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Pathomechanisms in IgA pemphigus are assumed to rely on Fc-dependent cellular activation by antigen-specific IgA autoantibodies; however, models for the disease and more detailed pathophysiologic data are lacking. In this study, we aimed to establish in vitro models of disease for IgA pemphigus, allowing us to study the effects of the interaction of anti-keratinocyte IgA with cell surface FcαRs. Employing multiple in vitro assays, such as a skin cryosection assay and a human skin organ culture model, in this study, we present mechanistic data for the pathogenesis of IgA pemphigus, mediated by anti–desmoglein 3 IgA autoantibodies. Our results reveal that this disease is dependent on FcαR-mediated activation of leukocytes in the epidermis. Importantly, this cell-dependent pathology can be dose-dependently abrogated by peptide-mediated inhibition of FcαR:IgA-Fc interaction, as confirmed in an additional model for IgA-dependent disease, that is, IgA vasculitis. These data suggest that IgA pemphigus can be modeled in vitro and that IgA pemphigus and IgA vasculitis are FcαR-dependent disease entities that can be specifically targeted in these experimental systems.

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INTRODUCTION

IgA pemphigus or intercellular IgA dermatosis, constitutes a group of rare intraepidermal autoimmune blistering skin diseases characterized by the presence of anti-keratinocyte (KC) cell surface IgA autoantibodies as determined by direct immunofluorescence (IF) microscopy of perilesional skin biopsies and indirect IF microscopy on epithelial substrates incubated with patient sera. Characteristic morphological manifestations include vesiculopustular rash overlying configurative plaques along the trunk and extremities (Kridin et al., 2020). The typical histopathological picture consists of intraepidermal clefts, slight epidermal acantholysis, and widespread neutrophilic infiltration, sometimes intermingled with eosinophils (Hodak et al., 1990; Nishikawa et al., 1991).

On the basis of the sites of intraepidermal clefts and pustules, IgA pemphigus is further subdivided into (i) a subcorneal pustular dermatosis type, whose target antigen is thought to be desmocollin (DSC) 1, and (ii) an intraepidermal neutrophilic IgA dermatosis type, whose target antigen remains to be fully elucidated (Ishii et al., 2004). Taken together with other types of IgA pemphigus targeting desmoglein (DSG) 1 (IgA pemphigus foliaceus) or DSG3 (IgA pemphigus vulgaris [PV]), it becomes clear that IgA autoantibodies from all these patients bind to desmosomal antigens, including DSGs and DSCs (i.e., two groups of epidermal adhesion proteins that are known to interact in a heterophilic manner in desmosomes), with histopathologies not always following the differentiation pattern mentioned earlier (Harrison et al., 2016; Hashimoto et al., 2017; Ishii et al., 2020; Męcińska-Jundziłł et al., 2016). However, IgA pemphigus should be strictly differentiated from cases of IgG-type pemphigus, which may also show additional IgA labeling by direct IF and/or by in indirect IF microscopy; in these cases, the IgA component is thought to be a manifestation of advanced class-switch recombination (Ellebrecht et al., 2018)

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Abbreviations: Ab, antibody; DSC, desmocollin; DSG, desmoglein; ETA, exfoliative toxin A; HSOC, human skin organ culture; IC, immune complex; IF, immunofluorescence; IgAV, IgA vasculitis; KC, keratinocyte; NH, normal human; PMN, polymorphonuclear leukocyte; PV, pemphigus vulgaris; scFv, single-chain variable fragment

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(resulting in IgG and IgA antibodies [Abs]), whereas the pathophysiologic mechanisms may differ greatly in IgA pemphigus, an exclusively IgA-mediated disease.

The molecular pathomechanisms by which anti-KC cell surface IgA autoantibodies lead to blister formation and characteristic skin lesions in IgA pemphigus are yet to be fully established. Although binding of specific IgA autoantibodies to the monocyte/granulocyte IgA Fc receptor (FcαR or CD89) was speculated to be critical to the pathophysiology of the disease (Wang et al., 1997), functional studies in IgA pemphigus are lacking. Delineating the mechanisms underlying this disease more precisely may be of great help to facilitate its early diagnosis and optimized treatment.

RESULTS

Production of a defined recombinant anti-DSG3 full IgG1 mAb in the human IgA1 format

The F779-IgG1 mAb is a well-defined, human anti-DSG3 mAb in the human IgG1 format previously cloned from peripheral blood cells of a patient with active, untreated PV (Cho et al., 2014). The epitope of this mAb was mapped to the EC1 domain of DSG3. Using published nucleotide sequences for the variable light and heavy chains of F779-IgG1, we engineered a fully human IgA1 version of this clone and recombinantly produced it in human embryonic kidney 293 cells. Binding of the F779-IgG1 and F779-IgA1 mAbs to the KC cell surface was confirmed by indirect IF staining on normal human (NH) skin and monkey esophagus substrates (Figure 1a) and by DSG3/1 ELISA showing binding of both F779 mAbs to DSG3 (Figure 1b). Because the F779-IgA1 mAb was derived from a patient with PV with IgG reactivity, we additionally checked for bona fide IgA autoantibodies. For this purpose, we utilized Ab phage display to specifically clone the IgA Ab repertoire from a representative, patient with clinically active IgA pemphigus. The female index case aged 58 years presented with typical clinical signs of IgA PV, that is, vesiculopustular eruptions and erosions on the scalp and oral mucous membrane lesions at the hard palate and the gums, and typical histopathology features with intraepidermal acantholysis and an inflammatory dermal infiltrate predominantly composed of neutrophils (Supplementary Figure S1a). Direct IF microscopy of perilesional skin revealed KC cell surface IgA deposition but no concurrent deposition of IgG or complement component C3 (Supplementary Figure S1b). Indirect IF microscopy on NH skin and monkey esophagus demonstrated cell surface binding of serum IgA but not IgG Abs (Supplementary Figure S1b). Indirect IF microscopy on DSG3-transfected human embryonic kidney cells showed positive reactivity for serum IgA but not for

