The Role of Antibody to Human $\beta_4$ Integrin in Conjunctival Basement Membrane Separation: Possible In Vitro Model for Ocular Cicatricial Pemphigoid

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PURPOSE. To demonstrate the specific binding of autoantibodies present in the sera of patients with ocular cicatricial pemphigoid (OCP) to human $\beta_4$ integrin present in the normal human conjunctiva (NHC) and to study the role of OCP autoantibodies and antibody to human $\beta_4$ integrin in the pathogenesis of subepithelial lesion formation in OCP.

METHODS. Indirect immunofluorescence assay and in vitro organ culture method using NHC were used. Sera and IgG fractions from 10 patients with OCP; immunoaffinity-purified OCP autoantibody; antibodies to human $\beta_4$, $\alpha_6$, and $\alpha_5$ integrins; and sera from patients with pemphigus vulgaris, bullous pemphigoid (BP), and chronic atopic and chronic ocular rosacea cicatrizant conjunctivitis; and normal human serum (NHS) were used.

RESULTS. Nine of 10 OCP sera or IgG fractions, immunoaffinity-purified OCP autoantibody, antibodies to human $\beta_4$ and $\alpha_6$ integrins, and sera from patients with BP showed homogenous, smooth linear binding along the basement membrane zone (BMZ) of the NHC. NHS, antibodies to other integrins, and sera from patients with chronic cicatrizant conjunctivitis from other causes showed no such binding. When NHC was first absorbed with OCP sera and then reacted with anti-$\beta_4$ antibodies or vice versa, the intensity of the BMZ binding was dramatically reduced or completely eliminated, indicating that there were autoantibodies in OCP sera specific for the $\beta_4$ integrin. BMZ separation developed 48 to 72 hours after addition of total OCP sera, IgG fractions from OCP sera, immunoaffinity-purified autoantibodies from sera of patients with OCP, or anti-$\beta_4$ antibodies to the NHS cultures, but not after addition of normal control sera, sera from patients with chronic cicatrizant conjunctivitis from causes other than OCP, or sera from patients with OCP in clinical remission.

CONCLUSION. Circulating anti-$\beta_4$ integrin antibody may have an important role in the pathogenesis of OCP. (Invest Ophthalmol Vis Sci. 1999;40:2283–2290)

Ocular cicatricial pemphigoid (OCP) is an uncommon, chronic, subepithelial, scarring systemic autoimmune disease that mainly affects the conjunctiva and other mucous membranes derived from stratified squamous epithelium and (occasionally) the skin.1–5 The pathologic process of chronic conjunctivitis and the accompanying progressive subepithelial fibrosis, results in trichiasis, distichiasis, conjunctival keratinization, xerosis, and, eventually, blindness secondary to corneal damage as a consequence of these changes in the ocular environment.

OCP has some pathophysiological features in common with other bullous diseases, such as linear IgA bullous disease, epidermolysis bullosa acquisita, bullous pemphigoid (BP), and cicatricial pemphigoid (CP). The process of identifying anti-BMZ antibodies in the sera of many patients with CP is difficult and variable. It is likely that a variety of anti-BMZ autoantibodies with different specificities recognize different target molecules present in the BMZ. This may account in part for the wide spectrum of clinical manifestations and clinical courses of these bullous diseases. For example, BP sera bind to 180-kDa hemidesmosome (BPAg2) and to 230-kDa desmoplakin (BPAg1) proteins. On direct immunofluorescence examination of perilesional conjunctiva of patients with OCP, deposition of immunoglobulin and/or complement along the BMZ, although pathognomonic, can often be difficult to demonstrate or inconclusive.

Circulating anti-BMZ antibodies have been observed in the sera of patients with OCP.5–8 The autoantigen against which these anti-BMZ antibodies are produced has not been well defined or fully characterized. In our earlier studies we demonstrated that sera of patients with OCP bind to a 205-kDa protein in human skin, conjunctiva, and tumor cell lysates in an immunoblot.6,7 In our further studies we observed that the

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antibody to a 205-kDa protein in OCP sera recognizes the cytoplasmic domain of human β4 integrin. These observations collectively suggest that sera of patients with OCP specifically contain antibodies to human β4 integrin. A subset of patients with OCP with clinical features similar to CP has been characterized by the presence of autoantibodies against epiligrin, which is now identified as the α3-subunit of laminin 5,10 a ligand for α6β4 integrin.11 Binding of oral pemphigoid autoantibodies to the α6 integrin subunit has been reported.12

The purpose of this study was to investigate whether the anti-BMZ autoantibodies in sera of patients with OCP specifically bind to the human β4 integrin present in human conjunctiva. We describe an in vitro model that may facilitate understanding of some of the events that produce vesicular lesions in conjunctival epithelium in OCP.

