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*J Immunol* 2009; 183:4088-4093; Prepublished online 28 August 2009;
doi: 10.4049/jimmunol.0800389
http://www.jimmunol.org/content/183/6/4088
A Novel Humanized Neonatal Autoimmune Blistering Skin Disease Model Induced by Maternally Transferred Antibodies

Wataru Nishie,²* Daisuke Sawamura,* Ken Natsuga,* Satoru Shinkuma,* Maki Goto,* Akihiko Shibaki,* Hideyuki Ujiie,* Edit Olasz,‡ Kim B. Yancey,§ and Hiroshi Shimizu*

All mammalian neonates receive maternal Abs for protection against pathogenic organisms in the postnatal environment. However, neonates can experience serious adverse reactions if the Abs transferred from the mother recognize self-molecules as auto-Ags. For example, neonatal lupus, which is clinically characterized by skin eruptions and fatal congenital heart block, is induced by auto-Abs against Ro(SSA), Ro(SSB), or U1 ribonuclear protein transferred from mothers affected with Sjögren syndrome or systemic lupus erythematosus (3, 4). In addition, maternally transferred auto-Abs against acetylcholine receptors can induce the characteristic features of myasthenia gravis in human neonates (5). This suggests that mothers, in experimental animal models, might be able to induce autoimmunity in their offspring.

One possible approach to using maternal Abs to produce disease models for autoimmune diseases is the use of gene-targeted mice (6). Immunizing Ag-knockout female mice with a targeted Ag can induce Abs against the antigenic molecule. Mating these immunized females with wild-type males could mimic autoimmune diseases in the neonates expressing antigenic peptides transcribed by paternal genes in the presence of circulating maternally transferred Ag-specific IgG (6). However, this approach has not achieved practical application, probably because gene-targeted mice often die soon after birth, especially when the targeted genes encode functionally important proteins (7–11). Consequently, another method that does not use lethal gene-deleted maternal mice is desirable. The difference in immune systems between humans and mice is another important problem underlying most of the current experimental autoimmune disease models. In fact, the auto-Ags in existing autoimmune disease models have been the mouse’s own proteins, which are expected to differ from those in the human autoimmune disease condition (12–14). Therefore, autoimmune disease models with human auto-Ag expression would be ideal.

In this study, we tried to produce a novel neonatal autoimmune disease model induced by passage of maternal IgG. We aimed at the most common and life-threatening autoimmune blistering skin disease, bullous pemphigoid (BP). In BP, circulating IgG auto-Abs are directed against type XVII collagen (COL17, formerly known as BP180 or BPAG2) in the skin (15, 16). COL17 is a type-II-oriented, 180KD hemidesmosomal transmembrane protein that anchors basal keratinocytes to the underlying epidermal basement membrane. The pathogenic epitope in COL17 is tightly clustered within the noncollagenous (NC) 16A stretch of its ectodomain (17, 18). Interestingly, due to significant differences between humans and rodents in the amino acid sequence in the NC16A region, mice that have received human IgG from BP patients fail to show any clinical, histological, or immunological findings consistent with BP (13, 14). We recently generated Col17al gene-targeted (mCol17/−/−) mice as well as COL17-humanized mice by introducing human COL17A1 cDNA (hCOL17+/+/+) transgene driven under keratin 14 promoter into mCol17/−/− mice (12, 19). Importantly, the mCol17/−/− mice were too fragile to mate with male mice, but reproductive ability was restored in COL17-humanized (mCol17−/−, hCOL17+/+/+) mice (12). In this study, we used these genetically manipulated COL17-humanized mice to produce a novel neonatal autoimmune disease model induced by passage of maternal IgG.

Abbreviations used in this paper: BP: bullous pemphigoid; COL17: type XVII collagen; NC: noncollagenous; Tg: transgenic; IIF: indirect immunofluorescence; DIF: direct immunofluorescence; GST: glutathione S-transferase.
Generation of neonatal BP mice

Two weeks after skin grafting, the immunized and the control mCol17+/− female mice were crossed with 6- to 8-wk-old COL17-humanized (mCol17−/−, hCOL17+/+) male mice (12). Half of their newborns (mCol17−/−, hCOL17+/+) were predicted to express only human COL17 and not mouse COL17 in the skin and the other half of the newborns (mCol17−/−, hCOL17−/−) to express both mouse and human COL17 in the skin (Fig. 1).