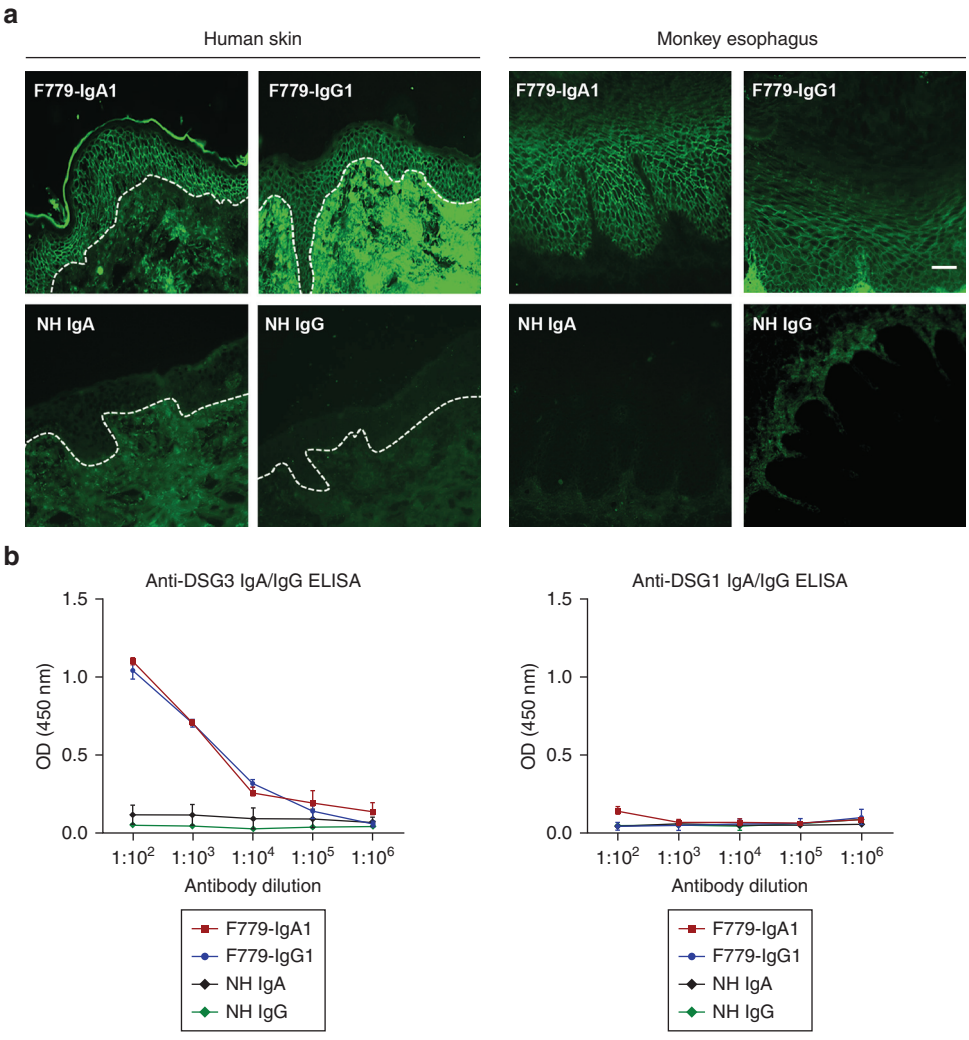

Figure 1. Immunoreactivity of the anti-DSG3 F779 mAbs. (**a**) The IgA1 and IgG1 variants of F779 mAb showed cell surface binding on human skin and on monkey esophagus by indirect IF microscopy. Magnification ×200; Bar = 100 μm. The dashed line indicates a dermal–epidermal junction. (**b**) ELISA showed specific binding of the F779 mAbs to DSG3 but not to DSG1. The concentration of each mAb was adjusted to 1 μg/μl. NH IgA and NH IgG were used as negative controls in both assays. n = 3. DSG, desmoglein; IF, immunofluorescence, NH, normal human; OD, optical density.

IgG (Supplementary Figure S1b). No serum IgA/IgG reactivity was detected by DSG1 ELISA or by IF on DSC1–3–transfected cells in this patient (not shown). From this patient, we isolated and genetically characterized three DSG3-specific IgA mAbs, in the form of single-chain variable fragments (scFvs). As expected, these scFv mAbs stained the cell surfaces of KCs by indirect IF and bound to DSG3 by ELISA (Supplementary Figure S1c). A representative scFv (i.e., mAbs 3–6) from this patient with IgA pemphigus was subjected to further testing to determine whether its binding ability was inhibited by patients' polyclonal anti-DSG3 Abs indicating shared epitopes. Indeed, sera from the same patient, from an unrelated patient with active IgA pemphigus, and from a patient with active IgG PV led to decreased binding of the scFv mAbs 3–6 in competition ELISAs (Supplementary Figure S2). Results from these competition ELISA studies indicated that both IgG-dominant classical PV sera and IgA pemphigus sera bind to the same epitopes on DSG3. A similar result was also obtained by competition ELISA of F779-IgA1 mAb with the scFv mAbs 3–6 (Supplementary Figure S3). Therefore, we concluded that the F779-IgA1 mAb is a valid reagent to study molecular mechanisms present in patients with IgA pemphigus in vitro.