**Materials and Methods**

**Serum Samples**

The method section confirms adherence to the Declaration of Helsinki. Sera used in this study were obtained from 10 patients with OCP in the acute and active phase of the disease before the institution of therapy. All patients were evaluated in the Immunology Service at the Massachusetts Eye and Ear Infirmary between July 1 and December 31, 1997. The diagnosis of OCP was confirmed in each patient by direct and indirect immunofluorescence (IIF) analysis of bulbar conjunctival biopsy specimens, as previously described.1 Four patients had involvement of other mucous membranes or the skin. Oral mucosa was involved in three patients. In one patient there was oral and skin involvement. All the sera studied bound to a 205-kDa protein (β4 integrin) on an immunoblot assay using human conjunctiva as substrate.6,13 Additionally, when tested on salt-split skin, the anti-BMZ autoantibody at 1:20 dilution bound to the epidermal side of the split.14

Sera were obtained from 10 normal healthy people, from 10 patients with established BP (cutaneous only, without oral involvement) who had anti-BMZ antibody of 1:1280 or greater titer, when tested by IIF with monkey esophagus as substrate;15 from 3 patients with pemphigus vulgaris (PV); from 2 patients with chronic active cicatrizing conjunctivitis secondary to rosacea or atopy; and from 3 patients with OCP in remission, monoclonal (10 μg/ml) and polyclonal antibodies to human β4 integrin (1:100 dilution), and monoclonal antibodies to α6, α5, and β1 integrins (10 μg/ml). Phosphate buffered saline (PBS) and normal human serum (NHS) and sera from patients with chronic active cicatrizing conjunctivitis secondary to rosacea or atopy served as negative controls. Positive controls for this assay included sera from patients with PV and BP. Four-micrometer sections of the conjunctiva were first incubated at room temperature with normal goat serum 1:20 dilution for 25 minutes and then washed three times with PBS. Primary antibodies were diluted in PBS-bovine serum albumin 1% in 1:10 to 1:320 dilutions and incubated at room temperature for 1 hour. The sections were then washed three times in PBS, incubated at room temperature with the appropriate fluorescein isothiocyanate (FITC)–conjugated secondary antibody for 30 minutes, and viewed under a fluorescence microscope. Incubation with the primary antibody was omitted in PBS control experiments.

**Absorption and Blocking Studies**

Blocking studies were performed as follows: Multiple sections of NHC were preabsorbed for 60 minutes repeatedly with the following six reagents: sera of patients with OCP that contained high titer (1:1000 by immunoblot) of anti-BMZ antibodies, the IgG fraction from OCP sera, sera from patients with BP containing antibodies to BPAG1 and BPAG2, monoclonal antibodies to human α6 integrin, monoclonal antibody to human β1 integrin, and normal human serum. These sections were then washed four times with PBS and incubated with monoclonal or polyclonal antibodies to the human β4 integrin. The sections were then stained with the appropriate FITC-conjugated secondary antibodies and viewed under a fluorescence microscope to assess binding at the BMZ. In the reverse experiment, multiple sections of NHC were repeatedly preabsorbed with monoclonal and polyclonal antibodies to human β4 integrin, washed four times with PBS, and reacted with the six reagents listed earlier in the paragraph. The sections were washed and then stained with the appropriate FITC-conjugated secondary antibodies and viewed under the fluorescence microscope for BMZ staining.

**In Vitro Culture of Normal Human Conjunctiva**

The in vitro model used to evaluate the effects of specific reagents in the pathogenesis of OCP was based on earlier brane. The band of choice was cut horizontally, incubated with sera from patients with OCP, and washed. Autoantibodies were eluted and recovered with retention of biologic activity and antigen specificity. This eluted antibody had been characterized to be anti-β4 antibody by an immunoblot assay.16 Mouse monoclonal anti-CJ1014 (human β4 integrin) antibodies, monoclonal antibodies to human α6 integrin (Ancell Corporation, Bayport, MN), and antibodies to human α5 and β1 integrin (ImmunoTech, Westbrook, ME) were purchased. Rabbit polyclonal antibody to human β4 integrin was kindly provided by Martin Hemler (Dana Farber Cancer Institute, Boston, MA).

