J Oral Pathol Med (2007) 36: 1–5

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# Relationship between cancer and oral pemphigoid patients with antibodies to α6-integrin

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**BACKGROUND: Mucous membrane pemphigoid is an autoimmune mucocutaneous blistering disease. A subset, known as anti-epiligrin cicatricial pemphigoid is associated with a high risk for malignancy. Oral pemphigoid (OP) is limited to the oral cavity. The purpose of this study was to determine the association between malignancy and patients with OP with antibodies to α6-integrin subunit.**

**METHODS: We determined the incidence of cancer in 72 patients with OP and compared it to the expected incidence using age and sex-specific rates of malignancy in the National Cancer Institute's Surveillance, Epidemiology, and End Results (NCI SEER) Registry.**

**RESULTS: During a mean observation period of 9.1 years (range: 2.8–40), for 70, three OP patients developed malignancies. The expected number of cancers based on the NCI SEER Registry was 8.83. The relative risk for cancer in OP patients, with autoantibodies to α6-integrin, was 0.34 (95% CI, 0.07–0.99, $P < 0.05$).**

**CONCLUSION: It appears that patients with OP, with antibodies to α6, may have a possible reduced relative risk for developing cancer.**

*J Oral Pathol Med* (2007) **36**: 1–5

**Keywords:** anti-α6-integrin (alpha6-integrin) antibody; cancer; mucous membrane pemphigoid; oral pemphigoid; relative risk

## Introduction

Mucous membrane pemphigoid (MMP), also known as cicatricial pemphigoid, is an autoimmune vesiculobullous disease of the mucous membranes and, rarely, the skin (1). In one of the earliest studies, published over three decades ago, the *in vivo* deposition of auto antibodies in oral tissues and the presence of circulating antibodies to basement membrane proteins were demonstrated (2). Autoantibodies are directed against several molecules in the basement membrane, including α6-integrin, β4-integrin, laminin 5, BPAg1, and BPAg2 (1). Various subsets of MMP are being recognized. A subset of MMP patients, in whom most mucosal surfaces and the skin can be involved, have antibodies to laminin 5 and 6 in their sera, and are referred to antiepiligrin cicatricial pemphigoid (AECP) (1). Oral pemphigoid (OP) is another subset of MMP, in which the disease activity is limited only to the oral cavity, as determined by clinical presentation and long-term follow-up. No other mucosae and the skin are involved. The sera of some patients with OP have antibodies to α6-integrin subunit (3). OP is characterized by subepidermal blisters in the oral cavity on histology. On direct immunofluorescence, deposition of IgG is observed along the basement membrane zone (BMZ). The histology and immunopathology of the oral lesions of AECP and OP are similar. On indirect immunofluorescence, using salt split skin as substrate, one of the differences between the two subsets is that antibodies in the sera of OP patients bind to the roof, while antibodies in AECP bind to the floor, of the lM NaC1 created blister (1).

In a recent study, Egan *et al.* demonstrated that patients with AECP, with antilaminin 5 antibodies in their sera, have an increased risk of malignancy (4). Several recent *in vitro* studies in animal models, and in humans, suggest that the overexpression or aberrant expression of α6 integrin subunit may promote the development of metastasis of certain malignancies (5–11).

These observations in the literature encouraged us to retrospectively review the records of 72 OP patients to determine the rate of malignancy in this sample, and compare it to the expected incidence based on age and sex-specific rates of malignancy from the National Cancer Institute's Surveillance, Epidemiology, and End Results (NCI SEER) Registry.

## Materials and methods

The medical records of patients with OP seen at the Center for Blistering Diseases, Boston, MA, USA, between 1990 and 2005 were reviewed. No specific

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Accepted for publication July 5, 2006

efforts or advertisements were made to recruit these patients. The patients were referred by their primary care physicians, oral surgeons, dentist or oral pathologists. The patients came from the general population that lives in the New England region of the US. These patients were most likely to be similar to patients with OP seen anywhere else in the US or perhaps the world.

In each of these 72 patients, the diagnosis of OP was based on the clinical presence of blisters or erosions in the oral cavity and was established by the presence of a subepidermal vesicle on histological examination of a lesion. Direct immunofluorescence of perilesional tissue demonstrated the deposition of IgG on the BMZ, in all patients to confirm the diagnosis of OP. Antibodies to α6-integrin subunit, determined by immunoblot assay, were present in the sera of the 72 OP patients, during the acute stage of the disease (3). In all the patients in this study, the anti-BMZ antibody demonstrated the binding of antibody to the epidermal side of salt split skin (12). All patients had only oral disease and the presence of blisters at other sites were excluded by a detailed and thorough physical examination and endoscopic evaluation of the upper airway by an otorhinolaryngologist. Each patient was thoroughly examined by an ophthalmologist to exclude conjunctival involvement. Female patients were evaluated by a gynecologist to exclude vulvar or vaginal involvement. During each follow-up visit, the patients were carefully and deliberately questioned, for extraoral involvement. If there was any suspicion of any other mucosal involvement, the specialists who had previously evaluated the patient was requested to evaluate the patient again.

The length of follow-up was defined as the duration of time, in years, from the date the pathological diagnosis of OP was made to the date of the last visit to the specialist. Some of the 72 patients presented in this report were described in earlier studies. Fifteen patients were described in one study (3), 29 in the second study (13) and 7 in the third study (14). The remaining 21 patients have not been described in any earlier study. The previous three studies (3, 13, 14), describing a total of 51 patients, did not address the association of OP and malignancy. The study was approved by the institutional review board.

