Vancomycin Mediates IgA Autoreactivity in Drug-Induced Linear IgA Bullous Dermatosis

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Vancomycin (VCM) is known to induce linear IgA bullous dermatosis (LAD). However, in contrast to conventional LAD, in which circulating IgA autoantibodies against basement membrane proteins are commonly detected, patient sera from VCM-induced LAD yields negative results in indirect immunofluorescence microscopy, and the targeted autoantigen remains undetermined. By using sera from a typical patient with VCM-induced LAD, we identified that co-incubation of sera with VCM resulted in linear IgA deposition at the basement membrane zone by indirect immunofluorescence. Patient sera reacted with the dermal side of 1 mol/L NaCl-split skin and with the recombinant noncollagenous (i.e., NC1) domain of type VII collagen by both immunoblot and ELISA in the presence of VCM. The investigation of an additional 13 patients with VCM-induced LAD showed that 10 out of the 14 sera (71.4%) reacted with the NC1 domain of type VII collagen by ELISA when spiked with VCM, whereas only 4 (28.6%) tested positive without it. The enhancement of reactivity to NC1 by VCM, as determined by optical density via ELISA, was observed in 10 out of the 14 sera (71.4%). These findings indicate that type VII collagen is a target autoantigen in VCM-induced LAD and that VCM mediates IgA autoreactivity against type VII collagen, providing an insight into mechanisms involved in drug-induced autoimmune disease.


INTRODUCTION

Linear IgA bullous dermatosis (LAD) is an autoimmune blistering disease characterized by subepidermal blisters with linear deposits of IgA along the basement membrane zone (BMZ) on direct immunofluorescence (DIF). Clinical manifestations of LAD vary, from patients with vesicular lesions, which often appear in a herpetiform arrangement on erythemas, to those with tense blisters indistinguishable from mucocutaneous lesions, and spontaneous resolution of the disease characterized by subepidermal blisters with linear deposits of IgA along the basement membrane zone (BMZ) on direct immunofluorescence (DIF). Clinical manifestations of LAD vary, from patients with vesicular lesions, which often appear in a herpetiform arrangement on erythemas, to those with tense blisters indistinguishable from mucocutaneous lesions, and spontaneous resolution of the disease.
lesions after drug withdrawal. Only a few cases of vLAD have been reported with supportive evidence for the cause-effect relationship, such as positive lymphocyte transformation tests and patch tests (Fortuna et al., 2012). However, such results need to be carefully interpreted, because they reflect immune responses limited to T cells, whereas IgA-mediated humoral immunity is the relevant mechanism involved in LAD.

Several basement membrane proteins, such as type XVII collagen (BP180) and its fragments, including LAD-1 and LABD97 (antigen with molecular weight of 97 kDa), lamin-332, and type VII collagen (COL7), have been reported as the target antigens of IgA autoantibodies in conventional LAD (Ishiko et al., 1998; Schumann et al., 2000; Tsuchisaka et al., 2015; Zenke et al., 2014). Autoantigens in conventional LAD can be determined by studying the autoantibodies that circulate in peripheral blood in approximately 70% of patients (Zone et al., 1990). Paradoxically, although DIF from vLAD patients shows linear IgA deposition, indirect immunofluorescence (IIF) results are usually negative, suggesting that circulating antibodies in LAD patients are either low in level or incapable of binding BMZ antigens in their native form. As such, targeted autoantigens, such as type XVII collagen and LAD285 (antigens with molecular weight of 285 kDa), have been identified in only a few cases (Palmer et al., 2001; Tashima et al., 2014). Thus, the lack of BMZ reactivity of patient sera and better definitions of targeted antigens are major issues that need to be explored in vLAD.

In this study, we discovered that co-incubation of VCM renders IgA from most vLAD patients reactive to COL7, as confirmed by IIF against normal human skin, ELISA, and immunoblotting analysis using recombinant COL7. Thus, COL7 is the major targeted autoantigen in vLAD, and the observation that the antigen-antibody reaction required VCM provides insight into the mechanisms involved in drug-induced autoimmune diseases.