The anti-DSG3 IgA1 mAb F779 induces IgA pemphigus pathology when coincubated with polymorphonuclear leukocytes

The pathogenicity of F779-mAb variants was investigated in vitro in the human skin organ culture (HSOC) model. As expected and described for classical, IgG-dependent PV, injection of anti-DSG3 mAbs F779-IgG1 and F779-IgA1 resulted in acantholysis only when coinjected with non–blister-inducing concentrations of exfoliative toxin A (ETA) (Figure 2a). Direct IF staining of the injected skin sections confirmed the binding of injected F779 mAbs but not that of injected polyclonal NH IgA or NH IgG controls to the cell surfaces of KCs (Figure 2b). Our data demonstrated an Fc-independent split formation of F779 mAbs in the presence of subclinical amounts of ETA. This result again suggests that in IgA PV cases where concurrent anti-DSG1 reactivity is absent (Hashimoto et al., 2017; Męcińska-Jundziłł et al., 2016; Mentink et al., 2007; Tajima et al., 2010), additional cell-/Fc-dependent mechanisms are likely to be involved in skin pathology. We confirmed this line of evidence by studying F779-IgG1 and F779-IgA1 mAbs in additional skin cryosection and skin injection assays. Coincubation of human skin cryosections with F779-IgA1 mAb and polymorphonuclear leukocytes (PMNs) led to intraepidermal acantholysis and typical pathology, whereas F779-IgG1 mAb was ineffective in the same assay under the same conditions (Figure 3a). In line with this observation, intraepidermal acantholysis occurred (without requiring concurrent DSG1 inactivation by ETA or an anti-DSG1 mAb) only when F779-IgA1 mAb was coinjected with PMNs in the HSOC-based skin injection assay (Figure 3b). Again, when F779-IgG1 mAb in combination with PMNs was injected into HSOCs, no pathology resulted. The binding of F779-IgA1 and F779-IgG1 mAbs to their surface Fc receptors on PMNs, identified as a prerequisite for blister formation in the absence of ETA, was confirmed by flow

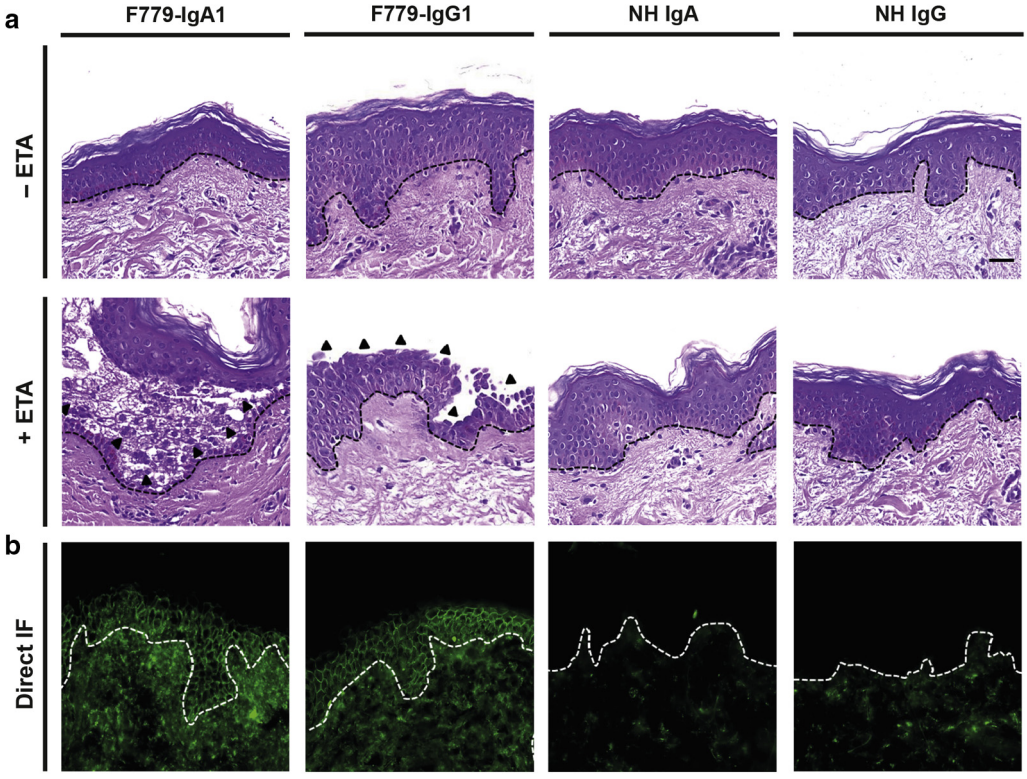

Figure 2. Anti-DSG3 F779 mAbs induce a pemphigus vulgaris–like phenotype in human skin organ cultures. (**a**) F779 mAbs were injected into the dermis of NH skin in organ culture in the presence or absence of ETA (80 ng, subclinical amount) to block DSG1 compensation. The H&E-stained sections showed typical acantholytic changes in the skin explants on mAb injection in the presence of non–blister-induced concentration of ETA. NH IgA and NH IgG were used as negative controls. (**b**) Direct IF staining of the skin sections confirmed antibody binding to the cell surface of epidermal cells. Magnification ×200; Bar = 100 μm. The dashed line indicates a dermal–epidermal junction. DSG, desmoglein; ETA, exfoliative toxin A; IF, immunofluorescence; NH, normal human.