**Indirect Immunofluorescence**

Standard IIF was performed using normal human bulbar conjunctiva as substrate to test for the presence of anti-BMZ antibodies, as described earlier.1,2,17 Test reagents included sera from 10 patients with active OCP, IgG fraction, immunofinity-purified OCP autoantibodies, sera from 3 patients with OCP in remission, monoclonal (10 μg/ml) and polyclonal antibodies to human β4 integrin (1:100 dilution), and monoclonal antibodies to α6, α5, and β1 integrins (10 μg/ml). Phosphate buffered saline (PBS) and normal human serum (NHS) and sera from patients with chronic active cicatrizing conjunctivitis secondary to rosacea or atopy served as negative controls. Positive controls for this assay included sera from patients with PV and BP. Four-micrometer sections of the conjunctiva were first incubated at room temperature with normal goat serum 1:20 dilution for 25 minutes and then washed three times with PBS. Primary antibodies were diluted in PBS-bovine serum albumin 1% in 1:10 to 1:320 dilutions and incubated at room temperature for 1 hour. The sections were then washed three times in PBS, incubated at room temperature with the appropriate fluorescein isothiocyanate (FITC)–conjugated secondary antibody for 30 minutes, and viewed under a fluorescence microscope. Incubation with the primary antibody was omitted in PBS control experiments.

**Conjunctiva Collection**

Normal human bulbar conjunctiva was obtained during cataract surgery from six patients after informed consent and ethical permission was obtained for their use in a human organ culture model. Conjunctiva was used immediately after surgical excision or snap frozen in liquid nitrogen, embedded in compound (Optimum Cutting Temperature; Tissue-Tek, Miles Scientific, Elkhart, IN), and stored at −70°C until sectioning.

**Reagents**

Immunofinity-purified OCP autoantibodies were eluted from nitrocellulose blots, as previously described.6,16 Briefly, protein samples were transferred from gels to nitrocellulose mem-
models that used skin in organ culture to study the effects of PV autoantibody on intercellular adhesion.\textsuperscript{18,19} Pieces of NHC (2–3 mm\textsuperscript{2}) obtained during cataract surgery were floated in 2 ml complete RPMI-1640 medium in a 12-well tissue culture plate immediately after surgical excision. Three different concentrations of the test reagent were studied. Total serum (10%, 20%, and 30% by volume); purified IgG fractions; 100, 200, and 300 \(\mu\)g of immunoaffinity-purified autoantibodies from sera of patients with OCP; and 10, 20, and 50 \(\mu\)g of anti-\(\beta_1\) and anti-\(\alpha_6\) integrin antibodies were added to the wells and incubated at 37°C with 5% CO\(_2\) for 12 to 72 hours. These optimal volumes and concentrations were determined by pilot experiments using three concentrations to titrate the ability of test reagents to produce BMZ separation. In these pilot experiments, the cultures were terminated at 12, 24, 48, 72, and 96 hours. The conjunctival sections were processed for routine histology using hematoxylin and eosin stain and were examined for subepidermal blister formation. There was some degree of variability in the BMZ separation, but the best results were seen in cultures that were incubated for at least 48 hours. NHS and normal human IgG and sera of three patients with PV and three patients with BP with high titers of antibodies to BPAg1 and BPAg2, two patients with chronic active cicatrizating conjunctivitis secondary to rosacea or atopy were used as controls. Sera from three patients with disease in prolonged clinical remission who had received no therapy for 3 years were also evaluated. Sera of the patients with OCP in clinical remission did not show the presence of anti-BMZ binding using immunoblot assays.\textsuperscript{13} After incubation, the tissue samples were examined by routine histology, using hematoxylin and eosin staining procedures. The experiment was repeated five times using the same panel of reagents.

**Blocking of Blister Formation in an In Vitro Model**

OCP sera, immunoaffinity-purified OCP antibody, antibody to human \(\beta_4\) integrin, and sera from patients with PV were absorbed with lysates from \(\beta_4\) integrin-expressing cell lines (UM-SC-22), as previously described.\textsuperscript{6,7} Thereafter, they were tested in the immunoblot assay for the presence of antibody to human \(\beta_4\) integrin.\textsuperscript{6} These antibodies were then used in the in vitro organ culture system for BMZ separation, as described earlier.

