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The Autoantibodies to $\alpha_6\beta_4$ Integrin of Patients Affected by Ocular Cicatricial Pemphigoid Recognize Predominantly Epitopes Within the Large Cytoplasmic Domain of Human $\beta_4$\textsuperscript{1}

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This study was undertaken to characterize the antigenic determinants recognized by the autoantibodies of patients with ocular cicatricial pemphigoid (OCP). OCP is a subepithelial, blistering, autoimmune disease that mainly affects the conjunctiva and other mucous membranes. We previously demonstrated that a cDNA clone, isolated from a keratinocyte expression library by using immunoaffinity-purified OCP autoantibody, encoded the cytoplasmic domain of $\beta_4$ integrin subunit. Our subsequent studies showed that sera from all the OCP patients that were tested recognize the human $\beta_4$ integrin subunit. To identify the prevalent epitopes of the anti-$\beta_4$ autoantibodies of OCP, we have used cell lines transfected with vectors encoding a wild-type $\beta_4$ subunit, a tailless $\beta_4$ subunit, or a $\beta_4$ subunit lacking the extracellular domain. Nontransfected cell lines were used as controls. Lysates from these cell lines were analyzed with OCP sera, IgG fractions from OCP sera, and immunoaffinity-purified OCP autoantibodies. Abs to extracellular and cytoplasmic domains of human $\beta_4$ integrin were used as positive controls, whereas normal human sera and normal human IgG fractions were used as negative controls. The reactivity of OCP Abs was determined by using immunoblotting, immunoprecipitation, and FACS analysis. The results of this study indicate that OCP sera, OCP IgG fractions, and immunoaffinity-purified OCP autoantibodies react with the intracellular and not the extracellular domain of human $\beta_4$ integrin subunit. In vitro cell culture experiments demonstrated that OCP autoantibody binds to the cytoplasm of the cells. The relevance of these findings to the pathogenesis of OCP is discussed. \textit{The Journal of Immunology}, 2000, 165: 2824–2829.

Ocular cicatricial pemphigoid (OCP)\textsuperscript{4} is an autoimmune vesiculobullous disease that affects the conjunctiva, and less frequently, other squamous epithelia (1). If untreated or inappropriately treated, OCP can result in blindness. OCP is a form of mucous membrane pemphigoid or cicatricial pemphigoid (CP), a heterogeneous disease with a wide spectrum of clinical phenotypes, depending upon the predominant area affected and the number of loci involved (mouth, eye, skin, genitals, nose, oropharynx, esophagus, and larynx). OCP can also have a diverse array of clinical courses and a spectrum of responses to systemic and topical therapy.

Some investigators have reported that like bullous pemphigoid (BP), mucous membrane pemphigoid or CP patients’ sera recognize 180-kDa proteins termed as BPAG2 when human epidermal lysate and BPAG2 fusion proteins were used as substrates (2, 3). This protein along with BPAG1 (BP230) and $\alpha_6\beta_4$ integrin are found in hemidesmosomes, which are structures that link the underlying basement membranes of stratified as well as other epithelia to the intermediate filament system. They are composed of an outer plaque, containing proteins such as BP180 and $\alpha_6\beta_4$, and an inner plaque containing BP230 and HD1/plectin, which then associate with the cytoskeleton. Defects in these proteins can lead to epithelial blisters in both humans and mouse models (4).

We have observed that after preabsorption of epidermal or conjunctival or tumor cell lysates with BP sera, OCP sera recognize a 205-kDa protein (5, 6), identified as the $\beta_4$ integrin subunit (7). These findings raised the possibility that a putative target Ag for OCP may be the $\beta_4$ integrin subunit (7). In fact, we have recently reported that OCP sera, immunoaffinity-purified OCP Abs eluted from the 205-kDa band on nitrocellulose, and anti-$\beta_4$ Abs cause blistering in an in vitro human conjunctival model (8). This result suggests that Abs from OCP sera against $\beta_4$ could be pathogenic in vivo.

A subset of CP patients has been reported to be characterized by the presence of autoantibodies against epiligrin, which is now identified as the $\alpha_3$ subunit of laminin-5 (9, 10). Interestingly, many of the OCP sera used in the present study and in our earlier reported studies (5, 6) did not bind to epiligrin (K. Yancey, unpublished observation).

