Complement-independent blistering mechanisms in bullous pemphigoid

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Abstract

Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease that clinically demonstrates tense blisters with widespread erythema, histologically demonstrates subepidermal blistering and immunologically demonstrates the presence of circulating autoantibodies against hemidesmosomal molecules. Complement activation has long been regarded as necessary for the generation of the BP. However, certain evidence has recently come to support non-complemental blistering mechanisms. The story of BP blistering mechanisms is a complicated one. This review mainly focuses on a specific blistering mechanism that highlights the role of complements in BP blistering.

1. Introduction

Complement activation has long been regarded as necessary to produce the blistering seen in bullous pemphigoid (BP). However, certain pieces of clinical and experimental evidence show the possibility that non-complement blistering mechanisms may also be important.

BP is an autoimmune subepidermal blistering disease that demonstrates characteristic clinical, histological, and immunological features (1). Clinically, it shows tense blisters with or without widespread erythema. Histologically, it shows blistering along the lamina lucida beneath the basal cells at the basement zone. Immunologically, it shows the presence of circulating autoantibodies against hemidesmosomal molecules. Mucous membranes, such as the oral and ocular membranes, are affected in 10-20% patients (2). Immunological analyses such as Western blotting, immunofluorescent staining and enzyme-linked immunosorbent assay (ELISA) reveal the presence of autoantibodies against two antigens type XVII collagen (COL17, also known as BP180, BPAG2) (3,4) and BP230 (5–8). Human COL17 consists of 15 collagenous domains and 16 non-collagenous (NC) domains, numbered from the COOH terminus (4). The NC16 domain is divided into 3 subdomains: one extracellular (NC16A), one transmembrane (NC16B) and one intracellular (NC16C).

The blistering mechanisms in BP have been well investigated and discussed; nevertheless, they remain unclear. Complement activation, however, has been reported to be critical for the development of clinical phenotype in experimental models of BP (9,10). Regarding BP blistering, the recruitment of inflammatory cells such as neutrophils, eosinophils and mast cells (18-22) have also been reported to be important to the development of clinical manifestations in experimental models (11–15). In particular, immune complexes are thought to initially activate the complement cascade, which may induce the activation of proteases and/or cytokines and cause dermal-epidermal separation (16). These pieces of evidence strongly suggest that the clinical phenotypes of BP are associated with inflammation.

Epidermolysis bullosa acquisita (EBA) is also an autoimmune subepidermal blistering disease (1). EBA shows tense blisters and erosions that histologically demonstrate the subepidermal separation of the skin or mucous membrane. The clinical features of EBA are quite similar to BP, except for autoantigens. The major autoantigen in EBA is type VII collagen, a 290 kDa protein that is a major component of anchoring fibrils, whereas the main autoantigen in BP is COL17. In general, EBA classified into inflammatory and non-inflammatory types, respectively called classical and mechanobullous. Complement deposits are not commonly detected by direct immunofluorescence in non-inflammatory type classical EBA (17). Similarly, recent studies have discriminated non-inflammatory BP from typical inflammatory BP (18) (Figure 1). However, it is still not clear the mechanisms in both inflammatory and non-inflammatory type EBA or BP. These different clinical manifestations may have distinct pathomechanisms, including complement activation.

This review focuses on relevance of complement activation as a blistering mechanism in BP pathogenesis.

2. The complement system in autoimmune diseases

The general complement system is mentioned in Dada S1.

Dysregulation of complements may be involved in the pathogenesis of autoimmune diseases (S1). However, whether complements are associated with autoimmune diseases remains controversial. The clinical manifestations of several autoimmune diseases, including systemic lupus erythematosus (SLE), anti-glomerular basement membrane disease and vasculitis, are related to complement activation. In such cases, complement deposits are detected in the affected lesions. Meanwhile, complement deficiency is thought to be associated with the pathogenesis of autoimmune diseases (S1). The individuals with complement deficiency are significantly higher incidence of SLE than those with complement sufficiency (S2). Furthermore, severe clinical course are observed in SLE

associated with C1q deficiency (S3). These indicates complement system are not crucial to develop SLE.