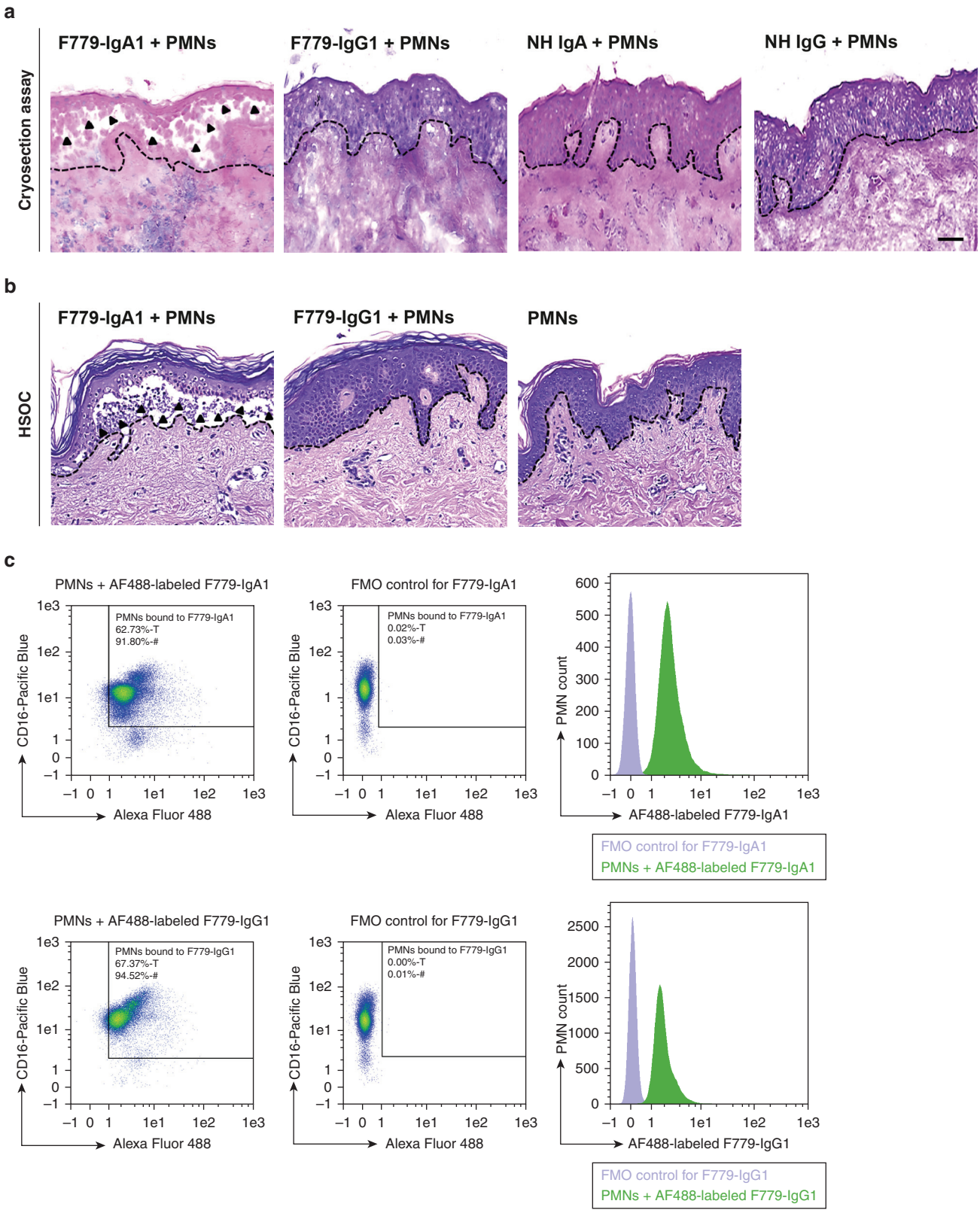

Figure 3. Anti-DSG3 F779 IgA1 in the presence of PMNs induces blister in the cryosections of human skin and in the HSOC model. (**a**) Ex vivo acantholysis is induced in the cryosection model by the addition of PMNs to the sections of human skin preincubated with F779-IgA1 mAb but not with F779-IgG1 mAb. NH IgA and NH IgG served as negative controls. (**b**) F779-IgA1 mAb was pathogenic in the HSOC when coinjected with PMNs. However, F779-IgG1 mAb was ineffective under the same conditions. PMNs alone served as a negative control. Each experiment in panels **a** and **b** was repeated three times. Magnification ×200; Bar = 100 μm. (**c**) Representative demonstration of the flow cytometric plots of human PMNs incubated with AF488-labeled F779 IgA1 or F779 IgG1. Cells were stained with an antibody cocktail for detection of $CD193^{-}CD14^{-}CD16^{+}$ PMNs. When cells were exposed to AF488-labeled F779 mAbs, >90% of PMNs were bound to labeled F779 IgA1 or F779 IgG1 (left column). The middle column indicates FMO controls, that is, PMNs incubated with

cytometric analysis (Figure 3c and Supplementary Figure S4). In summary, we found in two distinct assays that the F779-IgA1 mAb but not the IgG1 variant was capable of causing acantholysis, suggesting that IgA mAbs are able to activate PMNs and cause pathology. Similar results in both assays were obtained for polyclonal IgA anti-DSG3 serum Abs affinity purified from our index patient (not shown).

Confirmation of Fc-dependence of F779-IgA1–mediated pathology by an IgA-Fc:FcαR–blocking peptide

Using the same skin cryosection assay and a blocking peptide, designated as peptide M, we were able to demonstrate a dose-dependent inhibition of intraepidermal splitting mediated by coincubation of F779 IgA1 and PMNs in vitro. Peptide M is a 50-residue, synthetic IgA-binding peptide derived from the streptococcal Sir22 protein that recognizes the interdomain region of IgA Fc at the sites overlapping with that used by the human IgA receptor FcαRI (CD89) (Johnsson et al., 1999; Pleass et al., 2001; Sandin et al., 2002). In addition to a 29-residue IgA-binding region, peptide M includes a C-terminal cysteine residue that is not present in the bacterial Sir22 protein, known to allow for dimerization and strongly enhanced ligand binding (Supplementary Figure S5). As shown in Figure 4, peptide M fully blocked blister formation induced by F779 IgA1/PMN at concentrations ranging from 1 to 50 μg, with no significant inhibitory effect observed at concentrations at or below 0.5 μg. Dose-dependent inhibition was found to be highly significant at a broad range of doses (1–50 μg; adjusted $P = 0.0001$, Jonckheere–Terpstra test) (Figure 4). Blocking the interaction between IgA and FcαRI reduced tissue damage in this ex vivo model of the disease, serving as an additional confirmation for the dependence on IgA-Fc:FcαR interaction for pathology.

