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Experimental models reproducing an autoimmune response resulting in skin blistering in immunocompetent animals are lacking. Epidermolysis bullosa acquisita (EBA) is a bullous skin disease caused by autoantibodies to type VII collagen. In this study, we describe an active disease model of EBA by immunizing mice of different strains with murine type VII collagen. All mice developed circulating IgG autoantibodies that recognized type VII collagen and bound to the lamina densa of the dermal-epidermal junction. Importantly, subepidermal blisters developed in 82% of SJL-1, 56% of BALB/c mice, and 45% of FcγRIIb-deficient mice, but not in SKH-1 mice. In susceptible animals, deposits of IgG1, IgG2, and complement C3 were detected at the dermal-epidermal junction. In contrast, in the nondiseased mice, tissue-bound autoantibodies were predominantly of the IgG1 subclass and complement activation was weak or absent. This active disease model reproduces in mice the clinical, histopathological, and immunopathological findings in EBA patients. This robust experimental system should greatly facilitate further studies on the pathogenesis of EBA and the development of novel immunomodulatory therapies for this and other autoimmune diseases. The Journal of Immunology, 2006, 177: 3461–3468.

The immune system specifically recognizes and eliminates foreign Ags and thus protects the integrity of the host. Tolerance mechanisms that prevent or inhibit potentially harmful reactions to self Ags include clonal deletion of autoreactive T and B cells in the thymus, lymph nodes, and peripheral circulation or their active suppression by regulatory T cells. The breakdown of one or more of these mechanisms may result in autoimmunity (1). Autoimmune diseases are characterized by the activation of autoantigen-specific T and B lymphocytes and by their differentiation into autoreactive effector cells (2). The dissection of mechanisms of autoimmunity induction and tissue damage and the development of more effective therapeutic strategies require adequate disease modeling.

In a group of organ-specific autoimmune diseases, blistering of skin and mucous membranes is associated with autoantibodies against structural epithelial proteins. The blister-inducing capacity of Abs to various Ags, including desmosomal cadherins, acetylcholine receptors, type XVII collagen, laminin 5, BP230, and type VII collagen, has been characterized by passive transfer of (auto)antibodies into experimental animals (3–10). By this approach, significant information has accumulated on the mechanisms effective in the skin that, after binding of autoantibodies, mediate blistering. Interestingly, these mechanisms differ markedly. Although autoantibodies from patients with pemphigus and mucous membrane pemphigoid cause blisters just by binding to their targets, lesion induction by autoantibodies from patients with bullous pemphigoid and epidermolysis bullosa acquisita (EBA)3 appears to require subsequent activation of inflammation pathways (11–13).

Studies on the induction and modulation of the autoimmune response resulting in blistering have been hampered by difficulties in developing animal models reproducing both autoantibody production and skin pathology (active disease models) (14, 15). Recently, Amagai et al. (16) circumvented this problem by immunizing desmoglein 3-knockout mice with desmoglein 3 and subsequently transferred splenocytes of these mice into Rag-2−/− mice expressing this desmosomal cadherin. This led to production of anti-desmoglein 3 Abs in the immunodeficient mice and to subprabasilar cleavage like in pemphigus vulgaris (16, 17). However, active blistering disease has not been induced in immunocompetent mice by immunization with autoantigen (15, 16).

EBA, a severe autoimmune subepidermal blistering disease of skin and mucous membranes, is characterized by tissue-bound and circulating IgG Abs to the dermal-epidermal junction (DEJ) (12). Patients’ serum autoantibodies bind to the 290-kDa type VII collagen, the major component of anchoring fibrils (18). Epitopes recognized by the majority of EBA sera were mapped to the non-collagenous (NC) 1 domain of type VII collagen (19–21). The pathogenic relevance of Abs against type VII collagen is supported by compelling evidence. Autoantibodies against type VII collagen from EBA patients were shown to recruit and activate leukocytes in vitro, resulting in dermal-epidermal separation in cryosections of human skin (22, 23). Abs against type VII collagen induce subepidermal blisters when passively transferred into mice (8–10).

In the present investigation, immunization of mice with recombinant murine type VII collagen NC1 induced production of autoantibodies and resulted in a subepidermal blistering phenotype, duplicating the findings in the human disease. Using this animal model, we show that the production of autoantibodies binding to

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3 Abbreviations used in this paper: EBA, epidermolysis bullosa acquisita; DEJ, dermal-epidermal junction; IF, immunofluorescence; NC, non-collagenous.