RESULTS

Serum IgA in a typical case of vLAD acquires reactivity to the BMZ in the presence of VCM

A 73-year-old woman with mixed connective tissue disease undergoing treatment with 5 mg/day of prednisolone presented with a chronic ulcer on her left lower limb that was
associated with subcutaneous calcinosis due to her underlying disease. Routine culture showed superinfection with methicillin-resistant \textit{Staphylococcus aureus}, and the patient underwent treatment with intravenous VCM at a dose of 1.5 g twice daily. Six days into VCM treatment, she developed rapidly expanding annular, edematous erythemas with peripherally arranged tense blisters and erosions on the abdomen, buttocks, and groin (Figure 1a–c). Blisters and erosions were also seen on the oral mucosa and vulva (Figure 1d). Skin biopsy showed a subepidermal blister that was filled with numerous neutrophils (Figure 1e). DIF showed linear IgA deposition along the BMZ, and neither IgG nor complement component 3 deposition was observed (Figure 1f). Lymphocyte transformation test for VCM yielded a stimulation index of 3,980% (positive/C0 180%). These findings collectively led to the diagnosis of vLAD, and VCM was immediately discontinued. The woman was further treated with systemic administration of prednisolone (1 mg/kg/day) and dapsone (75 mg/day), which led to complete resolution of skin lesions. No relapse was seen after the discontinuation of dapsone and reduction of corticosteroid to a baseline dose.

Recent studies on the mechanisms of T cell-mediated drug hypersensitivities proposed that causative drugs are capable of directly binding to class I MHC or to the T-cell receptors (TCRs), thereby resulting in altered specificity of the preexisting T-cell repertoire (Chung et al., 2016; Illing et al., 2012; Watkins and Pichler, 2013). We therefore hypothesized that VCM might bind to vLAD patients’ IgA, modifying it to BMZ antigens. Indeed, VCM spiked into patient serum at a concentration of 2 μg/ml rendered serum reactivity against BMZ via IIF, showing IgA binding along the BMZ, a pattern identical to that obtained via DIF (Figure 2b). Serial dilution of VCM resulted in a loss of reactivity against BMZ at concentrations below 0.004 μg/ml, indicating that IgA reactivity is dependent on VCM concentration (Figure 2c–e). VCM levels peak as high as 25–50 μg/ml at 1–2 hours after administration, and trough levels are commonly maintained at 10–20 μg/ml. Thus, the concentration of VCM used for IIF reflects the in vivo condition.

### COL7 as the target antigen of IgA autoantibodies in this case of vLAD

To further characterize the target of IgA autoantibodies in vLAD, we performed IIF with 1 mol/L NaCl-split skin (salt-split skin) as the substrate. A serum with 0.5 μg/ml of VCM showed IgA binding to the dermal side of the split skin (Figure 3a), indicating that the autoantibodies reacted with an autoantigen below the lamina lucida, such as COL7, which form the anchoring fibrils. We then performed immunoblot analysis using the recombinant noncollagenous (i.e., NC1) domain of COL7 to determine whether the serum bound to COL7 with or without 0.5 μg/ml of VCM. The serum showed a positive band of 150 kDa corresponding to the NC1 domain of COL7 only in the presence of VCM (Figure 3b). Furthermore, we examined serum reactivity against COL7 containing the mixture of the recombinant NC1 and NC2 domains by ELISA. VCM enhanced ELISA reactivity (optical density at 280 nm) of IgA against COL7 from 0.031 to 0.125 (4-fold increase) at 0.004 μg/ml concentration (Table 1, Case 1). The binding activity of circulating IgA to BMZ via IIF was lost in sera that were obtained after vLAD remission (Table 1, patient 1). However, ELISA detected the enhancement of IgA reactivity against COL7 mediated by VCM, from 0.031 to 0.125 (4-fold increase) at 0.5 μg/ml concentration (Table 1, Case 2).
VCM. Taken together, IgA antibodies in this case of vLAD were significantly enhanced by VCM. To extend these findings, we investigated sera from an additional 13 patients with vLAD (Table 1). With the initial patient included, 12 out of the 14 patients were men, and their ages ranged from 38 to 84 (average = 67) years. The onset of vLAD since the administration of VCM ranged from 4 to 16 days. The diagnosis of vLAD in all patients was based on skin manifestations (erythema, blister, and erosion on the body), subepidermal blister formation with neutrophilic infiltration by histology, and linear IgA deposition along the BMZ by DIF.