Since CP is a heterogeneous disease, it is possible that clinical subsets may correlate with a variety of anti-BMZ autoantibodies with different specificities that recognize different target molecules within the complex of cell surface proteins and extracellular matrix proteins in the BMZ.

Given the possible pathogenic role of anti-$\beta_4$ Abs in OCP sera, the purpose of this study was to determine the region of $\beta_4$ recognized by these Abs. Surprisingly, we have found that the anti-$\beta_4$
Abs of OCP bind to the intracellular region of human \( \beta_4 \) integrin and we were unable to detect, using immunoblotting, immunoprecipitation, and FACS analysis, any binding to the extracellular domain of \( \beta_4 \) integrin subunit.

**Materials and Methods**

**Serum samples and Abs**

Sera were obtained from 15 patients with active OCP in the acute phase of the disease before the institution of therapy. The diagnosis of OCP was confirmed in each patient by clinical presentation, routine histology, and immunopathological analysis of biopsied conjunctiva. When tested on salt split skin, the anti-BMZ autoantibody bound to the epidermal side of the split.

Total IgG of OCP autoantibodies was prepared from 10 active OCP patients by EZ-SEP kit obtained from Middlesex Sciences (Foxborough, MA). Immunofluorescence-purified OCP autoantibodies were eluted from the nitrocellulose blots, as described previously (11). In another Western blot, it was confirmed that the immunofluorescence-purified OCP autoantibodies bound to a 205-kDa protein in normal human conjunctiva and epidermis in exactly the same manner as OCP sera. mAb to human \( \beta_4 \) (3E1) and polyclonal Ab to extracellular and cytoplasmic domains of human \( \beta_4 \) integrin were described elsewhere (12, 13). The 9E10 anti-\( \alpha_{IV} \) mAb was prepared by the hybridoma core facility at Memorial Sloan-Kettering Cancer Center (New York, NY). Control sera were obtained from 10 healthy individuals and from 1 patient each with active disease with pemphigus vulgaris (PV), BP, oral pemphigoid (OP), and dermatitis herpetiformis (DH). All the control and patient sera were stored at \(-80^\circ C\) until used. The study has been reviewed by our Institutional Review Board. Sera were obtained after appropriate informed consent procedures.

**Preparation of lyase from human skin and conjunctiva**

The epidermis of the human skin was separated from underlying dermis, and clear lysates of Ags were prepared as reported earlier (14). Briefly, the human epidermis and conjunctiva were homogenized on ice with 1.5% Triton X-100, 0.5 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, and 10 \( \mu \)g/ml each pepstatin A, aprotinin, and leupeptin. Lysates were clarified by centrifugation for 10 min at 14,000 rpm in a refrigerated centrifuge. The epidermis of the human skin was separated from underlying dermis, and clear lysates of Ags were prepared as reported earlier (14). Briefly, the human epidermis and conjunctiva were homogenized on ice with 1.5% Triton X-100, 0.5 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, and 10 \( \mu \)g/ml each pepstatin A, aprotinin, and leupeptin. Lysates were clarified by centrifugation for 10 min at 14,000 rpm in a refrigerated centrifuge.

**Cells and transfection**

The 804G rat bladder carcinoma cells and 293T human embryonic kidney were grown in DMEM containing 10% bovine calf serum. The 804G cells stably expressing recombinant human full-length \( \beta_4 \) (clone A), extracellular domain of \( \beta_4 \) (clone F), and intracellular domain of \( \beta_4 \) integrin (clone L) were described elsewhere (13, 15). The 293T cells, which do not express endogenous \( \beta_4 \), were cotransfected by calcium phosphate precipitation method with cDNAs of human \( \alpha_c \), and versions of \( \beta_4 \) (13, 15, 16) cloned into the EcoRI site of the PRK5 expression vectors. Typically, subconfluent cells were extracted for 30 min on ice in Triton X-100 lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM EGTA, 4 mM EDTA, 1% Triton X-100, 0.5 mM 4-(2-aminophenyl)benzenesulfonyl fluoride, and 10 \( \mu \)g/ml each pepstatin A, aprotinin, and leupeptin). Lysates were clarified by centrifugation for 10 min at 14,000 rpm in a refrigerated centrifuge.