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the epidermal basement membrane can be induced in mice of dif-
ferent strains. However, the blistering phenotype is restricted to
certain strains. Lesion formation is associated with autoantibodies
against type VII collagen that belong to a certain subclass and are
capable of activating complement in the skin of the mice.

Materials and Methods

Mice

Seven- to 8-wk-old SJL-1, SKH-1, C57BL/6, and BALB/c female and
male mice with a body weight of $\approx 20$ g were obtained from Charles River
Laboratories. FcRRIIb$^{-/-}$ female mice (on a C57BL/6 background) were
obtained from Taconic Farms. All injections and bleedings were performed
on mice narcotized by inhalation of isoflurane or i.p. administration of a
mixture of ketamine (100 $\mu$g/g) and xylazine (15 $\mu$g/g). The experiments
were approved by local authorities of the Animal Care and Use Committee
(Kiel, Germany; No. 66/08) and performed by certified personnel.

Immunizations and evaluation of mice

Mice were immunized at the tail base with 40 $\mu$g of recombinant murine
type VII collagen (GST-mCOL7C) (8) or human type XVII collagen
(GST-NC16A2-5) (24) emulsified in the nonionic block copolymer adjuvant
TiterMax (ALEXIS Biochemicals) and boosted at the same location
with 40 $\mu$g of Ag in TiterMax 3, 6, and 9 wk later. Mice were examined
every second day for their general condition and for evidence of cutaneous
lesions (i.e., erythema, blisters, erosions, and crusts). Blisters or erosions
were scored, and the extent of skin disease was scored as follows: 0, no
lesions; 1, 1–10 lesions or <1% of the skin surface; 2, >10 lesions or 1–5% of
the skin surface; 3, 5–10%; 4, 10–20%; and 5, >20% involvement of the
skin surface. All mice were observed for at least 12 wk. Twenty of each
SJL and BALB/c mice were followed up for 18 wk. For passive transfer
studies, 50 $\mu$(1 mg) of IgG purified from diseased SJL and nondiseased
SKH mice was injected every second day intradermally into the ears of
C57BL/6 mice. After four injections, mice were killed and the ears were
prepared for histological and immunopathological examination, as de-
scribed (8).

Serum samples were obtained from mice at different time points and
assayed by immunofluorescence (IF) microscopy and for autoantibody lev-
els by ELISA using recombinant protein. Biopsies of lesional and periles-
ional skin, esophagus, and colon were obtained at the end of the obser-
vation period and prepared for examination by histopathology, electron
microscopy, and IF microscopy.

Histological and electron-microscopical studies

Biopsies of lesional and perilesional skin, oral mucosa, esophagus, and
colon were fixed in 4% buffered Formalin. Sections from paraffin-embed-
ded tissues were stained with H&E. For electron microscopy, specimens
collected from six diseased mice and one control mouse were fixed in 3%
agarose, and frozen in liquid nitrogen. Ultrathin sections (60 nm in thickness)
were prepared from tissue biopsies using 100-fold diluted FITC-labeled
mouse serum with cell lysate of bacteria transformed with wild-type pGEX
was performed, as described (8). Rabbit Abs SA8009 and SA2954 gener-
ated against rGST fusion proteins containing sequences of human type
XVII collagen (GST-NC16A2-4) and murine type VII collagen (GSTM-
cOL7C), respectively, were described previously (8, 28).