The pathomechanisms of tissue injury in autoimmune diseases have been well studied using mouse models. Immunization of type II collagen, which is a major constituent protein of cartilage, can induce arthritis in mouse (collagen-induced arthritis, CIA) that resembles rheumatoid arthritis. The CIA mice produce anti-type II collagen antibodies, which bind to antigens in the joint and induce a destructive arthritis. In this process, the complement system is activated at the affected site, and complement deposits can be detected. According to several studies, alternative pathways play the most important role in autoimmune disorders such as rheumatoid arthritis and lupus nephritis (S4). In the CIA model, C3-deficient mice are protected from disease induction. In addition, the anti-C5 antibody prevents disease induction. The above evidence indicates that complement activation is essential for the induction and augmentation of severity in CIA. By contrast, there is evidence of complement-independent arthritis models (S5,S6).

In autoimmune blistering diseases, the evidence for the relevance of complements in BP is discussed later. EBA has been well studied for complement relevance using animal models (S7,S8). Mice injected with pathogenic antibodies or immunized with COL7 demonstrate clinical phenotypes and show deposits of IgG and complements at the dermal-epidermal junction. Furthermore, experimental EBA induced by repeated injections of anti-COL7 IgG was shown to be completely alternative pathway dependent (S9).

On the other hand, there are pieces of evidence to show complement independent in autoimmune blistering diseases. The pathogeneses of pemphigus diseases are well known to be complement-independent. IgA autoantibodies are detected in some autoimmune blistering diseases, such as linear IgA bullous dermatosis or IgA-type EBA (1). IgA is generally known to be hardly able for complement activation due to lacking the residues in the Fc regions that bind to C1q (S10).

3. The pathogenicity of COL17

Almost all BP-patients' sera react with 180 kDa and/or 230 kDa proteins, i.e., COL17 and BP230, respectively, as determined by Western blotting using epidermal extract of normal human skin or normal human keratinocytes. In addition, serum titers of anti-COL17-NC16A autoantibodies correlate with disease severity in BP patients (19–21). There are several options for studying BP pathogenesis *in vivo*, and they fall into two categories: 1) pathogenic IgG transfer into mice (so-called passive models), and 2) immunization-induced mouse models (so-called active models). Given the low homology between human and mouse COL17-NC16A (22), the passive transfer of IgG from BP patients into mice cannot induce disease (23). To overcome the issue of insufficient COL17 homology between humans and mice, Nishie et al. generated COL17-humanized mice (24). These COL17-humanized mice express human COL17 at the dermal-epidermal junction. Liu et al. generated a humanized mouse in which the mouse COL17-NC14A was replaced with the homologous human COL17-NC16A cluster region (25). These two humanized mice models develop skin fragility when injected with either whole IgG or affinity-purified IgG against COL17-NC16A from BP patients. In contrast, active mouse models of BP have also been reported to induce clinical manifestations immunized with mouse NC14A or human NC16A of COL17 (14,26). These several lines of evidence suggest that COL17 is the most likely pathogenic molecule for BP, and the NC16A domain of COL17 is considered to be the major pathogenic epitope for BP. On the other hand, it is lacking the evidence to demonstrate the pathogenicity of epitopes outside the NC16A region on COL17 which have been associated with the non-inflammatory variant of BP (18).

4. Evidence of complement relevance in BP

4-1) Clinical evidence of complement relevance

A typical clinical manifestation of BP is tense blisters associated with urticarial erythema (Figure 1). The complement activation results in inflammation and tissue damage. Direct immunofluorescence examination using patients' skin shows complement deposits at the dermal-epidermal junction in the

majority of BP cases. Our group previously evaluated 100 cases of BP, and 98% of these cases showed complement deposits at the dermal-epidermal junction (27). Romeijn et al. investigated 301 cases of BP, and found C3 deposits in 250 (83.1%) of the cases (28).

