Epitope-Dependent Pathogenicity of Antibodies Targeting a Major Bullous Pemphigoid Autoantigen Collagen XVII/BP180

Mayumi Wada[1,2], Wataru Nishie[1], Hideyuki Ujiie[1], Kentaro Izumi[1], Hiroaki Iwata[1], Ken Natsuga[1], Hideki Nakamura[1], Yoshimasa Kitagawa[2] and Hiroshi Shimizu[1]

In bullous pemphigoid, the common autoimmune blistering disorder, IgG autoantibodies target various epitopes on hemidesmosomal transmembrane collagen XVII (COL17)/BP180. Antibodies (Abs) targeting the extracellular noncollagenous 16th A domain of COL17 may be pathogenic; however, the pathogenic roles of Abs targeting non-noncollagenous 16th A regions are poorly understood. In this study using a pathogenic and a nonpathogenic monoclonal antibody (mAb) targeting the noncollagenous 16th A domain (mAb TS39-3) and the C-terminus domain (mAb C17-C1), respectively, we show that endocytosis of immune complexes after binding of Abs to cell surface COL17 is a key phenomenon that induces skin fragility. Passive transfer of IgG1 mouse mAb TS39-3 but not mAb C17-C1 induces dermal-epidermal separation in neonatal human COL17-expressing transgenic mice. Interestingly, mAb C17-C1 strongly binds with the dermal-epidermal junction of the recipient mice skin, suggesting that binding of Abs with COL17 is insufficient to induce skin fragility. In cultured normal human epidermal keratinocytes treated with these mAbs, mAb TS39-3 but not mAb C17-C1 internalizes immune complexes after binding with cell surface COL17 via macropinocytosis, resulting in reduced COL17 expression. This study shows that pathogenicity of Abs targeting COL17 is epitope dependent, which is associated with macropinocytosis-mediated endocytosis of immune complexes and finally results in the depletion of COL17 expression in basal keratinocytes.

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INTRODUCTION

Bullous pemphigoid (BP), a common autoimmune blistering disorder, mainly affects the elderly (Schmidt and Zillikens, 2013). The disorder clinically presents with tense blister formation and pruritic erythema on the entire body (della Torre et al., 2012). Immunologically, BP patients have IgG autoantibodies (autoAbs) that preferentially target two major hemidesmosomal components, collagen XVII (COL17)/BP180 and BP230, both of which are present at the dermal-epidermal junction (DEJ) of basal keratinocytes (Diaz et al., 1990; Labib et al., 1986; Nishie, 2014; Stanley et al., 1981, 1988). Regarding the autoimmunity of these molecules, COL17 is mainly involved in blister formation (Giudice et al., 1993).

[1]*Department of Dermatology, Hokkaido University, Graduate School of Medicine, Sapporo, Japan; and* [2]*Department of Oral Diagnosis and Medicine, Hokkaido University Graduate School of Dental Medicine, Sapporo, Japan*

Correspondence: Wataru Nishie, Department of Dermatology, Hokkaido University Graduate School of Medicine, N15W7, Kita-ku, Sapporo 060-8638, Japan. E-mail: nishie@med.hokudai.ac.jp

Abbreviations: Ab, antibody; autoAb, autoantibody; BP, bullous pemphigoid; COL17, collagen XVII; DEJ, dermal-epidermal junction; Dsg3, desmoglein 3; IC, immune complex; mAb, monoclonal antibody; NC16A, noncollagenous 16th A; NHEKs, normal human epidermal keratinocytes