IF microscopy and immunoblot analysis

Tissue-bound autoantibodies were detected by IF microscopy on frozen
sections prepared from tissue biopsies using 100-fold diluted FITC-labeled
Abs specific to mouse IgG (DakoCytomation), IgG1, IgG2a, IgG2b, IgG3
(all obtained from BD Pharmingen), and murine C5 (Cappel Organon-
Teknika). The staining intensity of immunoreactants in the skin of immu-
nized mice was assessed semiquantitatively using a score comprising 0, for
no staining; 1, faint staining; 2, medium; and 3, intense staining. Detection
of serum autoantibodies in mice followed published protocols with minor
modifications (8, 28). Briefly, after incubating with diluted mouse serum,
the frozen sections of murine and human skin were treated with 100-fold
dilution of Abs to mouse IgG and IgG subclasses. Expression and purification
of recombinant murine type VII and human type XVII collagen forms
followed published protocols (8, 24). Extracts of murine dermis were pre-
pared, as described (8, 29). Recombinant proteins or dermal extracts were
fractionated by 12% and 6% SDS-PAGE, respectively, transferred to nitro-
cellulose, and analyzed by immunoblotting (22). Alternatively, proteins
were separated by gradient 4–20% SDS-PAGE. Immunoadsorption of
mouse serum with cell lysate of bacteria transformed with wild-type pGEX
was performed, as described (8). Rabbit Abs SA8009 and SA2954 gener-
ated against rGST fusion proteins containing sequences of human type
XVII collagen (GST-NC16A2-4) and murine type VII collagen (GSTM-
cOL7C), respectively, were described previously (8, 28).

Detection of circulating autoantibody levels by ELISA

ELISA using recombinant murine type VII collagen was performed at
room temperature on 96-well microtiter plates. The optimal working con-
ditions of the assay were defined by checkerboard titrations with dilutions of
Ag and secondary Ab, as described (30). The optimized ELISA was run
under the following conditions. Each well was coated with 500 ng of pu-
rified protein rGST-mCOL7C or with an equimolar amount of GST in 0.1 M
bicarbonate buffer (pH 9.6). After blocking, wells were incubated with
a 200-fold dilution of mouse sera for 60 min. Bound Abs were detected
using a 10,000-fold dilution of an HRP-labeled rabbit anti-mouse IgG Ab
(DakoCytomation) and orthophenylene diamine (Sigma-Aldrich).

Statistical analysis

Data are expressed as mean ± SEM of n observations (i.e., the number of
animals for the in vivo studies). To compare the weight between diseased
and control mice, the independent-samples Student’s t test was used. For
comparing the frequency of IgG autoantibodies and diseased and nondis-
ased mice, the Fisher’s exact test was applied. Differences in disease se-
verity were calculated using the $\chi^2$ test (8). To estimate the correlation
between the IF staining intensity at the DEJ and disease activity, the Spear-
man’s rank correlation test was applied (8). To compute these tests, the
OpenStat2 free software for Linux (www.agrivisser.com/cgi-bin/English/
OpenStat2.htm) was used.

Results

Immunization against autologous type VII collagen induces
autoantibody production in mice of different strains

SIL-1 ($n = 29$), BALB/c ($n = 25$), FcRRIIb$^{-/-}$ ($n = 20$),
C57BL/6 ($n = 10$), and SKH-1 ($n = 20$) mice were immunized
against a rGST fusion protein containing a sequence of the type
VII collagen NC1 domain. Control SIL-1 ($n = 5$) and BALB/c
($n = 5$) mice were immunized with an irrelevant GST fusion
proein. Preimmune serum from none of the mice showed IgG re-
activity to the DEJ by IF microscopy. One month after the first s.c.
injection of autoantigen, circulating IgG binding to the DEJ was
detected in all mouse sera by IF microscopy using murine skin as
a substrate (Fig. 1a). Indirect immunoelectron microscopy analysis
demonstrated that mouse IgG Abs labeled the lamina densa of
mouse skin (Fig. 1b). By immunoblot analysis, IgG Abs from all
immune sera, in contrast to preimmune sera, targeted both cell-
derived and recombinant forms of type VII collagen (Fig. 1c).
Levels of IgG autoantibodies specific to type VII collagen were
measured at several time points by ELISA using recombinant Ag.
Although initially BALB/c mice showed significantly lower levels
of autoantibodies to type VII collagen, 12 wk after the first im-
munization, levels of autoantibodies were similar in all strains

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3462 EXPERIMENTAL EBA

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the sera from the immunized mice bind to recombinant murine type VII collagen. After preadsorption against GST, like the specific rabbit Ab (SA2954-PP), an Ab specific to an irrelevant GST fusion protein (SA8009), the immune (SA2954-PP), and preimmune serum (SA2954-PPI) of a rabbit immunized against skin (magnification, \( \times \)).

Mice of different strains show distinct susceptibility to the induction of blisters by immunization against type VII collagen.

