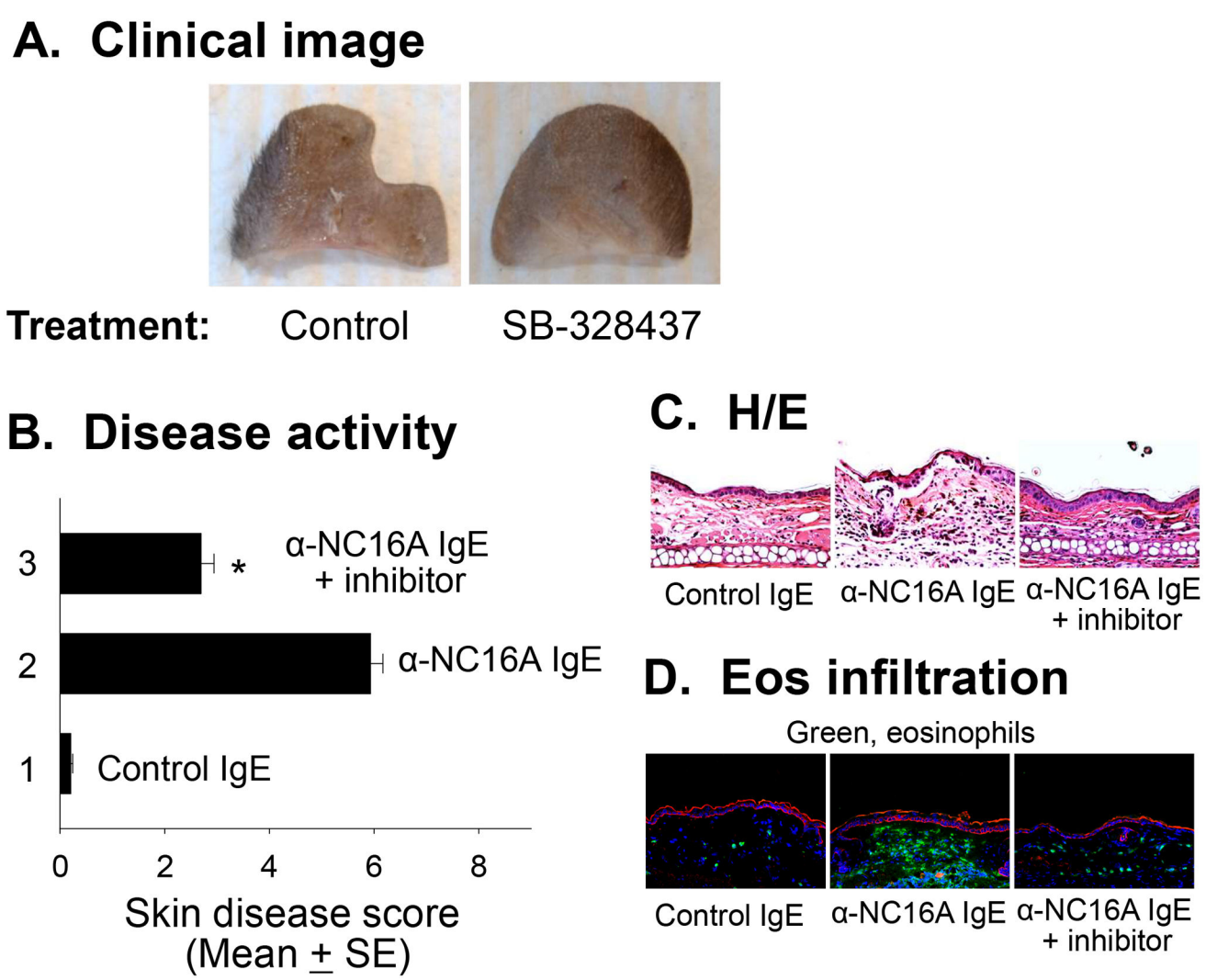

Figure 2: The eotaxin-1/CCR3 axis is critical in anti-NC16A IgE induced BP in FcεRI/NC16A mice.