Sera from patients with active PV and with high titers of PV autoantibody were absorbed with UM-SC-22 cell lines. Absorbed and nonabsorbed PV sera were used in the in vitro NHC culture model.

**RESULTS**

**Indirect Immunofluorescence**

NHC sections incubated with sera from patients with OCP showed homogenous, continuous linear BMZ staining indistinguishable from that observed during direct immunofluorescence analysis of conjunctiva from 9 of 10 patients with OCP who had active disease (Fig. 1). Strikingly similar observations were made when NHC was incubated with purified IgG fractions of sera from patients with active OCP, with immunoaffinity-purified OCP antibodies, with monoclonal and polyclonal antibodies to human \(\beta_4\) integrin, and with monoclonal antibodies to the \(\alpha_6\) integrin. The monoclonal antibody to \(\beta_4\) integrin exhibited more intense staining than did the polyclonal antibody to \(\beta_4\) integrin and OCP sera. Sera of patients with active PV showed binding to intercellular spaces between the epithelial cells of the NHC (data not shown). Sera of patients with active BP that contained antibodies to BPAg1 and BPAg2, determined by immunoblot analysis, showed smooth linear binding to BMZ of NHC sections (data not shown). The marked reduction in background was caused by prior absorption of the NHC sections with goat serum for the IIF assay and NHS for the blocking experiments. No binding was observed in sections of NHC incubated with normal human serum and PBS (Fig. 1) and normal human IgG (Fig. 2D). No staining was observed with monoclonal antibodies to the \(\alpha_5\) or \(\beta_1\) integrins or sera of patients with chronic active cicatrizign conjunctivitis other than OCP.

**Blocking Experiments with Indirect Immunofluorescence**

When the NHC sections were first repeatedly preabsorbed with OCP sera and then incubated with antibodies to \(\beta_4\) integrin, the intensity of the binding of the anti-\(\beta_4\) antibody to the BMZ was reduced, indicating that OCP sera contained antibodies binding to the same epitope as anti-\(\beta_4\) integrin antibodies. When the NHC sections were first repeatedly pre-absorbed with monoclonal or polyclonal antibodies to human \(\beta_4\) integrin and then incubated with sera or IgG from patients with OCP, BMZ binding was eliminated (Fig. 2F). When the NHC sections were preabsorbed with NHS or antibody to \(\beta_1\) integrin and then reacted with OCP sera or antibody to human \(\beta_4\) integrin, the BMZ binding was unaffected (Fig. 2). This suggests that the continuous linear BMZ staining produced by sera from patients with active OCP competed with monoclonal and polyclonal antibodies to \(\beta_4\) integrin. The absorption of NHS with either OCP sera or with antibodies to the \(\beta_4\) integrin reduced but did not eliminate the binding of antibodies to the \(\alpha_6\) integrin in the BMZ. Absorption of NHS sections with high-titer BP sera reduced but did not eliminate the binding of antibodies to \(\alpha_6\) and \(\beta_4\) integrins. Binding of the PV sera to the intercellular cement substance of the NHC was unaffected by earlier absorption of NHS sections with both OCP sera and antibodies to \(\beta_4\) integrin (data not shown).