Twenty-four of 29 (82%) SJL-1, 14 of 25 (56%) BALB/c, and 9 of 20 FcγRIIb−/− (45%), but none of the SKH-1 and C57BL/6 mice immunized against type VII collagen developed a blistering phenotype (Table I; Fig. 2). Four to 8 wk after the first s.c. injection of recombinant type VII collagen, all diseased SJL mice showed single blisters on their ears and tails, often accompanied by erythema (Fig. 2b). Blisters developed into erosions partially covered by crusts and with longer disease duration, diffuse erythema, and thickening, and scarring of the ears and tails occurred, such that the architecture and shape of these organs began to change (Fig. 2, a and c). In the majority of diseased mice, 1–2 wk after first lesions had developed, muzzles, periorcular skin, and paws, as well as ventral and dorsal aspects of the trunk were also affected. Blisters/erosions on trunk, perioricularly, and around the snouts resulted in alopecia after an average time of 8–12 wk after immunization (Fig. 2, a and g). In BALB/c and FcγRIIb−/− mice immunized against type VII collagen, initial skin lesions were observed 6–8 wk after the first injection (Fig. 2, f and h). The development and evolution of blistering in these mice had a similar pattern, although with a latency and at a significantly lower scale compared with lesions seen in SJL mice (Fig. 3; Table I). After ceasing s.c. injections of type VII collagen, disease activity decreased in diseased animals (Fig. 3). None of the SKH-1 and C57BL/6 mice or mice injected with control protein (Fig. 2i) developed skin lesions. No behavioral alterations or significant weight loss were recorded in immunized mice of different strains compared among each other or with nonimmunized littermates.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serum Autoantibodies</th>
<th>Skin Immunopathology</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IF (^a)</td>
<td>ELISA (^a)</td>
<td>Blisters</td>
</tr>
<tr>
<td>SJL-1</td>
<td>29/29</td>
<td>29/29</td>
<td>24/29</td>
</tr>
<tr>
<td>FcγRIIb−/−</td>
<td>20/20</td>
<td>20/20</td>
<td>9/20</td>
</tr>
<tr>
<td>SKH-1</td>
<td>20/20</td>
<td>20/20</td>
<td>0/20</td>
</tr>
</tbody>
</table>

\(^a\) Reactivity of IgG autoantibodies was detected by IF microscopy on murine skin sections using 10-fold-diluted mouse serum obtained 6 wk after immunization against type VII collagen.

† Serum autoantibodies to type VII collagen were detected by ELISA using recombinant murine Ag as described in Materials and Methods; serum samples were obtained 6 wk after immunization.

| b Serum autoantibodies to type VII collagen were detected by ELISA using recombinant murine Ag as described in Materials and Methods; serum samples were obtained 6 wk after immunization against type VII collagen. | 20/20 | 20/20 | 0/20 | 20/20 | 6/20 |

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FIGURE 2. Mice of different strains show distinct susceptibility to the induction of blisters by immunization against type VII collagen. a, Extensive skin lesions, including blisters, erosions, alopecia, and scarring, developed in an SJL-1 mouse 8 wk after the first immunization against type VII collagen. b, Close-up picture of a blister on the tail of an SJL-1 mouse 7 wk after the first immunization against type VII collagen. c, Erosions, crusts, skin atrophy, and scarring on the tail of an SJL-1 mouse 12 wk after the first injection of autoantigen. d, Erosions and crusts on the right ear of an immunized SJL-1 mouse. e, Upon tangential pressure, epidermal detachment could be induced and the epidermis could easily be lifted up from the dermis. f, Erosions and crusts on the right ear of an immunized BALB/c mouse. g, Periocular scarring and alopecia in an SJL mouse 10 wk after the first immunization against type VII collagen. Erosions and scarring in FcγRIIb−/− mouse immunized against murine type VII collagen (h); and no skin lesions in a BALB/c mouse immunized against an irrelevant GST fusion protein (i).

FIGURE 3. SJL mice develop an earlier and more severe blistering disease compared with BALB/c mice. SJL and BALB/c mice were given primary and booster injections (arrowheads) of murine type VII collagen and evaluated for skin lesions, as described in Materials and Methods. Disease activity is represented as means ± SEM in 17 SJL and 20 BALB/c mice. *p < 0.01 represents significant difference of disease severity between the two groups.