Peptide M is effective by inhibiting neutrophil extracellular trap release and may also be used in other IgA-mediated immune complex diseases

To further corroborate the inhibitory effects of peptide M, we used a model of IgA vasculitis (IgAV) where IgA immune complexes (ICs) can activate neutrophils by FcαRI (CD89), resulting in extracellular DNA release (i.e., NETosis) and tissue damage. The inhibitory effect of peptide M on IgA–IC–stimulated neutrophil extracellular trap release was evaluated by a NETosis assay, and it again proved effective because it inhibited IgA–IC–induced neutrophil extracellular trap release in a dose-dependent manner (time points 180 minutes and 240 minutes) (Figure 5). This in vitro finding indicates that peptide M may be used translationally in other IgA–IC–mediated autoimmune diseases as well, as in IgAV or IgA nephropathy, for example.

DISCUSSION

In this study, we present mechanistic data for the pathogenic effect of anti-DSG3 IgA in IgA PV, indicating that blister formation is dependent on FcαR-dependent activation of leukocytes in the epidermis and that the pathology can be abrogated by inhibition of this interaction.

Blister induction in typical IgG-mediated pemphigus diseases (with PV and pemphigus foliaceus) is primarily induced by direct effects after binding of specific IgG autoantibodies to desmosomal cadherins, independent of complement or leukocyte activation (Hammers and Stanley, 2020, 2016; Ishii et al., 2008; Payne et al., 2005; Schmidt et al., 2019; Yamagami et al., 2010). By contrast, in most pemphigoid diseases, mere binding of autoantibodies is not sufficient to induce blistering because IgG Fc-dependent mechanisms leading to complement and PMN activation were shown to be required for lesion formation (Schmidt and Zillikens, 2013). Strikingly, the pathogenic potential of IgA autoantibodies in IgA pemphigus has not yet been dissected in detail (Ali et al., 2016; Mentink et al., 2007; Tajima et al., 2010). It has been speculated that intraepidermal blister formation by autoantibodies from patients with IgA pemphigus requires activation of leukocytes, but so far, no experimental data have been provided to support this hypothesis (Porro et al., 2014; Tajima et al., 2010; Zone et al., 2004). To obtain insights into autoantibody-induced tissue damage in IgA pemphigus and to establish models of the disease, we cloned and validated a recombinant full anti-DSG3 IgA1 mAb, F779-IgA1, and used it side by side with the F779-IgG1 version in multiple assays.

At first, the biological validity of our F779-IgA1 mAb was confirmed by competition ELISA studies, suggesting similar epitopes targeted in both IgG and IgA pemphigus allowing for examination of the underlying disease mechanisms in vitro. In injection studies applying the HSOC model and the F779-IgA1 mAb, the presence of coinjected PMNs overcame the requirement of subclinical doses of ETA inactivating compensating DSG1. A similar outcome was observed for the F779-IgA1 mAb in the skin-based cryosection assay and for affinity-purified polyclonal anti-DSG3 IgA in both the HSOC model and the cryosection assay. Therefore, our data suggest that interaction of the anti-DSG3 IgA1-Fc with its cell surface receptor is required for pathogenicity in IgA pemphigus without necessitating the inactivation of compensatory DSG1 by ETA or anti-DSG1 autoantibodies.

Our results also resemble what is seen in vivo in patients with PV who have no skin lesions when only anti-DSG3 IgG Abs are detected in the circulation, resulting in the binding of these Abs to mucous membranes and skin: although likely to be exposed to FcγR-bearing cells, those anti-DSG3 IgG Abs do not likely lead to activation of PMN in tissues. From this point of view, we can currently only speculate on the mechanistic differences that are mediated by modifications of the Fc parts of our F779-IgA1 and F779-IgG1 Abs used in this study. For example, all IgG molecules contain a single-conserved *N*-linked glycosylation site (Asn297) in each of the heavy chain constant region 2 domains (Seeling et al., 2017). In contrast, IgA *N*-glycans are present at Asn144 and Asn131 in the heavy chain constant region 2 domains of IgA1 and IgA2, respectively, and at Asn340

nonlabeled AF488-labeled F779 mAbs. The right column exhibits histogram overlays of PMNs incubated with nonlabeled F779-IgA1 or F779-IgG1 (FMO control) onto the PMNs incubated with AF488-labeled F779 IgA1 or F779 IgG1. It easily allows identifying the positive population. Histograms display the fluorescence intensity on the x-axis and the cell counts (number of events) on the y-axis. n = 3. %-T indicates the percentage of gated events of the total events measured; %-# indicates the percentage of gated cells in the parent population. AF488, Alexa Fluor 488; DSG, desmoglein; FMO, fluorescence minus one; HSOC, human skin organ culture; NH, normal human; PMN, polymorphonuclear leukocyte.