**Western blotting**

SDS-PAGE and Western blot were performed as previously described (17, 18). A sensitive Western blotting assay was used in this study, as described previously (7). In brief, blotted nitrocellulose membrane was blocked with 3% skinned milk. Blotted proteins (nitrocellulose strips) after extensive washing with TBS-containing 0.05% Tween 20 were incubated with diluted test sera or Abs. After 4× wash, nitrocellulose strips were incubated with HRP-conjugated secondary Abs (anti-human, anti-rabbit, and anti-mouse), and then the final step was detected by using the ECL Western blotting kit (Amersham Life Sciences, Arlington Heights, IL), according to the manufacturer’s protocol.

**Immunoprecipitation of OCP-Ag/\( \beta_4 \) integrin**

Immunoprecipitation was performed as described earlier (16). Briefly, 50 \( \mu \)l of packed protein G-agarose washed three times with lysis buffer, re-suspended in 500 \( \mu \)l of lysis buffer, then 10–50 \( \mu \)l of OCP sera or Abs to \( \beta_4 \) integrin was added to it and incubated for 2 h at 4°C. The protein G was washed three times with lysis buffer. The cell lysates (500 \( \mu \)g/500 \( \mu \)l) suspended in lysis buffer were added to the OCP sera or \( \beta_4 \) Ab-coated protein G and incubated overnight at 4°C. The immunoprecipitates were washed five times in cold lysis buffer and then resuspended in 50 \( \mu \)l of sample buffer boiled for 5 min and analyzed by immunoblotting.

In these experiments, we used 804G (rat bladder) and 293T fibroblast cell lysates (untransfected and transfected with full-length, extracellular, and intracellular domains of \( \beta_4 \) integrin) and immunoprecipitated with OCP autoantibodies.

**FACS**

The 293T cells expressing full-length \( \beta_4 \) were detached briefly with 0.02% trypsin and incubated for 20 min in ice with either 10 \( \mu \)g/ml of 3E1 mAb, or diluted OCP Abs, or normal human IgG, followed by FITC-conjugated donkey anti-mouse or anti-human secondary Abs. After fixation in 3.7% formaldehyde, cells were analyzed by flow cytometry using a Becton Dickinson FACS machine and Cell Quest Ware.

**Cytoplasmic localization of OCP autoantibody in cultured cells by direct immunofluorescence**

This study was done according to the methods described by other investigtaors (19, 20). Briefly, \( \beta_4 \)-expressing cell lines (MDA-435) were grown on chamber slides (Nunc, Naperville, IL) and allowed to adhere and proliferate. After 48 h, the cells were nearly confluent and washed with RPMI 1640 medium, then incubated with OCP sera, Abs to cytoplasmic, and extracellular domains of \( \beta_4 \) integrin and normal human serum for 24 h. After incubation, the slides were washed three times with PBS, then fixed in methanol acetone at \(-20^\circ C\) for 15 min. After fixation, the cells were washed in PBS and blocked with 3% BSA in PBS for 30 min. Then the cells were washed and incubated with fluorescein-labeled goat anti-human or anti-rabbit IgG (Sigma) for 45 min, then washed, mounted, and observed by fluorescent microscope.

**Results**

**Reactivity of OCP autoantibodies with wild-type and mutant human \( \beta_4 \) integrin subunits expressed in bladder epithelial OCP cells**

The 804G cells are derived from a rat bladder carcinoma and are widely used for studies on hemidesmosomes. These cells were transfected with cDNA constructs encoding wild-type \( \beta_4 \), a \( \beta_4 \) subunit lacking the ectodomain (headless), or one lacking the cytoplasmic domain (tailless). Stable clones expressing comparable surface levels of each recombinant protein were analyzed by immunoblot and immunoprecipitation experiments. Untransfected 804G cells were used as controls.