Blister localize at the sublamina densa of the basement membrane zone

From each mouse, three skin biopsies were obtained for histopathological examination at the end of the observation period. In all mice that showed skin lesions at the time of tissue collection, light microscopic analysis of skin biopsies revealed extensive dermal-epidermal separation and different degrees of inflammatory infiltrates dominated by neutrophils (Fig. 4a). Histological examination of skin biopsies from mice immunized with control protein (Fig. 4b) and from nondiseased mice immunized against type VII collagen demonstrated no blisters and no inflammatory infiltrate at the DEJ. In one SKH mouse, dermal-epidermal separation and a low inflammatory infiltrate were observed. In five SJL mice with skin lesions, biopsies were also obtained from oral mucous membranes, esophagus, and colon. In all five mice, subepithelial blisters were found in mucous membranes, whereas no blisters were detected in esophagus and colon (data not shown). Electron microscopy of lesional skin biopsies from diseased SJL-1 mice (n = 5) demonstrated split formation in the uppermost dermis. The basal lamina was found in the blister roof (dermolytic blister formation) and adhered to basal keratinocytes that showed intact hemidesmosomes (Fig. 4c). By immuno-electron microscopy of a perilesional skin biopsy, IgG deposits localized to the sublamina densa of the DEJ. IF analysis of lesional skin of diseased mice demonstrated IgG deposits predominantly on the epidermal side of the cleavage (Fig. 4d). Based on the binding of IgG to the sublamina densa, this IF analysis confirms the dermolytic split formation.

The blistering phenotype in mice is associated with local deposits of complement

IF microscopy of perilesional mouse skin revealed linear deposits of mouse C3 at the DEJ in all diseased animals that were immunized with type VII collagen (Table I; Fig. 5b). No or weak deposits of murine C3 were found by direct IF microscopy at the DEJ of nondiseased animals (Table I; Fig. 5, d and f). Grading of the relative intensity of C3 deposits in 41 diseased and 31 nondiseased mice demonstrates that complement deposition was significantly higher in diseased compared with nondiseased mice (1.65 ± 0.12 and 0.37 ± 0.13; p < 0.001). The relative intensity of C3 deposits in diseased mice was significantly higher compared with nondiseased mice (1.65 ± 0.12 and 0.37 ± 0.13; p < 0.001).

FIGURE 4. Blisters localize below the lamina densa of the epidermal basement membrane. a, Histological examination of skin biopsies from a diseased SJL-1 mouse reveals extensive subepidermal cleavage (magnification, ×400). b, No histological changes in the skin of a mouse immunized against the control protein (magnification, ×200). c, Electron-microscopic examination of a lesional skin biopsy from a diseased mouse demonstrates that the blister roof contains the lamina densa bordered by basal keratinocytes with hemidesmosomes. Dermal connective tissue represents the blister floor (magnification, ×11,000). d, IF analysis of lesional skin of diseased mice demonstrated IgG deposits predominantly on the epidermal side of the cleavage.
of C3 deposits correlates with the extent of the skin disease ($r = 0.88$; $p < 0.01$).

Subepidermal blistering is associated with IgG2 autoantibodies against type VII collagen

The IgG subclass of autoantibodies bound at the DEJ was assessed by IF microscopy of perilesional skin. The blistering phenotype in mice is associated with local deposits of complement. Skin biopsies from mice immunized against type VII collagen were assessed by IF microscopy for the presence of IgG and C3 deposits. Strong IgG deposition was found at the epidermal basement membrane in an SJL (a), SKH (c), and FcγRIIb−/− (e) mouse. Staining for complement C3 was strong in SJL (b), weak in SKH-1 (d), and almost absent in FcγRIIb−/− (f) mice. The patterns of reactivity are representative of the patterns revealed in the other mice, as shown in Table I.

Table II. IgG subclass distribution of autoantibodies bound at the epidermal basement membrane in immunized mice

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>IgG1</th>
<th>IgG2a</th>
<th>IgG2b</th>
<th>IgG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL</td>
<td>20/21</td>
<td>7/21</td>
<td>21/21</td>
<td>3/21</td>
</tr>
<tr>
<td>BALB/c</td>
<td>22/22</td>
<td>9/22</td>
<td>6/22</td>
<td>1/22</td>
</tr>
<tr>
<td>FcγR2b−/−</td>
<td>20/20</td>
<td>10/20</td>
<td>12/20</td>
<td>1/20</td>
</tr>
<tr>
<td>SKH</td>
<td>20/20</td>
<td>2/20</td>
<td>4/20</td>
<td>0/20</td>
</tr>
</tbody>
</table>

* Perilesional skin biopsies were analyzed for deposition of IgG isotypes at the epidermal basement membrane by IF microscopy.