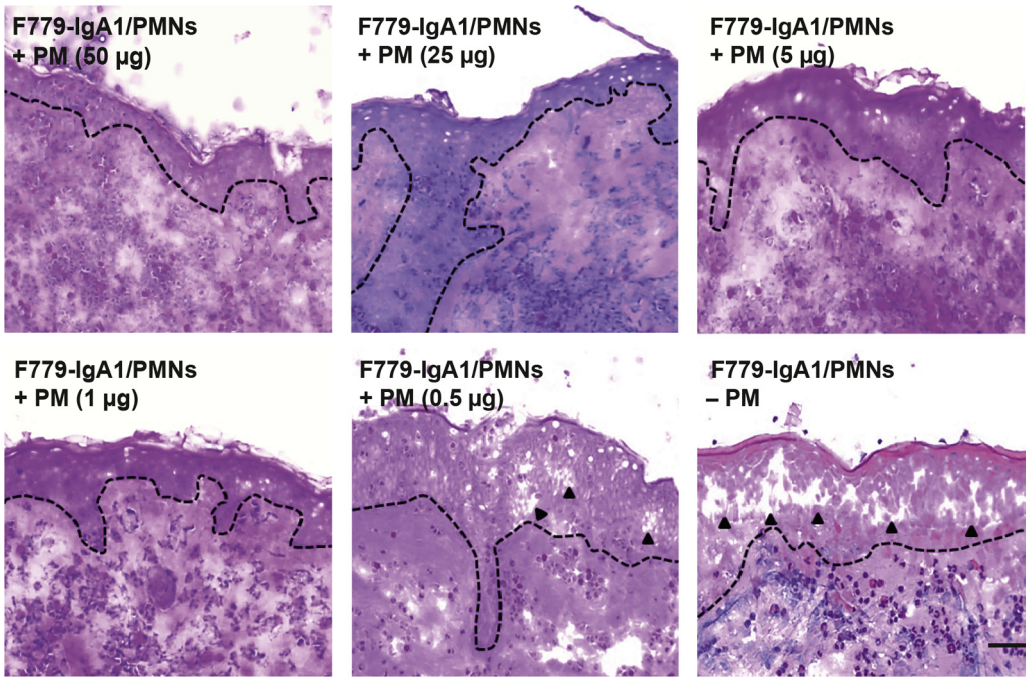

Figure 4. PM blocks F779-IgA1–induced pathology in cryosections of human skin. The IgA-binding PM abrogates the ex vivo pathology induced by anti-DSG3 F779-IgA1 mAb in cryosections of human skin (dosage range = 0.5–50 µg). Amounts of PM <0.5 µg had no significant inhibitory effects. Data are representative of the results obtained from three experiments. Magnification ×200; Bar = 100 µm. The Jonckheere–Terpstra test showed a significant correlation ($P = 0.0001$) between the PM concentration and the degree of split formation. DSG, desmoglein; PM, peptide M; PMN, polymorphonuclear leukocyte.

and Asn327 in the tailpiece domains (Toraño et al., 1977). IgA2 possesses two additional *N*-linked sites on the heavy chain constant region 1 (Asn47) and constant region 2 (Asn205) domains (Chandler et al., 2019). Furthermore, the hinge region of IgA1 is the site of attachment of usually up to six *O*-linked glycan chains (Wada et al., 2010). Disease-associated IgA *O*-glycosylation changes have been shown in several other IgA-related diseases, such as IgA nephropathy, Henoch–Schönlein purpura, Wiskott–Aldrich syndrome, rheumatoid arthritis, and X-linked thrombocytopenia (Bondt et al., 2017).

Currently, the mainstay for the treatment of IgA-mediated immunobullous diseases such as IgA pemphigus is the general suppression of immune responses by oral and topical corticosteroids. Some patients show high resistance to standard immunosuppressive therapies (Beasley and Sluzevich, 2015; He et al., 2015), as also seen for our patient with IgA PV, and to rituximab (an anti-CD20 antibody) (Lamberts et al., 2018). Because the interaction between IgA and CD89 (FcαRI) likely initiates PMN activation, interfering with this binding may be a highly specific avenue of therapy potentially minimizing side effects when compared with the currently used regimes. As a proof of concept, we were able to show a direct and immediate inhibitory effect of a synthetic IgA-binding peptide on blister induction triggered by F779 IgA1 and PMNs in skin cryosections. The general efficacy and mechanistic action of our blocking peptide M were further evaluated in another, independent in vitro model for IgA-mediated disease, that is, IgAV. We confirmed that peptide M significantly impaired IgA-induced NETosis and activation of neutrophils, corroborating a recently proposed model for IgAV pathogenesis. This model includes the interaction between IgA and FcαRI, resulting in neutrophil recruitment and activation and ultimately in processes such as NETosis and production of ROS that result in damaged vascular endothelial cells (Heineke et al., 2017).

IgA has been implicated in the pathogenesis of several other autoimmune diseases besides IgAV and intercellular IgA dermatosis, including linear IgA dermatosis, dermatitis herpetiformis (Duhring disease), Henoch–Schönlein purpura, and IgA nephropathy. The mechanisms in these IgA-dependent diseases range from IC deposition to IgA-mediated damage (Matsumura et al., 2004). In this study, we present, to our knowledge, previously unreported, mechanistic data on IgA PV, a disease entity that was difficult to study in the past because (i) IgA PV is rare and (ii) defined reagents, such as anti-KC antigen mAbs of different isotypes but with the same variable chain regions (i.e., with identical epitope binding specificities) have been lacking (Supapannachart and Mutasim, 1993). Further mechanistic and translational research may build on the insights presented in this study and benefit from the unique reagents and in vitro models established.

MATERIALS AND METHODS

Human material

This study was approved by the Institutional Review Board of the University of Luebeck, Luebeck, Germany (06-109, 12-178, 15-051) and the University Hospital of Muenster, Muenster, Germany (2015-439-f-S), and written informed patient consent was obtained following the principles of the Declaration of Helsinki. A patient with clinically active IgA PV was diagnosed at Luebeck, and serum, EDTA blood, and PBMCs were isolated from 50 ml of peripheral blood before treatment was initiated. Additional sera from patients with IgA pemphigus were obtained from coauthors TH, HHP, KMJ, and RC. Human skin was obtained from elective plastic surgeries. Healthy blood donors were recruited to allow for the isolation of PMNs. If not stated otherwise, all experiments were performed in triplicate.