Adult FcεRI/NC16A mice were injected in the convex pinnae with anti-NC16A IgE in the presence or absence of the selective CCR3 antagonist SB-328437 and were examined 48h post-injection. Blockade of CCR3 significantly reduced disease severity clinically (A, B) and histologically (C), and reduced the number of eosinophils that infiltrated the skin (D). α-NC16A IgE + inhibitor was compared to α-NC16A IgE. n=4 for bar 1, n=8 for bar 2, n=6 for bar 3, *p<0.01, paired t-test. Green, anti-MBP for eosinophils; red, anti-BP180; blue, DAPI.

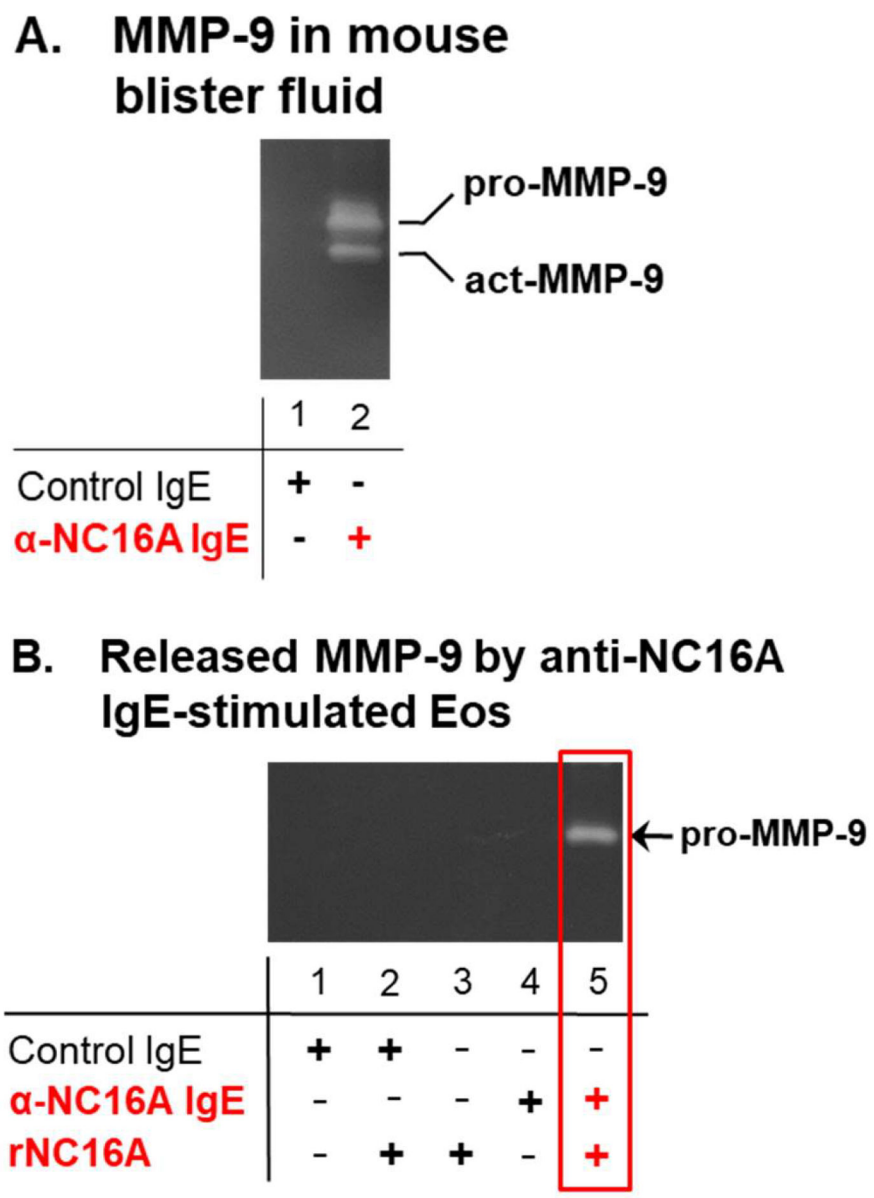

Figure 3: Anti-NC16A IgE induce the release of MMP-9 *in vivo* and *in vitro*.