**COL7 is a major autoimmune target in vLAD**

To extend these findings, we investigated sera from an additional 13 patients with vLAD (Table 1). With the initial patient included, 12 out of the 14 patients were men, and their ages ranged from 38 to 84 (average = 67) years. The onset of vLAD since the administration of VCM ranged from 4 to 16 days. The diagnosis of vLAD in all patients was based on skin manifestations (erythema, blister, and erosion on the body), subepidermal blister formation with neutrophilic infiltration by histology, and linear IgA deposition along the BMZ by DIF.

Most patients were treated with systemic corticosteroid and/or dapsone, but in two patients vLAD resolved without any treatment other than the discontinuation of VCM. The disease severities varied from mild to severe, and the duration of treatment ranged from one to several weeks.

Although IIF using normal human skin as a substrate did not show IgA binding to the skin in any of the samples tested, the presence of VCM, 3 (21.4%) out of 14 serum samples displayed IgA reactivity to BMZ with spiked VCM concentrations of 0.5 μg/ml. IIF with sodium-split skin showed that all three sera reacted with the dermal side of the skin in the presence of VCM (Table 1, patients 1, 7, and 12). We then tested the reactivity of vLAD sera to COL7 by ELISA. Only 4 of the 14 sera (28.6%) showed positive reactivity (optical density at 280 nm > 0.06) in the absence of VCM. In contrast, the addition of VCM resulted in positivity in 10 of the 14 sera (71.4%) (Table 1). Furthermore, the ratios of optical density values in the absence or presence of VCM increased in 10 of the 14 sera (71.4%) tested (P = 0.009) (Table 1 and Figure 4). The higher rate of positivity by ELISA than by IIF likely reflects differences in the sensitivities of the two assays.

It is possible that VCM binds to and modifies the antigenicity of COL7, rather than binding to IgA antibodies. To address this, we pretreated frozen human skin sections with 0.5 μg/ml of VCM or vehicle and then used these sections as substrates for IIF. However, incubation of VCM with the substrate did not result in IgA reactivity to BMZ with any of the three positive sera (data not shown). We also pretreated COL7-coated ELISA plates with 0.5 μg/ml of VCM, but IgA reactivity to COL7 was not observed (see Supplementary Table S1 online). These results suggest that VCM mediates IgA autoreactivity by modifying IgA rather than the antigen, COL7.

Several drugs other than VCM have been reported to cause drug-induced LAD, such as sulfamethoxazole and trimethoprim, which belongs to the same class of glycopeptide antibiotics that share similar structures to VCM. To determine whether such drugs were also capable of mediating IgA reactivity to COL7, we co-incubated these drugs with vLAD sera and performed IIF and ELISA. However, IgA binding to the BMZ was not observed upon the addition of any of these drugs in IIF and ELISA, indicating that the acquired or enhanced reactivity of IgA to COL7 was mediated specifically by VCM in the tested cases (see Supplementary Table S2 online).

In aggregate, these findings identify COL7 as a major autoimmune target in vLAD and show that IgA antibody autoreactivity to COL7 was mediated by VCM.