**In Vitro Culture of Normal Human Conjunctiva**

No morphologic changes were observed before 24 hours of culture. NHC cultured for 48 to 72 hours showed subepithelial separation from the BMZ in the presence of OCP sera. IgG fractions from sera of patients with OCP, immunoaffinity-purified antibody from OCP sera, but not in the presence of NHS (Fig. 3), sera from patients with chronic active cicatrizating conjunctivitis secondary to rosacea or atopy, or sera from patients with OCP in clinical remission. The effect of treatment with OCP sera, immunoaffinity-purified OCP antibody, and antibody to \(\beta_4\) integrin on induction of BMZ separation is summarized in Table 1. The data presented are the mean results of five experiments, using the same panel of reagents. In treatment with 10% volume of OCP sera, only 5% to 10% of explants showed BMZ separation after 48 to 72 hours of incubation. The maximum explants showing BMZ separation (60%–65%) were observed in treatment with a 30% volume of OCP sera during 48 to 72 hours of incubation. In treatment with 100, 200, and 300 \(\mu\)g of immunoaffinity-purified OCP antibody,
no BMZ separation was observed during a 12- to 24-hour incubation period. However, BMZ separation was observed in 45% to 50% of explants when treated with 300 μg immunoaffinity-purified OCP antibody and incubated for 48 to 72 hours. In treatment with 10, 20, and 50 μg of the monoclonal antibody to human β4 integrin, no BMZ separation was observed in the explants incubated for 12 to 24 hours. Using 20 μg, only 15% to 20% of explants showed BMZ separation during 48 to 72 hours' incubation. Forty percent to 50% of explants showed BMZ separation when treated with 50 μg of antibody to β4 integrin and incubated for 48 to 72 hours. Prominent tissue necrosis was observed in explants incubated for 96 hours. BMZ separation was observed in sections of organ culture of NHC incubated with antibodies to human β4 integrin (Fig. 4). The morphologic and histologic characteristics of the BMZ separation in the organ culture sections were indistinguishable from those observed in clinical OCP, except for the absence of infiltration of inflammatory cells in the submucosa. Such pathology was not observed in sections of NHC cultured with antibodies to α5, α6, and β1 human integrins or antibodies to BPAg1 or BPAg2. Sections of NHC cultures with serum from patients with acute PV showed typical acantholysis (Fig. 4). This positive control indicates the validity of the experimental design and technique. No BMZ separation was observed in the presence of NHS, normal human IgG, and sera of patients with OCP in prolonged clinical remission.

Blocking of Blister Formation in an In Vitro Model

OCP sera, immunoaffinity-purified OCP antibodies, and antibody to human β4 integrin absorbed with lysates of β4 integrin-expressing cell lines (UM-SC-22) did not produce blister formation in the in vitro model. These antibodies did not bind to the 205-kDa protein in an immunoblot assay using normal human conjunctiva and skin as substrate.

Sera from patients with PV demonstrated binding to 130-kDa protein in the same substrates. When sera from patients with PV were similarly absorbed with lysate of β4 integrin-expressing cell lines and used in the in vitro model, it resulted in production of acantholysis of conjunctival epithelial cells, identical with that produced by unabsorbed sera. No BMZ separation was seen in NHC cultures treated with absorbed or unabsorbed PV sera.
In this study, we focused on two specific issues in the pathogenesis of OCP. First is the observation that the autoantibody in OCP was targeted against human β4 integrin in the conjunctival BMZ. Second, an in vitro organ culture model provided histology fairly identical with or similar to that observed in the conjunctiva of patients with OCP early in the course of the disease. Interestingly, antibody to β4 integrin was specific in its action. Further evidence for the genic role of anti-β4 antibodies in OCP comes from the observation that such antibodies were not present in the sera of patients with OCP presented herein may be a distinct subset, because they had a high frequency of extraocular involvement and universal presence of immunoreactants on conjunctival BMZ. Therefore, it appears that the possibility of such patients having a higher concentration of circulating autoantibodies in their sera to different specificities was more likely to be detected in our assay system than from other patients with OCP.

Interestingly, several investigators have demonstrated that sera of patients with OCP contain antibodies to BPAG1 and BPAG2. However, our studies clearly indicated that the antibodies to BPAG1 and BPAG2 did not block the binding of the antibodies to conjunctival BMZ by OCP sera. The binding sites for BP antibodies and CP antibodies were on the different epitopes of BPAG2.20

Studies indicate that β4 and α6 form a heterodimeric molecule associated with hemidesmosomes.21 Yet, our blocking experiments demonstrated that, within the limitations of the IIF assay, OCP sera and anti-β4 antibodies did not block the binding of anti-α6 integrin antibody to conjunctival BMZ. This observation further supports the hypothesis that production of anti-β4 antibodies in patients with OCP is a specific, early, disease-related event and is not a consequence of damage or immune injury. Additional support for this notion of a pathogenic role of anti-β4 integrin antibodies in OCP comes from the observation that such antibodies were not present in the sera of patients with disease in prolonged clinical remission who were receiving no systemic therapy.

In our in vitro conjunctival organ culture model, antibodies to β4 integrin were capable of causing BMZ separation that was histologically similar to that produced by incubation of NHS with OCP sera, IgG from patients with OCP, and immunoaffinity-purified OCP autoantibodies. These microvesicles created in vitro had histology fairly identical with or similar to that observed in the conjunctiva of patients with OCP early in the course of the disease. Interestingly, antibody to α6 integrin did not produce any microvesicles, indicating that antibody to β4 integrin was specific in its action. Further evidence for the opportunity to study the mechanism of separation of epithelial cells from underlying subepithelial structures and in so doing to provide indirectly information on factors that contribute to the integrity of conjunctival BMZ.