(A) Adult FcεRI/NC16A mice were injected in the pinnae with either anti-NC16A IgE or normal human control IgE and examined 48h post-injection. Gelatin zymography examination revealed both pro- and active MMP-9 in the lesional skin of mice injected with anti-NC16A IgE, but not control IgE. (B) Purified eosinophils from FcεRI/NC16A mice were incubated with either anti-NC16A or control IgE, in the presence or absence of recombinant NC16A (rNC16A), for 1 hour. Pro-MMP-9 was only detected in the cell culture supernatants of eosinophils that were incubated with anti-NC16A IgE in the presence of rNC16A by gelatin zymography (lane 5).

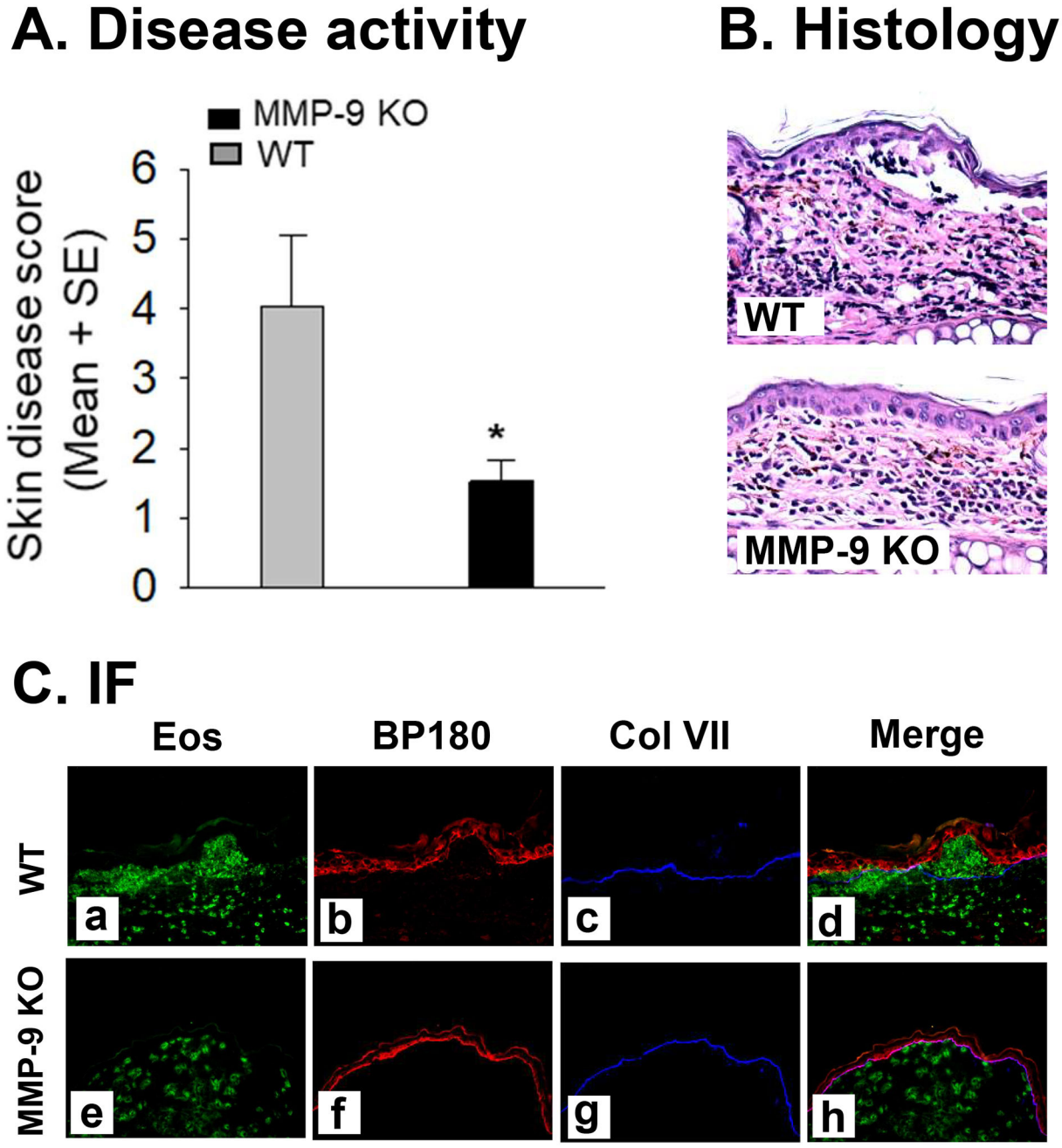

Figure 4: MMP-9 is critical in anti-NC16A IgE-induced BP in FcεRI/NC16A mice.