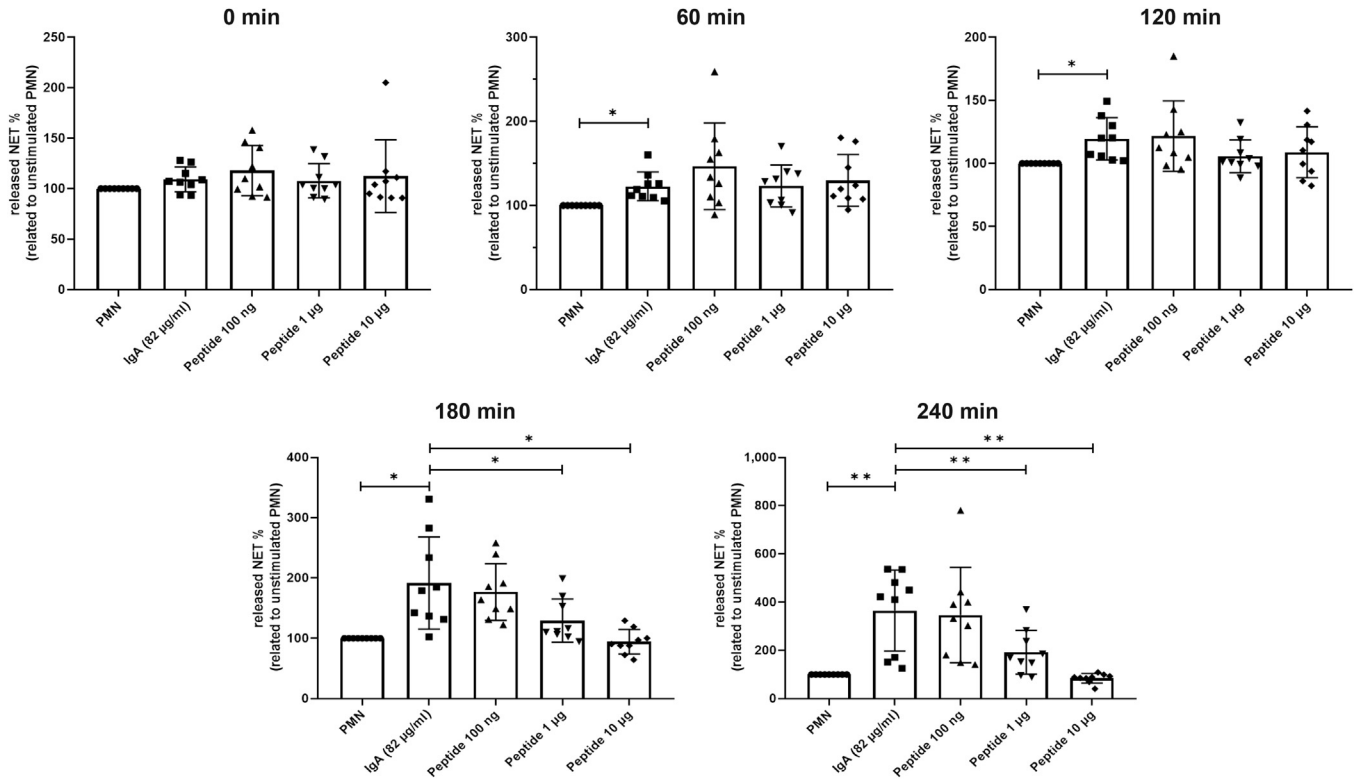

Figure 5. Inhibition of NET release by peptide M on IgA stimulation of PMNs. Human PMNs were incubated with IgA stimuli (82 µg/ml) and peptide M (100 ng, 1 µg, and 10 µg). Subsequently, IgA-induced NETosis was measured at different time points (0, 60, 120, 180, and 240 min) by SYTOX Green assay. NET release was inhibited after 180- and 240-min incubation with peptide M. Nonstimulated PMNs and IgA-stimulated PMNs served as negative and positive controls, respectively. The measurement was done in triplicates, and mean values of triplicates were plotted. Data were analyzed using one-way ANOVA followed by the Dunnett test; $*P < 0.05$, $**P < 0.005$. n = 3. All data were normalized to PMN = 100%. min, minute; NET, neutrophil extracellular trap; PMN, polymorphonuclear leukocyte.

Characterization of F779 anti-DSG3 IgA1 and IgG1 mAbs through direct and indirect IF microscopy

F779-IgA1 and F779-IgG1 mAbs against DSG3 were recombinantly produced by EUROIMMUN (Luebeck, Germany) in human embryonic kidney 293 cells, with proprietary expression vectors and published variable region sequences deposited at National Center for Biotechnology Information GenBank accession codes HQ338093 and HQ338094 (Cho et al., 2014; Lo et al., 2016). Binding specificities of recombinantly expressed and purified F779 mAbs were confirmed by anti-DSG ELISA (Medical & Biological Laboratories, Nagoya, Japan) and indirect IF on human skin and monkey esophagus substrates following standard protocols (Hofrichter et al., 2018) and with Ab concentrations adjusted to 1 µg/µl. NH IgA and NH IgG (1 µg/µl each; both from Sigma-Aldrich, Darmstadt, Germany) were used for negative controls. Direct IF staining of perilesional skin biopsies and indirect IF staining of serum antibodies on human skin and monkey esophagus were performed using goat anti-human IgA-FITC (1:100 dilution, SouthernBiotech, Birmingham, AL) and F(ab)2 anti-human IgG FITC (1:100 dilution, Bio-Rad Laboratories, Munich, Germany). Indirect IF on BIOCHIPs with human embryonic kidney 293 cells expressing recombinant DSG3, DSC1, DSC2, or DSC3 (EUROIMMUN) was performed as described previously (Mindorf et al., 2017; van Beek et al., 2012). All stained slides were examined under a fluorescence microscope (BZ-9000E, Keyence, Neu-Isenburg, Germany).