Evaluation of serum anti-humanCOL17 IgG in the immunized mother mice and their neonates

Sera from immunized mCol17+/− females (before immunization and 1 to 4 wk after immunization) and their neonates (at birth and 1 to 4 wk after birth, respectively) were sampled, followed by ELISA and indirect immunofluorescence (IFF) to evaluate the circulating mouse IgG Abs directed against human COL17 (12, 19). The ELISA index value against the human COL17 NC16A domain peptide was measured using BP180 ELISA kit (MBL) with minor modifications. In brief, this kit is designed to detect human IgG against human COL17; therefore, HORP-conjugated goat polyclonal anti-mouse IgG (1/200,000 dilution, Jackson ImmunoResearch Laboratories) was used as a secondary Ab substitute for prepared HRP-conjugated anti-human IgG. The absorbance was measured at 450 nm by microtiter plate readers (Bio-Rad). For IFF studies, serum from the mice was serially diluted in PBS. Normal or 1 M NaCl split human skin samples were obtained from a healthy volunteer and incubated with the sera for 30 min at 37°C, following by staining with FITC-conjugated polyclonal goat anti-mouse IgG (1/100 dilution, Jackson ImmunoResearch Laboratories) as described previously (12, 19).

Immunopathological analysis of neonatal BP

For histological investigations, back skin of the mice was obtained at birth and 1 to 4 wk after birth, and processed for H&E staining and direct immunofluorescence (DIF) microscopy. For DIF study, FITC-conjugated goat polyclonal anti-mouse IgG (1/100 dilution, Jackson ImmunoResearch Laboratories), rat monoclonal anti-mouse IgG1, IgG2a, IgG2b (1/100 dilution, Bio-Rad). For IF studies, serum from the mice was serially diluted in PBS. Normal or 1 M NaCl split human skin samples were obtained from a healthy volunteer and incubated with the sera for 30 min at 37°C, following by staining with FITC-conjugated polyclonal goat anti-mouse IgG (1/100 dilution, Jackson ImmunoResearch Laboratories) as described previously (12, 19).

Passive transfer of maternal IgG with or without immunoadsorption against human COL17 NC16A protein into neonatal COL17-humanized (mCol17−/−, hCOL17+/+) mice

Total IgG was purified from pooled sera obtained from 5 immunized mCol17+/− females (10 wks after skin grafting) using HiTrap Protein G HP (GE Healthcare) according to the manufacturer’s instructions. Recombinant human COL17 NC16A (amino acid: 490–566) protein was generated as a gulutathione S-transferase (GST) fusion protein as previously described (12, 19), and 6 mg of the purified protein was coupled with 1 ml of GSTrap FF (GE Healthcare). Half of the purified total IgG was coupled with the human COL17 NC16A-GST protein in the column to eliminate mouse COL17 IgG when grafted with human COL17 transgenic (Tg) mouse skin (19). We first immunized mCol17+/− mother mice with skin grafts obtained from human COL17 Tg mice, and then we mated the immunized mCol17+/− mother mice with COL17-humanized (mCol17−/−, hCOL17+) male mice. Neonatal COL17-humanized (mCol17−/−, hCOL17++) mice retained skin stability against mechanical friction (12); similarly, neonatal COL17-humanized mice heterozygously carrying human COL17 cDNA transgene (mCol17−/−, hCOL17−/+ ) showed none of the skin abnormalities seen in mCol17−/− mice, although it was possible to detach the epidermis by moderate mechanical friction (our unpublished data). We hypothesized that immunized mCol17−/− mother mice would produce circulating anti-human COL17 IgG that would be transferred into their neonates including those whose skin expressed only human and not mouse COL17 (mCol17−/−, hCOL17−/+ ), resulting in natural blistering that replicates human BP disease (Fig. 1).

Immunization of the heterozygote mCol17−/− female mice

Four- to 6-wk-old heterozygous null mCol17−/− females (F, mouse was 129/SvEv × C57BL/6 background, back-crossed with C57BL/6 over 10 generations) were immunized against human COL17 as previously described (19), with minor modifications. In brief, 1 × 1 cm of back skin obtained from gender-matched, syngeneic human COL17 CDNA Tg mice was grafted onto the back of the recipient mCol17−/− female mice. As a control, back skin obtained from wild-type C57BL/6 was grafted onto recipient mCol17−/− female mice (n = 5). The grafted skin was sutured, and bandages were removed 7 days after skin grafting.