**Immunoblot experiments**

Extracts from various cell lines were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with either OCP sera or control Abs against either the cytoplasmic or extracellular domains of \( \beta_4 \) (Fig. 1). OCP sera reacted with wild-type (205 kDa) and headless \( \beta_4 \) (140 kDa), but did not react with tailless \( \beta_4 \) (100 kDa). Immunoblotting with rabbit polyclonal Abs against the cytoplasmic and extracellular domains of \( \beta_4 \) confirmed the expression of the transfected constructs. Wild-type \( \beta_4 \) (205-kDa band) is observed in all lanes because it is endogenously expressed by 804G cells.

**Immunoprecipitation experiments**

Lysates from the various 804G cell lines were immunoprecipitated with OCP sera. These immunoprecipitates were then subjected to SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted with polyclonal Ab to extracellular and cytoplasmic domains of \( \beta_4 \) integrin (Fig. 2). OCP sera was able to immunoprecipitate the wild-type and headless versions of \( \beta_4 \), but did not immunoprecipitate with the tailless \( \beta_4 \). Wild-type \( \beta_4 \) is observed in...
all lanes because 804G cells express endogenous $\beta_4$. Similar results were seen using IgG fractions of OCP sera and immunopurified OCP Abs (data not shown). Sera from patients with PV, BP, OP, DH, or normal human sera did not immunoprecipitate with $\beta_4$ (data not shown). These experiments demonstrate that autoantibodies from OCP sera react predominantly with the cytoplasmic, and not extracellular domain of $\beta_4$ integrin.

To avoid the complexity from the reaction of OCP autoantibodies with the endogenous $\beta_4$ in 804G cells, we decided to confirm our results in 293T cells, which do not express endogenous $\beta_4$.

**Reactivity of OCP autoantibodies with wild-type and mutant human $\beta_4$ integrin subunits expressed in 293T cells**

**Immunoblot experiments.** Human embryonic kidney 293T cells were transiently transfected with cDNA constructs encoding the wild-type $\beta_4$, the $\beta_4$ subunit lacking the ectodomain (headless), or the $\beta_4$ subunit lacking the cytoplasmic domain (tailless). Extracts from these cells were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with IgG fractions from OCP sera or immunopurified OCP autoantibodies, as well as normal human IgG as a negative control or rabbit polyclonal Abs against $\beta_4$ cytoplasmic and extracellular domains as positive controls (Fig. 3). Immunopurified OCP autoantibodies and IgG fractions of OCP sera recognized wild-type $\beta_4$ and headless $\beta_4$, but not tailless $\beta_4$. Similar results were observed using whole sera from OCP patients (data not shown). Normal human IgG did not react with $\beta_4$. Expression of wild-type $\beta_4$ and $\beta_4$ mutants was confirmed by immunoblotting with rabbit polyclonal Abs to the cytoplasmic or extracellular domains of $\beta_4$. Lysates from untransfected 293T cells did not show any reactivity with OCP sera or control anti-$\beta_4$ Abs (data not shown).

**Immunoprecipitation experiments.** Lysates from 293T cell lines untransfected or transfected with wild-type or mutant versions of $\beta_4$ were immunoprecipitated with OCP sera. These immunoprecipitates were then subjected to SDS-PAGE, transferred to nitrocellulose, and immunoblotted with polyclonal Abs to the extracellular or cytoplasmic domains of human $\beta_4$ molecule (Fig. 4). The results demonstrate that OCP sera can immunoprecipitate the wild-type and headless $\beta_4$, but not tailless $\beta_4$. When the same lysates were immunoprecipitated with sera from PV, BP, OP, DH, or normal human serum, no reactivity with $\beta_4$ was observed (data not shown).

Both the immunoblotting and immunoprecipitation experiments, in which OCP autoantibodies fail to react with the tailless $\beta_4$, suggest that the predominant epitopes recognized by the anti-$\beta_4$ autoantibody in OCP sera are in the cytoplasmic rather than the extracellular domain of $\beta_4$ integrin.