Adult FcεRI/NC16A (WT) and MMP-9-deficient FcεRI/NC16A (MMP-9 KO) mice were injected in the convex pinnae with anti-NC16A IgE and examined 48h post-injection. (A) MMP-9 KO mice developed significantly less severe skin disease as compared to WT mice. n=8, bar 1 vs. bar 2, *p<0.05, paired t-test. (B) Routine histology revealed typical dermal-epidermal separation in the skin of WT mice, but not MMP-9 KO mice. (C) Indirect IF identified reduced numbers of eosinophils infiltrating the skin of MMP-9 KO mice compared to WT mice. Green, anti-MBP (eosinophils). Red, anti-BP180; Blue, anti-Col VII.

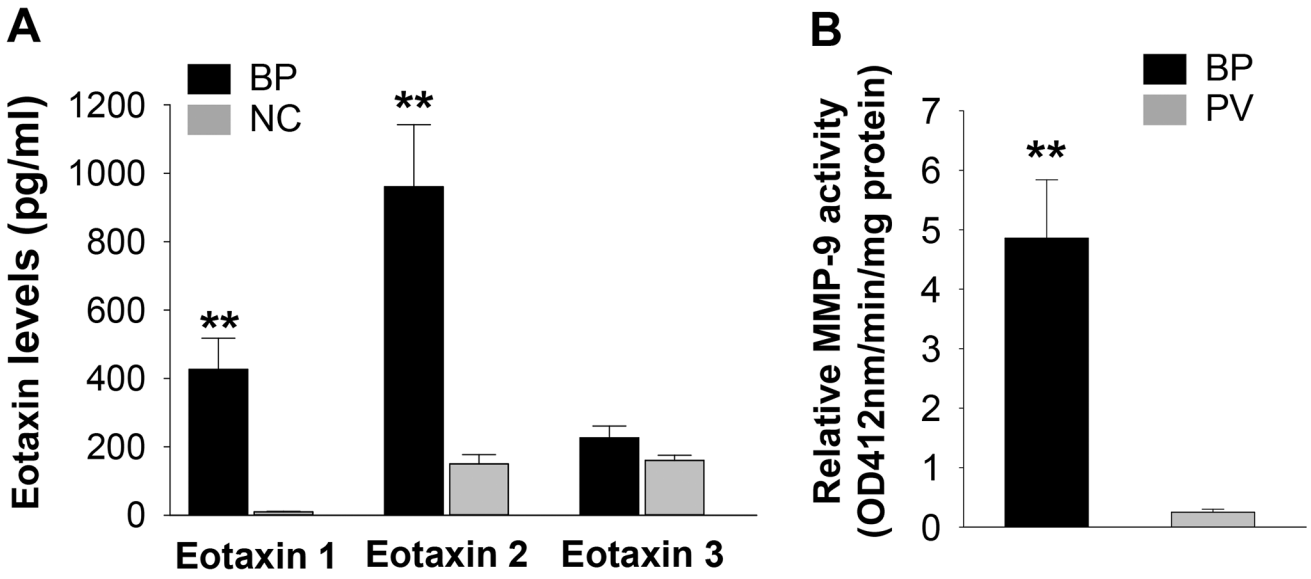

Figure 5: Levels of eotaxin-1, eotaxin-2, and MMP-9 are increased in the blister fluids of BP patients.

(A). Levels of eotaxin-1, −2, and −3 were quantified in blister fluids from BP patients and suction blister fluids from normal human controls (NC) by ELISA. Significantly higher levels of eotaxin-1 and −2 were present in BP blister fluids as compared to NC suction blister fluids. BP was compared to NC for each of the evaluated chemokines, n=12 for BP and n=16 for NC. **p<0.01, paired t-test. (B). Levels of MMP-9 were quantified in the blister fluids of patients with BP and pemphigus vulgaris (PV) using an enzymatic assay. Significantly higher levels of MMP-9 activity were detected in the blister fluids of BP patients as compared to the blister fluids of PV patients. n=3/group, **p<0.01, paired t-test.