4-2) Experimental evidence of complement relevance

Mice adaptively transferred with human BP-IgG, rabbit anti-COL17 IgG or human IgG1 mAb against COL17 demonstrate clinical blistering or epidermal detachment by gentle friction (25,29,30). These mice show complement deposits at the dermal-epidermal junction. Liu et al. reported significant insights regarding blistering mechanisms using a BP mouse model (9). They injected rabbit anti-mouse COL17 IgG into C5 deficient mice, and the mice developed neither clinical signs nor histological dermal-epidermal separation. Moreover, F(ab')2 fragments from pathogenic rabbit anti-mouse COL17 IgG induced neither clinical disease nor complement deposits at the dermal-epidermal junction. From these two results, the blistering mechanisms in BP are thought to principally depend on complement activation by Fc fragments of pathogenic IgG. To elucidate the contribution of complements, the same group investigated the complement activation pathways in a BP model (10). C4 deficient mice injected with anti-mouse COL17 IgG showed no clinical or histological changes. C4 is required for the activation of both classical and lectin pathways. Mice pretreated with anti-mouse C1q antibodies also were protected from disease induction. This implies that the classical pathway is required in experimental BP. Furthermore, the classical pathway plays a major role throughout disease in concert with the alternative pathway to increase the severity.

5. Evidence of non-complement relevance

5-1) Clinical evidence of non-complement relevance

A case of BP associated with C4 deficiency was reported (31). Romeijn et al. found no significant correlations between C3 deposits, erythema, itch or inflammatory infiltrates based on an analysis of 301 BP cases (28). Furthermore, a non-bullous pemphigoid subgroup was found to have

significantly fewer C3a deposits than those seen in typical BP. Recently, we reported that some BP patients have autoantibodies against parts of COL17 outside the NC16A domain (hereinafter: the non-NC16A domain) (18). Non-NC16A BP is frequently associated with an oral medication history of dipeptidyl peptidase-4 inhibitor (DPP-4i) and presents less severe inflammatory manifestations (Figure 1). In addition, we often face the cases whose urticarial erythema disappears soon after systemic steroid treatment despite a high serum antibody titer.

Complements are activated in many skin diseases (32), so complement deposition can be seen in some inflammatory skin diseases, such as lichen planus (33), lupus (34) and even atopic dermatitis (35). In addition, a previous report showed that the C3b degradation product C3d was observed at the dermal-epidermal junction even in normal human skin (36). As mentioned above, direct immunofluorescence studies show complement deposits at the dermal-epidermal junction in almost all cases of BP. The predominant IgG subclass at the dermal-epidermal junction in BP is IgG4 (37–39). Human IgG4 is the least abundant IgG subclass in healthy individuals, and it fails to fix C1q in the classical pathway (40). Deposits of C3 in BP lesional skin may occur via an alternative pathway, or small amounts of IgG1-3 subclass autoantibodies may activate the classical pathway (38). However, this conflicts with evidence from experimental BP, in which the classical pathway predominates. Recently, the IgG4 subclass has been reported to have inhibitory potential in experimental BP (41).

Based on these clinical pieces of evidence, we can conclude that complements may be involved in the pathogenesis of BP, but they are not all that is involved.

5-2) Experimental evidence of non-complement relevance

Hemidesmosomes are multiprotein complexes with dynamic structures (42–45). COL17 is distributed on the ventral plasma membrane of hemidesmosomes bound to keratin filaments as well as on the lateral-apical plasma membrane in pools that are not bound to keratin filaments (46,47). The distribution of desmoglein 3 is dynamically changed by a calcium-shift from low to high

concentration (48). This cell culture system may be employed to demonstrate that hemidesmosome are supposed to actively respond to calcium-shift and BP-IgG. Keratinocytes are stimulated with BP-IgG during calcium switching, and the binding of BP-IgG causes the internalization of COL17 from the lateral-apical plasma membrane in keratinocytes (47,49,50). In addition, COL17 is internalized by means of a macropinocytic pathway via PKC activation (Figure 2a) (51–53). Finally, BP-IgG depletes keratinocytes of COL17 after internalization (COL17-depletion assay, Figure 2b, left) (54). When normal human keratinocytes are treated with BP-IgG, COL17 is significantly decreased. In this process, stimulation with BP-IgG depletes cells of COL17 but not α6 and β4 integrins, which are hemidesmosomal components. After keratinocytes are stimulated with BP-IgG, the adhesion of cells to the culture plate is also significantly reduced in concert with COL17 depletion. During COL17 depletion, non-hemidesmosomal COL17, but not hemidesmosomal multiprotein complexes of COL17, preferentially internalizes into cells under BP-IgG stimulation (52). The amount of COL17 in the TritonX-100-soluble fraction (i.e., non-hemidesmosomal COL17) was found to be decreased at 6 hours after BP-IgG stimulation, and that in the TritonX-100-insoluble fraction (i.e., hemidesmosomal COL17) was found to be decreased at 48 hours. COL17 depletion was observed in the setting of stimulation with anti-NC16A BP-IgG, but not with non-NC16A BP-IgG (51,55) (Figure 2b, right).