Ab phage display library construction, Ab selection and validation, and competition ELISA

To obtain scFv mAbs, we used peripheral blood from the patient with active IgA PV mentioned earlier to construct an IgA–Ab phage display library, following published protocols (Hammers and Stanley, 2014). The binding of isolated scFv mAbs to human skin was confirmed by indirect IF microscopy (see Supplementary Materials and Methods for details). To examine whether other pemphigus sera target the epitopes defined by the isolated anti-DSG3 scFv mAbs, competition ELISA was performed as previously described (Payne et al., 2005) and as described in the Supplementary Materials and Methods in more detail. In all assays, the scFv mAb concentrations were adjusted to 1 µg/µl.

Flow cytometric assessment of binding of F779 mAbs to human PMNs

Using flow cytometric analysis, we showed the propensity of F779 mAbs to bind human PMNs. A detailed protocol is provided in the Supplementary Materials and Methods. In brief, F779-IgA1 and F779-IgG1 mAbs were fluorescently tagged using an Alexa Fluor 488 Protein Labeling Kit (Thermo Fisher Scientific, Dreieich, Germany) according to the manufacturer's instructions. Blood samples were collected from three healthy donors, and PMNs were isolated using density gradient centrifugation. Labeled mAb was added to purified PMNs. PMNs incubated with unlabeled mAb were used as fluorescence minus one control. After incubation for 90 minutes, a live and dead exclusion marker, Zombie-NIR (BioLegend, Fell, Germany), was added to the cell pellet. After 15 minutes, cells were washed and centrifuged, and human Fc blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) was added. After 15 minutes, the following Ab cocktail was added to the cell suspension: BV510 anti-CD45, pacific Blue anti-CD16, phycoerythrin anti-CD193, and phycoerythrin/Cy7 anti-CD14 (all from BioLegend). After 20

minutes of incubation on ice, the cell suspension was washed and analyzed on a MACSQuant Analyzer 10 Flow Cytometer (Miltenyi Biotec) with subsequent data analysis using MACSQuantify Software (Miltenyi Biotec). All concentrations of Abs used are provided in Supplementary Materials and Methods.

Organ-cultured skin model for mechanistic study of IgA pemphigus

HSOC was conducted as reported previously (Burmester et al., 2019) and as detailed in Supplementary Materials and Methods. In all assays, the mAb and control IgG/IgA concentrations were adjusted to 1 μg/μl.

Ex vivo cryosection assay disease models of IgA pemphigus and confirmation by HSOC

To dissect the role of PMNs in the blister formation induced by anti-DSG3 autoantibodies, an in vitro cryosection assay was conducted following published protocols (Gammon et al., 1984; Recke et al., 2014; Sitaru et al., 2002) and as detailed in Supplementary Materials and Methods. PMNs were used as a source of neutrophils in the following studies because neutrophils are the most abundant cells in peripheral PMNs. Before incubation with PMNs, cryosections of NH skin were preincubated with F779 IgA1 or F779 IgG1 for 1 hour at 37 °C. NH IgA and NH IgG were used as negative controls. mAb and control IgG/IgA concentrations were adjusted to 1 μg/μl. Experiments were repeated three times using different healthy blood donors. To confirm the results obtained from this ex vivo cryosection assay, F779 IgA1 or F779 IgG1 was injected twice into organ-cultured skin (maximum volume of each injection was 50 μl), followed by human PMN injection (1×10^6 cells in a final volume of 50 μl) 60 minutes after the first Ab injection. Each experiment was performed in three independent skin organ cultures.

Inhibitory effects of peptide M, a specific IgA-Fc binding synthetic peptide of defined sequence (Johnsson et al., 1999; Pleass et al., 2001; Sandin et al., 2002), on the blister formation induced by F779-IgA1 mAb was assessed in the cryosection model. Cryosections of human skin were exposed to F779-IgA1 mAb preincubated with different doses of peptide M (50, 25, 5, 1, and 0.5 μg; in a total volume of 50 μl per skin section) for 1 hour at 37 °C, and then the assay was carried out as described earlier and in Supplemental Materials and Methods. Finally, H&E-stained slides were examined under a microscope to evaluate the inhibition of intraepidermal split formation.

Affinity purification of polyclonal anti-DSG3 IgA Abs from serum

For details, see Supplementary Materials and Methods.

Measurement of extracellular DNA release by SYTOX Green

For details, see Supplementary Materials and Methods.

Statistical analysis

If not otherwise indicated, data are presented as mean ± SD. For statistical analysis, Prism (version 8, GraphPad Software, San Diego, CA, RRID:SCR_002798) and Gnu R open-source software, version 2.15, were applied. The tests used are indicated in the figure legends. For statistical analysis by the Jonckheere–Terpstra test, R with the additional package clinfun with function jonckheere.test and Jonckheere–Terpstra test, one sided (decreasing signal by increasing inhibitor concentration) with permutation (10^4 samples, as permutation test), was applied.

Data availability statement

No datasets were generated or analyzed during this study.

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CONFLICT OF INTEREST

CMH is a consultant to Argenx and viDA Therapeutics. CMH has a provisional patent for using BP mAbs to deliver biologic agents to the basement membrane zone. CP and LK are employees of the EUROIMMUN AG, a company that develops, produces, and manufactures immunoassays for the detection of disease-associated antibodies. DZ declares consultants honoraria from UCB, Almirall, and Argenx; grants from Biotest, EUROIMMUN, and Fresenius; and speakers' honoraria/travel support from Biotest, Fresenius, Miltenyi, Roche, Biogen, AbbVie, UCB, Janssen, and Novartis. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: CMH; Formal Analysis: CMH, SE, SG, SMH, AR; Investigation: SE, SG, SMH, LK, CP, CMH; Resources: SMH, LK, CP, TH, HHP, KMJ, RC, CS, JEH, CMH; Supervision: CMH, RJL; Writing - Original Draft Preparation: CMH, SE; Writing - Review and Editing: CMH, SE, SG, SMH, KK, TH, AR, CS, SWS, JEH, DZ, ES, RJL

SUPPLEMENTARY MATERIAL

Supplementary Data

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.06.007.

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