Indirect immunofluorescence and blocking experiments using IIF assays clearly indicated that OCP sera, IgG fractions from OCP sera, and immunoaffinity-purified OCP autoantibodies from OCP sera bound to the target antigen identical. The IIF assay also showed that conjunctival BMZ contained α6, BPAG1, and BPAG2, along with other integrins and adhesion molecules, and that OCP sera did not cross-react with them. This experimental strategy was similar to that previously published in studying the process of acantholysis in PV. In those studies, sera from patients with PV bound to the intercellular cement substance (desmoglein III) of the skin in a pattern identical with that seen in vivo in patients with PV. The validity of our model is further enforced by the observation that when NHS was incubated with serum from patients with PV, the pemphigus autoantibody bound in a manner identical with that seen on the normal human epidermis or in vivo in patients with PV. Furthermore, BP sera bound to conjunctival BMZ in a smooth linear manner identical with that of the skin. Normal human sera and sera of patients with other cicatrizing ocular diseases did not bind to the conjunctiva in a similar pattern. Observations in this report may suggest that the subset of patients with OCP presented herein may be a distinct subset, because they had a high frequency of extraocular involvement and universal presence of immunoreactants on conjunctival BMZ. Therefore, it appears that the possibility of such patients having a higher concentration of circulating autoantibodies in their sera to different specificities was more likely to be detected in our assay system than from other patients with OCP.
specificity of antibody to human $\beta_4$ integrin–producing blister came from the blocking experiments. OCP sera, immunoaffinity-purified OCP antibodies, and antibody to human $\beta_4$ integrin absorbed with lysate of $\beta_4$ integrin–expressing cell lines did not produce lesions seen in incubation with unabsorbed antibodies. These experiments, therefore, within the limitation of the organ culture system, provide additional potent evidence that anti-$\beta_4$ integrin antibody may play an important role in the production of vesicles or bullae in the conjunctiva of patients with OCP.

The concept that this in vitro model is a reasonable mechanism to study events that may mimic the in vivo phenomenon in tissue dysadhesion is valuable. Evidence for such a hypothesis comes from the experiments in which typical acantholysis, which is a histologic hallmark of PV, was observed in conjunctiva incubated with PV sera. The results observed in the conjunctiva were identical with previously published observations when normal human skin was incubated with PV sera. Absorption of PV sera with $\beta_4$-expressing cell lines did not affect the ability of PV sera to produce acantholysis. The earlier in vitro model culture for the study of PV eventually provided valuable insights in understanding the molecular pathogenesis of PV.

In our conjunctival organ culture, we did not observe any vesicle formation when NHC was incubated with BP sera. Using normal human skin, Gammon et al. have advanced our understanding of the pathogenesis of BP, showing that BP sera alone is incapable of causing skin BMZ separation but requires the addition of complement and polymorphonuclear leukocytes for lesion production. The data on the relationship between the duration of incubation and concentration of antibodies indicate that these events require time for the biologic processes to evolve. The process appeared to be dose dependent, indicating that an appropriate concentration of antibody is required to bind to available antigen sites to accomplish the separation of the epithelium from the submucosa. Similarly, the process of blister formation was time dependent, because no separation was seen in 12 to 24 hours. Our organ culture model permits the evaluation of the specific roles of polymorphonuclear leukocytes, complement, cytokines, and other bi-

### Table 1. Relationship of Duration of Incubation and Concentration of Autoantibody in Subepithelial Blister Formation in an In Vitro Culture Model

<table>
<thead>
<tr>
<th>Incubation Time (h)</th>
<th>OCP SERA*</th>
<th>Immunoaffinity-Purified OCP Antibody*</th>
<th>Antibody to Human $\beta_4$ Integrin*</th>
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<td>5</td>
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Data are represented as percentage of sections showing presence of vesicles.

* Twenty histologic sections were prepared from each explant and examined for presence of vesicles.
† Concentration (vol/vol).
‡ Concentration per ml.
The authors thank Tong-zhen Zhao in the Hilles Laboratory of the Massachusetts Eye and Ear Infirmary for valuable help.

References