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COL17 is a type II-oriented, 1,497-amino acid transmembrane protein whose N-terminus is in the cytoplasm and whose C-terminus is in the extracellular matrix (Franzke et al., 2003; Nishie, 2014). COL17 has 15 collagenous domains in the extracellular matrix, and epitopes cluster tightly within the juxtamembranous noncollagenous 16th A (NC16A) domain (Franzke et al., 2003; Schmidt and Zillikens, 2013). More than 90% of the IgG autoAbs from BP patients (BP-IgG) react with this region (Di Zenzo et al., 2008; Kobayashi et al., 2002; Nakatani, 1998; Zillikens et al., 1997), and such reactivity correlates closely with BP disease severity and activity (Di Zenzo et al., 2008; Kobayashi et al., 2002). The pathogenicity of IgG autoAbs with respect to the NC16A domain has been proven by the passive transfer of BP-IgG into neonatal human COL17-expressing transgenic (COL17-humanized) mice, which leads to skin fragility (Liu et al., 2008; Nishie et al., 2007). In addition to targeting the NC16A domain, BP autoAbs may target other parts of COL17 (Di Zenzo et al., 2004, 2008; Hofmann et al., 2002; Perriard et al., 1999; Schmidt and Zillikens, 2013), including the C-terminal region, which is mainly targeted by IgG autoAbs from patients with mucous membrane pemphigoid, another autoimmune blistering disorder involving COL17 (Hayakawa et al., 2014; Schmidt et al., 2001). Although previous studies have tried to address the pathogenic roles of Abs that target the non-NC16A regions of COL17, including the C-terminal region (Natsuga et al., 2012), the pathogenicity of such antibodies (Abs) has not been fully elucidated.

At the DEJ of perilesional skin in BP patients, activated complements are commonly observed (Nishie, 2014). In previous studies using experimental BP models in which skin fragility is induced by the passive transfer of rabbit anti-mouse COL17 Abs into neonatal wild-type mice (Liu et al., 1993), complement activation has been proven to be essential for blister formation (Liu et al., 1995; Nishie, 2014). Complement activation is thought to initiate mast cell degranulation, neutrophil recruitment and proteinase release, the last of which causes COL17 to degrade (Chen et al., 2001; Leighty et al., 2007; Liu et al., 1995, 1997, 2000, 2005, 2008; Nelson et al., 2006). In contrast, the passive transfer of polyclonal rabbit Abs directing the NC16A domain of human COL17 into COL17-humanized mice induces susceptibility to mechanical blistering without complement activation (Natsuga et al., 2012; Ujiie et al., 2014). In addition, treatment with BP-IgG in cultured normal human epidermal keratinocytes (NHEKs) reduces COL17 expression (Iwata et al., 2009; Messingham et al., 2011), a reduction that is associated with the internalization of immune complexes (ICs) via macropinocytosis (Hiroyasu et al., 2013). These observations indicate that blister formation in BP may be, at least in part, induced by autoAbs targeting the NC16A domain of COL17 in a complement-independent pathomechanism (Mihai et al., 2007; Nelson et al., 2006; Nishie, 2014). However, the role of complement-independent pathways in the pathogenesis of BP in vivo is not yet established. Moreover, the pathogenic roles of Abs targeting the non-NC16A region of COL17 are poorly understood.

In this study, we show that the pathogenicity of Abs targeting COL17 can be epitope dependent. The passive transfer of IgG1 mouse monoclonal antibodies (mAbs) targeting the NC16A domain of human COL17 but not targeting its C-terminal region induces dermal-epidermal separation in neonatal COL17-humanized mice. Interestingly, nonpathogenic mAbs targeting the C-terminal region strongly bind with the DEJ of the recipient mice skin, suggesting that binding of Abs with COL17 is not sufficient, in itself, to induce skin fragility. The pathogenicity of mAbs targeting the NC16A domain of COL17 has been proven to be associated with a cellular response: macropinocytosis-mediated endocytosis of ICs after binding of Abs with cell surface COL17 in basal keratinocytes.