In the passive transfer model mentioned previously, BP-IgG induces skin detachment by mechanical stress. Complement deposits are observed at the dermal-epidermal junction in these mice. The experimental BP model seems to be dependent on the classical pathway of the complement system. This means that the Fc portion is essential for the disease induction by pathogenic antibodies. Nevertheless, the passive transfer of F(ab')2 into mice induces skin fragility without complement deposits (56). This result indicates that the experimental BP model is not completely complement-dependent. Moreover, we generated C3-deficient COL17-humanized mice (57). The transfer of BP-IgG into C3 deficient COL17-humanized mice induced dermal-epidermal separation. Direct immunofluorescence studies revealed IgG deposits but not C3 deposits at the dermal-epidermal

junction in the diseased mice (Figure 2c). In addition, human IgG4 mAb against human NC16A was produced to confirm the complement-dependency in experimental BP. The human IgG4 subclass has restricted Fc receptor-activating ability and does not activate the classical pathway of the complement system via C1q (58,59). The transfer of IgG4 mAb against human NC16A clearly induced dermal-epidermal separation *in vivo*, and IgG4 mAb had much lower binding activity to C1q than that of IgG1 mAb in *in vitro* assays (57).

6. The hypothesis of non-complement blistering mechanisms

Here, we hypothesize about the blistering mechanisms involved after the formation of immune complexes. Initially, autoantibodies bind to COL17, which is distributed on the plasma membrane of basal cells, and COL17 immune complexes are internalized, causing the depletion of COL17 from the cell surface. This depletion of COL17 from the lateral-apical plasma membrane generates a significant shortage of COL17-supplementation during hemidesmosomal remodeling. Finally, it results in the formation of COL17-deficient hemidesmosomes. This depletion of COL17 from hemidesmosomes may weaken the adhesional strength in the patient's skin. This is quite a similar condition to congenital junctional epidermolysis bullosa, generalized intermediate type, which is caused by mutations in *COL17A1* (60) and in which the separation occurs intra-lamina lucida largely without complement deposits.

Taken these lines of evidence together, we can conclude that BP-IgG may be sufficient to induce skin fragility without complement activation. Complements are required to induce inflammation and exacerbation of the disease. Thus, it can be noted that complements and inflammatory cells are involved in the disease severity.

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8. Author contribution

HI and HU wrote the manuscript and designed the figures. HI and HU revised the manuscript and approved the final versions.

9. Conflicts of interest

The authors have no conflicts of interest to declare.

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Figure legends

Figure 1

Inflammatory BP (left) and non-inflammatory BP (right)

(a) The inflammatory BP phenotype shows tense blisters with widespread erythema. (b) The non-inflammatory BP phenotype shows clinically tense blisters without erythema on the skin. Closer picture shows tens blister without erythema.

Figure 2 (a) NHEKs are stimulated with either normal IgG or BP-IgG for 2 hours. Human IgG are detected by FITC-conjugated anti-human IgG. (b) COL17-depletion assay (left). NHEKs are stimulated with BP-IgG (NC16A-BP and non-NC16A-BP), and then total cell lysates are subjected to SDS–PAGE in 6% polyacrylamide gel. Blotting is performed by anti-COL17 and anti-β tubulin as an internal control. (c) Passive transfer model using COL17-humanized mice and $C3^{-/-}$/COL17-humanized mice. BP-IgG are injected i.p. into neonatal mice, and skin detachment is evaluated by gentle rubbing 48 hours after injection. Deposits of IgG and complement are detected by direct immunofluorescence.