**DISCUSSION**

Drug-induced autoimmunity is a well-recognized condition manifesting in a wide range of autoimmune diseases, but evidence for mechanisms by which drugs induce autoimmunity is scarce. Certain drugs such as hydralazine, minocycline, procainamide, and others have been associated with drug-induced lupus erythematosus. These drugs induce the production of autoantibodies, such as antinuclear antibodies, anti-single strand DNA antibody, and anti-histone...
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age in Years</th>
<th>Sex</th>
<th>Onset From VCM Administration in Days</th>
<th>Past History</th>
<th>Treatment</th>
<th>Duration of Treatment</th>
<th>Remission Period</th>
<th>IIF (SS) VCM⁻</th>
<th>Type VII Collagen ELISA (OD Value)</th>
<th>Immunoblot with NC1 of Type VII Collagen</th>
<th>Ratios VCM⁻/VCM⁺</th>
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<tr>
<td>1</td>
<td>73</td>
<td>F</td>
<td>6</td>
<td>MCTD</td>
<td>PSL 1 mg/kg/day, dapsone 75mg</td>
<td>5 weeks</td>
<td>4 weeks</td>
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<td>+ (dermis) 0.243 0.636</td>
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<td>2</td>
<td>81</td>
<td>M</td>
<td>12</td>
<td>Unknown</td>
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<td>Unknown</td>
<td>-</td>
<td>-</td>
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<td>3</td>
<td>76</td>
<td>M</td>
<td>12</td>
<td>ASO</td>
<td>Dapsone</td>
<td>Unknown</td>
<td>Unknown</td>
<td>-</td>
<td>-</td>
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<td>4</td>
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<td>M</td>
<td>5</td>
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<td>6 days</td>
<td>-</td>
<td>-</td>
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<td>5</td>
<td>82</td>
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<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
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<td>-</td>
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<td>6</td>
<td>83</td>
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<td>13</td>
<td>DM</td>
<td>MINO 200 mg/day</td>
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<td>Several days</td>
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<td>-</td>
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<td>-</td>
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<td>M</td>
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<td>BC, after artificial valve replacement</td>
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<tr>
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<td>73</td>
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<td>Bronchial asthma</td>
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<td>None</td>
<td>Several days</td>
<td>-</td>
<td>-</td>
<td>0.023 0.932</td>
<td>41.39</td>
</tr>
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</table>

Positive ELISA results are underlined. Ratios of VCM⁺/VCM⁻ more than 2 are underlined and in boldface.

Abbreviations: ASO, arteriosclerosis obliterans; BC, breast cancer; CHF, chronic heart failure; DM, diabetes mellitus; F, female; HD, hemodialysis; IIF (SS), indirect immunofluorescence with salt-split skin; IVIG, intravenous immunoglobulin; M, male; MCTD, mixed connective-tissue disease; MINO, minocycline; mPSL, methylprednisolone; PSL, prednisolone; RF, renal failure; VCM, vancomycin; vLAD, vancomycin-induced linear IgA bullous dermatosis.
In this study, we identified that VCM either mediates or enhances vLAD patient IgA reactivity against COL7. The hypothesis that vLAD patient IgA autoreactivity might be mediated by VCM was prompted by mechanistic studies on drug hypersensitivity. A series of studies on sulfamethoxazole hypersensitivity showed that direct high-affinity binding of the drug to the complementarity-determining region 2β domain of the TCR containing variable domain Vβ20-1 could result in an allosteric effect, which may directly enhance reactivity of TCR to HLAs that present endogenous peptides (von Greyerz et al., 1999; Watkins and Pichler, 2013).

The observation that a drug could directly bind to a complementarity-determining region domain of the TCR, together with the fact that immunoglobulins are similar in structure to TCRs (including complementarity-determining region domains), led us to hypothesize that VCM might have direct effects on vLAD patient IgA that results in modified antigen specificity (Ilbing et al., 2013; Pichler et al., 2015). This hypothesis was also inspired by the fact that onset of vLAD is usually rapid, well before adaptive immunity against VCM could take place, and by the paradoxical observation that despite IgA deposition in the BMZ, IIF with vLAD sera usually resulted in negative findings. Indeed, co-incubation of patient sera, but not co-incubation of the substrate (healthy volunteer skin), resulted in the deposition of IgA in the BMZ, showing that VCM modifies IgA and not the antigen itself. This approach enabled us to identify the targeted autoantigen in most of the studied vLAD patients as COL7, as shown by immunoblot and ELISA analyses.