Discussion
Animal models replicating the active disease are needed to study the regulation of autoreactive T and B cells in autoimmune blistering diseases. Our study describes an active disease model for EBA by immunization of mice against autologous type VII collagen. These mice developed blisters of skin and oral mucous membranes that were associated with circulating autoantibodies against type VII collagen as well as deposits of IgG and complement at the epithelial basement membrane. Dermal-epidermal separation localized to the sublamina densa. Thus, the blistering phenotype induced in the mice reproduces the clinical, histological, immunopathological, and ultrastructural features of human EBA. In addition, the disease in these animals mimics subepidermal blistering induced in mice by passive transfer of Abs against type VII collagen (8–10).

A major difficulty in developing animal models of autoimmune diseases has been to overcome self-tolerance. However, for some autoimmune conditions, experimental models reproducing active disease have been successfully generated by immunizing animals with the (auto)Ag (31–37). In our study, we immunized BALB/c, C57BL/6, and SJL animals that are inbred strains commonly used to study autoimmune immunity in mice. In addition, we used outbred SKH-1 mice because their hairless condition facilitates skin evaluation. FcγRIIb is thought to be an epistatic modifier of autoimmunity; its deletion results in a strain-specific enhancement of systemic lupus erythematosus (38, 39) or in the development of arthritis and Goodpasture disease on nonpermissive H-2 background (40, 41). Based on these data, we hypothesized that FcγRIIb−/− mice on C57BL/6 background might be prone to develop clinically overt autoimmunity against type VII collagen too.

Interestingly, we were able to induce an autoimmune response to type VII collagen in all mice immunized against autologous protein. However, although all immunized mice exhibited both comparable levels of circulating autoantibodies and tissue-bound IgG, skin blistering was restricted to SJL, BALB/c, and FcγRIIb−/− mice. In contrast, SKH-1 and C57BL/6 animals showed no blistering clinically or histologically. This strain dependence strongly suggests a genetic predisposition to the induction of skin blistering in mice by autoantibodies to type VII collagen. In addition, our observations demonstrate that the presence of circulating autoantibodies as well as the mere binding of autoantibodies to the Ag are not sufficient to induce skin blisters. A similar phenomenon has been observed in other models for autoimmune diseases. Experimental animals, either passively transferred with specific autoantibodies or immunized against the autoantigen, despite in vivo IgG deposition, failed to acquire a disease phenotype (42–45). Although local deposition of IgG has been considered a major criterion for the diagnosis of an autoimmune disease, our present findings and previous data demonstrate that tissue-bound autoantibodies may well be detected in the absence of specific clinical findings.

In the skin of EBA patients, deposition of complement components is found with an incidence ranging from ~40 to 100% (46–48). However, in patients included in these studies, diagnosis of EBA was based only on clinical and histological features, while...
reactivity to type VII collagen has not been confirmed (47). Therefore, in these patients, EBA may have been confounded with other subepidermal autoimmune blistering diseases (e.g., anti-p200 pemphigoid or anti-epiligrin cicatricial pemphigoid). In the active disease model described in this study, we found IgG deposition at the DEJ in all mice immunized against type VII collagen. In contrast, staining for complement C3 was significantly increased in diseased compared with nondiseased mice, suggesting that activation of complement is important for blister formation. To further explore this hypothesis, we analyzed the IgG subclass distribution of tissue-bound and circulating autoantibodies in mice. IgG1 autoantibodies were detected in all animals immunized against type VII collagen. However, deposits of complement-fixing IgG2a and IgG2b autoantibodies were significantly more frequent and intense in diseased compared with resistant mice. In addition, IgG from diseased, but not resistant mice induced skin blistering by passive transfer into mice. The resistance of mice to skin blistering is therefore most likely related to the lack of induction of complement-fixing autoantibodies; this resulted in absence/major reduction of complement activation in nondiseased mice. Consistent with this hypothesis are the observations that rabbit Abs to murine type VII collagen are not pathogenic in C5-deficient mice (8) and that F(ab’).2 of pathogenic Abs against type VII collagen fail to induce subepidermal blisters in mice (8, 10). Nevertheless, a direct, Fc-independent, pathogenic role of autoantibodies against type VII collagen (e.g., by interfering with its ligand function) cannot be excluded. However, in vivo experimental evidence supporting this hypothesis is still lacking.