Materials and Methods

Gross summary of strategy

We selected a breeding pair consisting of heterozygote Col17a1-deficient (mCol17+/−) female mice and COL17-humanized (mCol17−/−, hCOL17+/+) male mice (Fig. 1). Theoretically, half of the pups from this pair should express only human COL17 in the skin while the other half should express both mouse and human COL17 (Fig. 1). Wild-type mice can develop quite high titers of circulating anti-human COL17 IgG when grafted with human COL17 transgenic (Tg) mouse skin (19). We first immunized mCol17−/− mother mice with skin grafts obtained from human COL17 Tg mouse skin, and then we mated the immunized mCol17+/− mother mice with COL17-humanized (mCol17−/−, hCOL17++) male mice. Neonatal COL17-humanized (mCol17−/−, hCOL17++) mice retained skin stability against mechanical friction (12); similarly, neonatal COL17-humanized mice heterozygously carrying human COL17 cDNA transgene (mCol17−/−, hCOL17−/+ ) showed none of the skin abnormalities seen in mCol17−/− mice, although it was possible to detach the epidermis by moderate mechanical friction (our unpublished data). We hypothesized that immunized mCol17−/− mother mice would produce circulating anti-human COL17 IgG that would be transferred into their neonates including those whose skin expressed only human and not mouse COL17 (mCol17−/−, hCOL17−/+ ), resulting in natural blistering that replicates human BP disease (Fig. 1).
transferred IgG against human COL17 showed severe skin fragility and the epidermis easily detached with minor mechanical friction (Nikolsky phenomenon, Fig. 3a). Notably some mice developed spontaneous small blisters and pustules (Fig. 3, a and c). These skin lesions gradually disappeared in the first week after birth, leaving small, round, crusty lesions similar to those seen in BP patients (Fig. 3b). Although epidermal detachment could be induced by moderate (but not minor) friction in humanized mice heterozygously carrying the human COL17 cDNA transgene (mCol17+/−, hCOL17+/−), the skin fragility observed in neonatal BP mice was obviously more severe, and minor friction easily produced extensive epidermal detachment. In contrast, none of the other neonates (n = 13) that expressed both human and mouse COL17 in skin (mCol17+/−, hCOL17+/−) demonstrated any distinct skin abnormalities following exposure to maternal IgG, including spontaneous blister formation or Nikolsky phenomenon (data not shown).

**Neonatal BP mice showed histological and immunological features identical with those seen in patients with BP**

This system is characterized by complete humanization of the Ag in neonatal mice with ensuing inflammatory cascades that are completely mouse-derived. Therefore, the system is able to induce specific IgG-Ag reactions and lead to skin inflammation consistent with BP in humans. Notably, histological examinations demonstrated distinctive subepidermal blister formation with numerous inflammatory cell infiltrates predominately consisting of neutrophils (Fig. 3c). DIF studies of BP model mice skin revealed deposition of mouse IgG and of mouse complement (C3) in epidermal basement membrane until the third and the first to second weeks after birth, respectively (Fig. 3d). Subclass analysis of in vivo deposition of IgG showed that IgG1 and IgG2c predominated at the dermal-epidermal junction (Fig. 3e). This characteristic of IgG subclass deposition was the same for immunized mCol17+/− females as for their neonates, as shown by IIF on the normal human skin as a substrate (data not shown).

**IgG Abs to the NC16A domain of human COL17 play a major role in inducing blistering skin disease**

We previously demonstrated that IgG Abs to the NC16A domain of human COL17 play a major role in inducing blistering disease; this was demonstrated by passive-transfer experiments using IgG autoAbs from BP patients in neonatal COL17-humanized (mCol17+/−, hCOL17+/−) mice (12). To assess and characterize the role of IgG Abs in immunized mCol17+/− female mice in the current model, we performed passive-transfer experiment using IgG Abs obtained from immunized female mice with or without immunoabsorption against human COL17 NC16A protein (n = 2, respectively). By IIF study using normal human skin as a substrate, both immune-adsorbed and without immunoabsorption purified IgG reacted to the dermal-epidermal junction until 5120 and 20480 times dilution respectively (data not shown). The passive-transfer experiment showed that purified total IgG without immunoabsorption with human COL17 NC16A protein resulted in skin fragility associated with IgG deposition along the dermal-epidermal junction (Fig. 4, a and b). In contrast, treatment of IgG with COL17 NC16A protein resulted in no blistering phenotype, although slight deposition of IgG could be observed along the dermal-epidermal junction (Fig. 4, a and b). These results clearly suggest that IgG Abs to NC16A domain of human COL17 played the major role to induce blistering skin disease in vivo.
Maternal IgG to human COL17 was transmissible into neonatal circulation via milk even after birth