**FACS analysis**

Although the OCP autoantibodies do not bind to the $\beta_4$ extracellular domain by immunoblot or immunoprecipitation analysis, it is possible that a small, but potentially pathogenic pool of OCP autoantibodies can also recognize the $\beta_4$ extracellular domain in its native conformation at the cell surface. Therefore, we tested the ability of the OCP autoantibodies to bind the extracellular domain of $\beta_4$ in intact cells by flow cytometry with nonpermeabilized cells. Under these conditions, we could not detect any binding of OCP autoantibodies to the cell surface, although the control Ab 3E1 against $\beta_4$ extracellular domain bound strongly (Fig. 5).

**Cytoplasmic localization of OCP autoantibody in cultured cells by direct immunofluorescence**

When MDA-435 cells, which endogenously produce full-length $\beta_4$, were incubated with OCP sera, Abs to cytoplasmic and extracellular domains of $\beta_4$ integrin, and normal human serum for 24 h, the following observations were made. OCP sera bind only to the cytoplasm of the cultured cells (Fig. 6C). Ab to cytoplasmic domain of $\beta_4$ integrin binds in a similar pattern to only the cytoplasm of the cultured cells (Fig. 6B). No extracellular binding was seen in cells cultured with OCP sera or Ab to cytoplasmic domain of $\beta_4$...
integrin. Ab to extracellular domain of $\beta_4$ integrin demonstrated binding to the cell surface only (Fig. 6A). No cytoplasmic binding was seen. No binding to either the cell surface or cytoplasm was seen when the cells were cultured with normal human serum (Fig. 6D).

**Discussion**

This study provides evidence that the large majority of epitopes recognized by OCP autoantibodies reside in the cytoplasmic domain of human $\beta_4$ integrin, thus confirming and extending our previous observations. We have used immunoblotting and immunoprecipitation to analyze 804G cells stably transfected with cDNAs encoding a wild-type, a tailless, or a headless form of $\beta_4$. Selective binding of OCP sera to the cytoplasmic domain of $\beta_4$ was observed. However, OCP sera and rabbit polyclonal Abs to the extracellular and cytoplasmic domains of $\beta_4$ also showed background binding to the endogenous $\beta_4$ expressed by these cells. We have therefore also used 293T cells, which do not produce endogenous $\beta_4$. Extracts from 293T cells transfected with cDNAs encoding wild-type, tailless, and headless $\beta_4$ were analyzed by immunoblotting and immunoprecipitation with OCP sera. The results of these experiments confirmed that OCP sera bind selectively to the intracellular portion of $\beta_4$. No binding to the extracellular portion was detected by immunoblot, immunoprecipitation, and using even the most sensitive FACS analysis.

The ability of the OCP sera and Ab to cytoplasmic domain of $\beta_4$ to bind the cytoplasmic tail of $\beta_4$ was studied in vitro using cells in culture. We showed that OCP sera and Ab to cytoplasmic domain of $\beta_4$ demonstrate intracellular binding only.

No extracellular binding was observed. The control Ab to the extracellular domain of $\beta_4$ bound only to the cell membrane and showed no binding to the cytoplasm.

We preferred to use MDA-435 cell lines instead of the 293T-transfected cells because it produced full-length $\beta_4$, and any cross-reactivity between these Abs to the extracellular and cytoplasmic domains or OCP sera would have been detected. Although we have used all means available to detect the presence of Abs against extracellular domain of $\beta_4$ integrin, it is important to note that they may exist as a small and undetectable, yet potentially pathogenic pool of such autoantibody. Advances in technology or evaluation of patients in preclinical states may detect them.

The intracellular portion of $\beta_4$ integrin is large and consists of 1000 aa, and contains four type III fibronectin-like modules (4, 21). The cytoplasmic portion of $\beta_4$ has been shown to interact with two other hemidesmosomal proteins, BP180 and HD1/plectin (4).
and is required for hemidesmosome formation and stable adhesion of stratified epithelia, such as the skin and pyloris (22).

The basic pathology in OCP is a subepithelial vesicle or blister formation. In recent studies, we have demonstrated that when normal human conjunctiva is placed in organ culture with OCP sera, immunoaffinity-purified OCP autoantibodies, or Abs to human \( \beta_4 \) integrin, but not normal human sera, a separation between the conjunctival epithelium and underlying submucosa is observed. This separation is remarkably similar to that observed in human conjunctival pathology in vivo in OCP patients (8). These observations suggest that the autoantibodies to \( \beta_4 \) in OCP can contribute to the initiation or progression of the disease.