Complement-fixing autoantibodies are potent mediators of tissue damage. Th1 cells have been implicated in the pathogenesis of most Ab-mediated autoimmune diseases, because they help B cells to produce complement-fixing autoantibodies (49, 50). In our study, autoantibodies that bound in the skin of diseased mice were essentially of IgG1 and IgG2a/b isotypes. These findings demonstrate that the autoimmune response in diseased mice rather shows a Th1/Th2 polarization than a pure Th2 or a T-independent Ag-driven reaction. IgG2 autoantibodies have been implicated as mediators of tissue injury also in other autoimmune diseases, including myasthenia gravis, experimental autoimmune encephalomyelitis, and vasculitis (44, 51, 52). In contrast, in the active mouse model for pemphigus, disease expression is not dependent on complement activation. In desmoglein 3−/− mice, following immunization against desmoglein 3, Ag-specific B cells developed. When transferred into Rag-2−/− mice, these B cells predominantly produced IgG1 Abs that bound to the Ag

FIGURE 6. Subepidermal blistering is associated with IgG2b and IgG2a autoantibodies against type VII collagen. Depicted are the results of IF microscopy for IgG subclass distribution of autoantibodies in four mice immunized against type VII collagen. Strong IgG1 deposition was found at the epidermal basement membrane in SJL (a), BALB/c (e), FcγRIIb−/− (i), and SKH (m) mice. IgG2a and IgG2b deposits were found in the SJL (b and c) and BALB/c (f and g), but not in the FcγRIIb−/− (j and k) and SKH (n and o) mice. d, h, l, and p. None of the mice demonstrated cutaneous deposits of IgG3 (magnification, ×400). Analysis of the IgG subclass distribution of autoantibodies at the DEJ in immunized mice is summarized in Table II.

FIGURE 7. IgG purified from diseased mice induces dermal-epidermal separation when passively transferred into mice. A total of 50 μl (1 mg) of IgG purified from three diseased SJL and three nondiseased SKH mice was injected every second day intradermally into the ears of a total of six C57BL/6 mice. After four injections, histopathological analysis shows subepidermal blisters in mice injected with IgG purified from SJL (a), but not from SKH (b) mice. IF microscopy of perilesional skin reveals linear deposition of C3 at the DEJ in mice injected with IgG purified from SJL (c), but not from SKH (d) mice. Deposition of IgG1 was observed in mice injected with IgG from both SJL (e) and SKH (f), whereas deposits of IgG2b were found only in mice injected with IgG purified from SJL (g), but not from SKH (h) mice.
in the skin of the mice and induced acantholytic blisters. These Abs did not activate complement or recruit leukocytes (16, 53). These data are strengthened by work using the passive transfer model of pemphigus showing susceptibility of C5-deficient mice to the experimental pemphigus (54) and induction of blisters in mice by Fab(’2) (54), Fab (55), and single-chain variable fragment (56) fragments of pemphigus autoantibodies. In contrast, the active animal model of EBA described in this report will facilitate studies on autoantibody-induced tissue damage mediated by complement and inflammatory cells.

Our animal model allows for the evaluation of the autoimmune response and skin blistering by simple serological assays and clinical inspection, respectively. Therefore, this robust experimental system can be used as a model for organ-specific autoimmunity to investigate the immunogenetics of EBA and the contribution of T and B lymphocytes for both the initiation and regulation of autoantibody production. In addition, this active disease model should greatly facilitate the development of new therapeutic strategies modulating the autoimmune response.

In conclusion, this study demonstrates that an autoimmune response against type VII collagen can be readily induced by immunization of mice, resulting in induction of skin blisters. The blistering phenotype of the mice recapitulates the clinical, histological, and immunopathological changes in the skin of patients with EBA. This experimental system should greatly facilitate the further dissection of the cellular and molecular pathogenesis of EBA. In addition, it should help in the development of novel, more specific therapies for EBA, which will also have implications for the management of other autoimmune diseases.

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Disclosures
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References