Interestingly, some of the mice showed elevated IgG Ab titers to human COL17 by ELISA around 1 to 2 wk after birth (Fig. 2d). It has been reported that mouse IgG can be transferred from milk via neonatal FcR expressed in gut, which is different from humans (2, 21, 22). To investigate this possibility, COL17-humanized (mCol17+/−, hCOL17+/+) neonatal mice delivered from unrelated pairs were moved soon after birth to a lactating preimmunized mCol17+/−/H11002 mouse (ELISA index value to human COL17 of 1.33). As a result, it was found that, at 1 wk of breast-feeding from the immunized female mouse, serum IgG in these pups to human COL17 was markedly elevated (ELISA index titer: 0.73 ± 0.20, n = 4), and mouse IgG reacted positively to the dermal-epidermal junction in the skin until 1/1280 dilution (Fig. 5a). In contrast, IgG Abs to human COL17 of the pups breast-fed from the nonimmunized female mouse were not increased (ELISA index titer: 0.02 ± 0.03, n = 4), and mouse IgG did not react to the normal human skin (Fig. 5a). These results clearly indicate that maternally anti-human COL17 IgG Abs were transmitted from milk.

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FIGURE 3. a, Neonatal BP mice showed severe skin fragility, with the epidermis easily detaching from mechanical friction (Nikolsky phenomenon, arrow). Spontaneous small blisters and pustules were scattered over the entire body (arrowheads). b, Small, round, crusted lesion developed around the arm in 4-day-old neonatal BP mice. c, Histological finding of blistering lesion on the tail. Subepidermal blister formation associated with numerous infiltrations of neutrophils (arrowheads) was observed. d, DIF study revealed in vivo skin deposition of mouse IgG (yellow arrows) until 3 wk after birth, and activated mouse C3 (red arrows) was detected within 1 to 2 wk after birth. Note the Abs to mouse C3 strongly cross-reacted to the corneal layer of the epithelium (star). e, In vivo deposition of IgG1 and IgG2c was detected at the dermal-epidermal junction of a neonatal BP mouse soon after birth (arrows).

FIGURE 4. IgG Abs to the NC16A domain of human COL17 play a major role in inducing blistering skin disease. a, Neonatal COL17-humanized (mCol17−/−, hCOL17+/+) mice that received IgG Abs without immunoadsorption with human COL17 NC16A protein from immunized mCol17−/− females resulted in skin fragility (positive Nikolsky sign, arrows), whereas no epidermal detachment could be observed in mice that received immunoadsorbed IgG (50 μl of 2.1 μg/μl IgG Abs, respectively). b, In vivo deposition of mouse IgG was more intense in the skin obtained from mice that received IgG Abs without adsorption with human COL17 NC16A protein (arrows) compared with that being adsorbed with the protein (arrowheads).
IgG2c does fix mouse complement (26); therefore, activation of neonates. Mouse IgG1 Abs do not fix complement (20), whereas induced both IgG1 and IgG2c autoAbs which were transferred to complement. Finally, immunized heterozygous subsequent inflammation cascade, including activation of the mouse complement system does not work as efficiently during the neonatal procedure. Second, the pathogenic IgG remains in circulation longer in the new model than in conventional models that use injected procedure. Using maternally transferred pathogenic Abs and introducing human Ags in neonates, we succeeded in inducing autoimmune disease model in neonates whose Ags are functionally important. However, this system does not truly represent autoimmunity in human patients, because Abs to human COL17 in diseased neonates are transferred Abs. In addition, for immunized heterozygote Col17-deficient (mCol17+/−) female mice, human COL17 is not an autoAg but alloantigen therefore, pathogenic Abs to human COL17 in this system is not strictly an autoAbs. Nevertheless, maternally transferred Abs in genetically transformed Ag-humanized neonates will be useful in the study of autoimmune diseases as a novel method for generating diseases in neonates. **Acknowledgments**

We thank Ai Hayakawa, Yuka Hayakawa, and Akari Nagasaki for their technical assistance, and Dr. James R. McMillan and Dr. Heather Ann Long for their language editing and proofreading.

**Disclosures**

The authors have no financial conflict of interest. **References**