RESULTS

IgG1 mAb C17-C1 targets the C-terminal domain of human COL17 spanning amino acid Gly^{1316} to Gly^{1342}

Western blotting shows that mAb C17-C1 reacts with 180-kDa full-length protein and with COL3 but not with NC6, COL11, or COL15 recombinant proteins, suggesting that the mAb targets the end-most C-terminal region of COL17 (Figure 1a and b). The mAb C17-C1 reacts with C-22K but not with C-16K (Figure 1a and c) or GST-NC4 (data not shown), suggesting that an epitope of mAb C17-C1 is present within amino acid Gly^{1316} to Gly^{1340} (Figure 1a). Indirect immunofluorescence studies showed that mAb C17-C1 (1 mg/ml) reacts with the DEJ of the normal human skin until a dilution of 1:51,200 (Figure 2a and Supplementary Figure S1 online), which is comparable with that of the same concentration of mAb TS39-3 (Ujiie et al., 2014). Loss of reactivity of these mAbs to COL17-lacking skin of non-Herlitz junctional epidermolysis bullosa patient argues for the specific reactivity of the mAb to human COL17 (Figure 2b). Indirect immunofluorescence using 1 M NaCl-split human skin shows that mAb C17-C1 strongly binds to the epidermal side (Figure 2c). Indirect immunofluorescence using subclass-specific secondary Abs reveals that the mAb C17-C1 is IgG1 (Figure 2d).

Passive transfer of mAb C17-C1 fails to induce skin fragility in neonatal COL17-humanized mice

To address the pathogenicity of mAbs, we performed intraperitoneal injection of mAbs C17-C1 (50 μg), mAb TS39-3 (25 μg), or normal mouse IgG1 as a control (50 μg) into neonatal COL17-humanized mice. Forty-eight hours after injection, mAb TS39-3 targeting the NC16A domain of COL17 induced skin detachment ($n = 3$). In contrast, mAb C17-C1 targeting the C-terminal region failed to induce skin fragility ($n = 4$) (Figure 3). Direct immunofluorescence showed deposits of IgG but not C3 at the DEJ, similar to deposits for mice skin injected with mAb C17-C1 and mAb TS39-3 (Figure 3). Regarding the tongue, no epithelial detachment was observed clinically or histologically, although direct immunofluorescence showed IgG deposits in both mAb C17-C1 and mAb TS39-3-injected mice tongue (Figure 3). Although weak signals of C3 were observed at the DEJ of the tongue mucosa, similar signals were also observed in control mice that had received normal mouse IgG1 (Figure 3). Hematoxylin and eosin staining revealed dermal-epidermal detachment only in the mice that had received mAb TS39-3 (Figure 3).

mAb C17-C1 does not reduce COL17 expression in NHEKs

To address why TS39-3 induces skin fragility but mAb C17-C1 does not, even though both mAbs strongly react with the DEJ of mice skin, we treated cultured NHEKs with these mAbs. The experiments revealed that mAb TS39-3 reduced the expression of COL17 in treated NHEKs, whereas no effects were observed in NHEKs treated with mAb C17-C1 or control mouse IgG1 (Figure 4). The reduction of COL17 by mAb TS39-3 was detectable starting 3 hours after treatment (Figure 4).

Endocytosis of immune complexes is not induced in NHEKs treated with mAb C17-C1

COL17 expression was reduced by treatment with mAb TS39-3 but not with mAb C17-C1 in NHEKs. To address why mAb C17-C1 failed to reduce COL17 expression after binding with cell surface COL17, NHEKs cultured to 60% confluence were incubated with Alexa Fluor 488-conjugated mAbs. Within 5 minutes after incubation, mAb TS39-3 and mAb C17-C1 both started to bind to COL17 on the cell surface of NHEKs. Approximately 20–30 minutes after treatment, ICs of COL17 and mAb TS39-3 were internalized into the cytoplasm from the cell surface. In sharp contrast, internalization of ICs was not observed in the NHEKs treated with mAb C17-C1 (Figure 5a). Numerous internalized ICs were observed in the cytoplasm within 1 to 2 hours only in cells treated with mAb TS39-3 and not in cells treated with mAb C17-C1 (Figure 5a). These findings