After the recovery of patient 1 from vLAD, the reactivity of serum IgA to COL7 was dramatically decreased and was only minimally detectable via ELISA, even when co-incubated with VCM. This suggested that VCM does not broadly target IgA and that it may have some specificity, perhaps to a certain domain of the variable region, similar to the specific binding of sulfamethoxazole to TCR (Watkins and Pichler, 2013). Such an IgA repertoire may have transiently expanded in response to the acute methicillin-resistant Staphylococcus aureus infection that occurred before VCM administration, the contraction of which after infection may explain why not all sera from vLAD patients in this study showed positive results in COL7 ELISA and IIF. It remains possible that BMZ molecules other than COL7 were targeted in these cases. Because of the transient nature of vLAD, the amount of COL7-reactive sera was limited. Together with limitations in the analytical techniques that we currently have access to, the possibility of non-COL7 autoantigens will need to be further determined in future studies.

The precise mechanism by which VCM mediates or enhances IgA reactivity to COL7 remains to be determined. This may require the isolation of peripheral B cells that express IgA that VCM binds to or the generation of phage display single-chain antibodies (Payne et al., 2005) to obtain sufficient amounts of antibodies to allow determination of the specific domain(s) VCM binds to. We postulate that the mechanisms that lead to autoimmunity in conventional LAD, given the continuous autoantibody production, likely reflects a bona fide loss of tolerance and therefore is distinct from that of vLAD, in which autoimmunity is dependent on continuous presence of VCM with no true loss of tolerance against COL7.

In conclusion, we have shown that IgA from vLAD patients acquires reactivity to COL7 in the presence of VCM. This observation represents a mechanism by which a drug induces autoimmunity. Further examination on precise mechanisms may provide insight into the pathogenesis involved not only in autoimmunity but also in drug hypersensitivity.
MATERIALS AND METHODS

The study protocols were reviewed and approved by the institutional review board of the Keio University School of Medicine and were conducted following the principles established by the Declaration of Helsinki. Written informed consent was obtained from the patients.

Subjects and sera

We examined sera from a patient with vLAD treated in the Keio University Department of Dermatology and 13 patients reported by other institutes. All patients showed positive IgA reactivity along the BMZ of the skin by DIF in the active phase of the disease.

Indirect immunofluorescence

Each serum sample was subjected to IIF analysis using cryosections of normal human skin and 1 mol/L NaCl-split skin. The cryosection slides were washed in phosphate buffered saline three times for 5 minutes, and the cryosections were incubated with serial dilutions of tested sera for 1 hour at room temperature or overnight at 4°C. Deposition of human IgA antibodies was detected with a 1:100 dilution of FITC-labeled polyclonal rabbit anti-human IgA (DAKO, Copenhagen, Denmark).

COL7 ELISA

The reactivity of IgA in sera against COL7 was measured by ELISA kit (Medical and Biological Laboratories Company, Nagoya, Japan) according to the manufacturer’s protocol, instead using horseradish peroxidase-conjugated anti-human IgA antibody as the secondary antibody (DAKO). We incubated serum samples with ELISA plates at 4°C for 24 hours to increase the sensitivity. A cutoff value (optical density at 280 nm) was defined as the average value plus three standard deviations of control sera from 20 healthy individuals.

Immunoblot

Recombinant NC1 domain of COL7 was produced by mammalian expression system, and recombinant NC2 domain was produced by bacterial expression system, as previously reported (Saleh et al., 2011). The NC1 and NC2 proteins were dissolved in SDS sample buffer, fractionated by SDS-PAGE, transferred to a nitrocellulose membrane, and detected using horseradish peroxidase-conjugated anti-human IgA or IgG antibody at a dilution of 1:100. After washing the strips three times in phosphate buffered saline washing buffer, proteins were visualized using Western Lightning Chemiluminescence Reagent (PerkinElmer LAS, Shelton, CT) and autoradiography.

Statistics

All parameters were compared by Fisher exact test, as appropriate, with P less than 0.05 considered significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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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.2017.12.035.

REFERENCES