The experiments in this study strongly suggest that autoantibodies to \( \beta_4 \) contribute to the pathogenesis of OCP. The observation in this study presents the dilemma of the role of the extracellular domain of \( \beta_4 \) in the pathogenesis of OCP and access of OCP Ab to its intracellular target Ag. Three hypothetical but plausible explanations deserve consideration. First, the disease could be initiated by Abs directed to the ectodomain of \( \beta_4 \). However, we did not detect such Abs in the sera of the patients we studied with active disease. Hence, it is likely that such Abs could be transient and are present only in the preclinical stage of the disease, and therefore not detected.

Second, the inciting Ag initially involved may be irrelevant, but epitope spreading may involve epitopes that have homology to \( \beta_4 \). Alternatively, autoantibodies could enter the cell to initiate injury that is subsequently propagated by Abs to exposed Ag on the cell surface. Nonetheless, Abs to the cytoplasmic epitopes of \( \beta_4 \), BP180, and BP230 are generated and cause disruption of hemidesmosomal assembly-resultant blister formation due to BMZ separation and clinical disease.

Our direct immunofluorescence studies using cultured cells demonstrate that OCP autoantibodies and Ab to cytoplasmic domain of \( \beta_4 \) integrin bind intracellularly to cells. A number of previous observations demonstrate that autoantibodies can penetrate into living cells, bind to their intracellular targets, and influence cellular function (23). For example, autoantibodies to ubiquitous intracellular Ags are commonly found in the sera of patients with systemic lupus erythematosus, poly and dermatomyositis, progressive systemic sclerosis, and Sjögren’s syndrome (24). Investigators have demonstrated that specific ant-DNA Abs can penetrate cells in culture (19, 20).

Furthermore, when these anti-DNA antibodies are injected into normal mice, they produce glomerular damage and proteinuria, indicating the functional capability to the autoantibodies (19). Anti-nuclear and anti-cytoplasmic Abs have been detected in vivo in human skin biopsies from the patients with connective tissue diseases (25, 26).

There are several mechanisms by which autoantibodies could penetrate cells and gain access to the intracytoplasmic target Ags. The mechanisms are probably different in different diseases and may be different in different subsets of the same disease. Based on the work of several investigators, one hypothesis has emerged and has gained some acceptance. Intracellular molecules that contain sites targeted by autoantibodies may be presented on cell membranes by surrogate molecules (27). The surrogate membrane molecule mimics the structure of the intracellular Ag (28). The complex containing the autoantibody and surrogate Ag is internalized within the cell. The autoantibody is then released and binds to the pathogenic intracellular Ag and initiates the events that may eventually lead to autoimmunity (29, 30).

The observations made in this study may help in providing an explanation for the two divergent observations made by different investigators studying immunoelectron microscopy (IEM) of CP. In the first group of studies, authors report deposition of immunoreactants on the lower lamina lucida and lamina densa (31–33). The sera used by two investigators bound to the dermal side of salt split skin on indirect immunofluorescence assay and lamina densa on IEM contained Abs to laminin-5 (34). In the second group, investigators demonstrate that immunodeposits occur on hemidesmosomes and basal keratinocyte cytoplasm or the junction between hemidesmosomes and the inner plasma membrane of keratinocytes (33–35). These investigators observed that sera that produced such immune deposition did not contain Abs to laminin-5 or BP (33). In this study, using transfected cell lines, we demonstrated that patients with CP whose autoantibody binds to the epidermal side of salt split skin have Abs that bind to the cytoplasmic domain of \( \beta_4 \) integrin. Hence, we propose that the sera studied by the second group contained Abs to cytoplasmic domain of \( \beta_4 \) integrin, which accounts for their IEM pattern.
A definitive role for Abs to human β4 integrin in the pathogenesis of OCP can come from in vivo animal model studies. Further studies on the role of β4 in the generation of autoimmunity are important since β4 is the first integrin to be implicated in the pathogenesis of an autoimmune epithelial blistering disease. In addition, OCP may provide a model to study the possible role of integrins in initiation of progression or disease process.

References