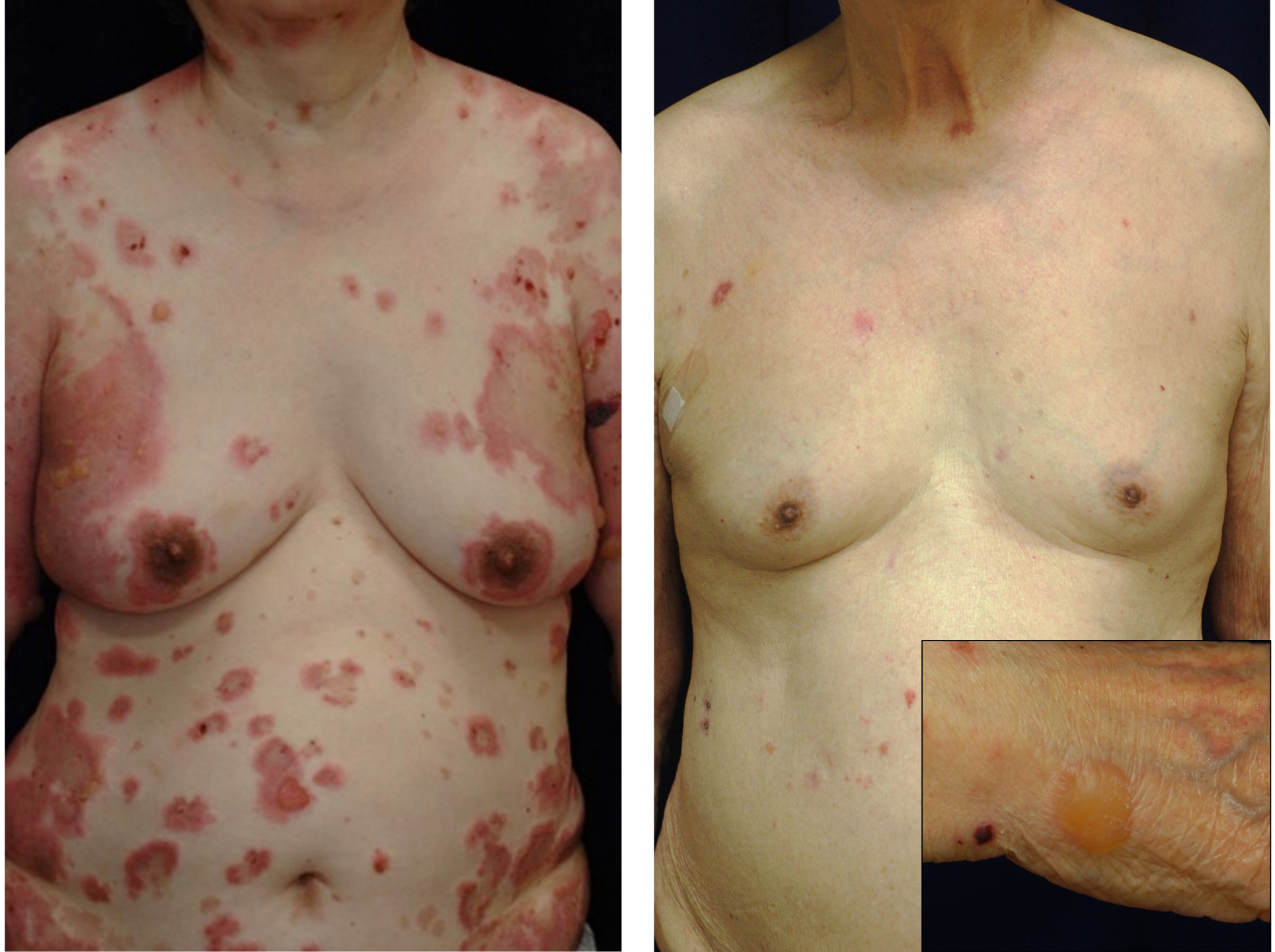

Fig. 1

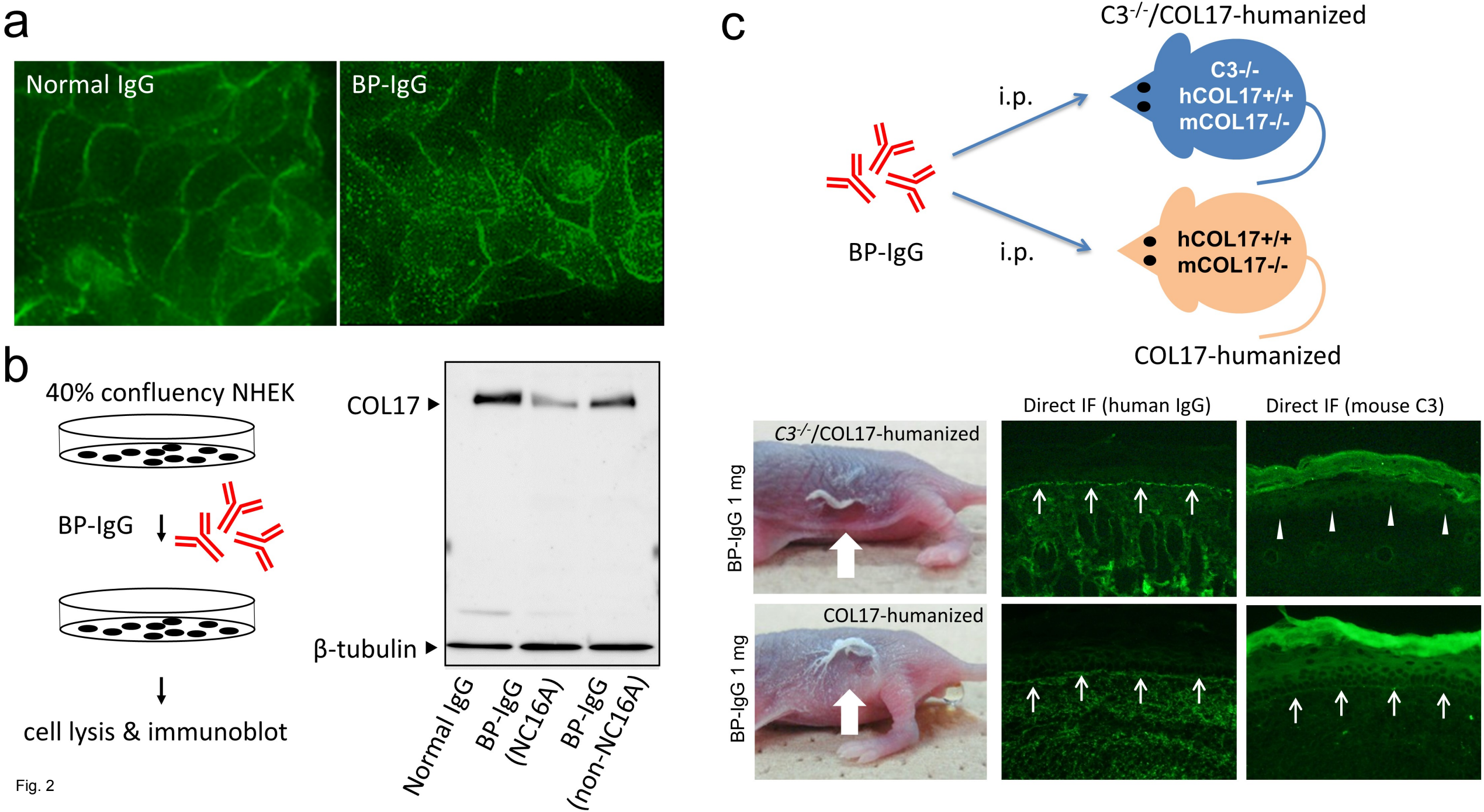
a
Normal IgG
BP-IgG
b
40% confluency NHEK
BP-IgG
cell lysis & immunoblot
COL17
β-tubulin
Normal IgG
BP-IgG (NC16A)
BP-IgG (non-NC16A)
c
C3-/-/COL17-humanized
i.p.
C3-/- hCOL17+/+ mCOL17-/-
BP-IgG
i.p.
hCOL17+/+ mCOL17-/-
COL17-humanized
Direct IF (human IgG)
Direct IF (mouse C3)
C3-/-/COL17-humanized
BP-IgG 1 mg
COL17-humanized
BP-IgG 1 mg

Fig. 2