Controls for serological detection of antibodies to the α6-integrin subunit included sera from 100 healthy volunteers, 50 patients with known malignancies selected from a local oncology clinic, 20 patients with known pemphigus vulgaris, 15 patients with known bullous pemphigoid, and five with toxic epidermal necrolysis of the 50 cancer patients. Approximately half had a variety of solid tumors and remaining half had lymphoproliferative malignancies of a wide spectrum. Antibodies to α6-integrin subunit were detected by an immunoblot assay, as previously described (3). The antibodies used as positive/control for the α6-integrin subunit immunoblot assay, included CD49f, BQ16 and GoH3 (3). These are monoclonal antibodies known to bind exclusively to the α6-integrin subunit.

Entry into the cohort was defined as the point at which the diagnosis of MMP was made by histology and immunopathology. Data on the diagnosis of cancer were collected for the entire duration of the follow-up period for each patient. Patients with cancer diagnosed 12 months or longer before the diagnosis of MMP was made, were excluded from the study. Two patients had cancer, 16 and 21 months before the diagnosis of MMP and were excluded from the study group. Thus the study group had 70 patients only. The diagnosis of cancer in the patients in the study group was made based on histological characteristics of the tumor.

The number of cancers expected in this group of OP patients was calculated as the sum of the products of the number of person-years for each 5-year age specific and sex specific group and the corresponding age- and sex-specific incidence rates reported by the NCI SEER Registry (15). Relative risk (RR) was calculated as the ratio of cancers observed in this cohort compared with those excepted from the NCI SEER Registry. The 95% CI was calculated using the Poisson distribution (16).

## Results

Seventy patients with OP met the inclusion criteria and were the basis of this cohort of patients. Forty-five patients were female and 25 patients were male. The mean age of onset of OP was 54 years (range: 26–76). The mean length of follow-up was 9.1 years (range: 3.9–15.8).

Cancers were detected in three of the 70 patients after the diagnosis of OP (95% CI 0.62–8.77). One patient had malignant melanoma, one had non-Hodgkin's lymphoma, and one had squamous cell carcinoma of the lung. These diagnoses were based on histologic evaluation of the tumors. Two patients who had developed cancer before the diagnosis of OP were excluded. Using age- and sex-specific incidence rates for all cancers from NCI SEER Registry, the expected number of cancers in this group of patients was 8.83. The relative risk for cancer in these OP patients was 0.34 (95% CI 0.07–0.99), which was significantly lower ($< 0.0l$) than predicted by the NCI SEER Registry.

The sera of all the 70 OP patients, in this study, had detectable levels of antibodies to human α6-integrin subunit. None of the sera from the various disease control group contained antibodies to the α6-integrin subunit. The positive control antibodies CD49f, BQ16, and GoH3 demonstrated binding to α6-integrin subunit, in a pattern similar to the sera of the OP patients.

## Discussion

We studied 70 patients with OP, who were followed for a mean of 9.1 years. Only three patients developed cancer during this period. This incidence is significantly fewer than the 8.83 cancers, which would be expected to develop based on data from the NCI SEER Registry. The RR was 0.34. In contrast the RR was 6.8 in the AECP patients reported by Egan *et al.* (4). The experimental design and statistical analysis in the two studies were identical.

The three patients with OP in this study who developed cancer were similar to other patients with OP. They had no distinguishing clinical features, histological findings, laboratory abnormalities, responses to treatment, or long-term outcomes, compared to other patients with OP. OP was treated in all the patients by topical cortieosteroid therapy with or without low-dose intermittent systemic oral corticosteroids. The overall prognosis in patients with OP is good (13). No deaths have been reported in patients with OP, because of the disease or its therapy.

The α6-integrin subunit co-localizes with β4 subunit to form the α6β4-integrin heterodimer (3). Amongst other locations, it is found in the hemidesmosomes and involved in the adhesion between the basement membrane and the basal epidermal cells. The α6β4 integrin heterodimer is the ligand for laminin 5 and 6. Unlike patients with OP, who have disease limited to the oral cavity, patients with AECP have disease involving multiple mucosae and the skin (1). Their histology and immunopathology are indistinguishable from OP or MMP in general. The difference is that, in AECP patients, antibodies bind to the floor of the Salt-Split Skin (SSS), because they have antibodies to laminin 5/6. The prognosis in the patients with AECP who had antibodies to laminin 5 is poor, the majority of patients do not respond to therapy, and the mortality rate is 40% (4).

This study and the study by Egan *et al*. (4), puts into focus the relationship between α6-integrin subunit and its ligand, laminin 5, and cancer. Laminin 5 is produced only by epithelial cells and secreted in their basement membranes (17). There is an interesting and complex relationship between α6-integrin subunit, Laminin, anti-α6 antibody, antilaminin antibody and cancer. Emerging evidence suggests that expression of laminin 5 plays a role in epithelial tumor progression and may be a marker of invasion (18). Recently Natarajan and coworkers have demonstrated a consistent increase of coexpression of p16 and γ2 chain of laminin 5 in keratinocytes in areas of micro invasion and superficial margins of squamos cell carcinomas (19). Their studies indicate that this molecular coexpression may act as a tumor suppressor and its loss may result in an invasive growth of SCCs of the skin and the oral mucosa (20). In addition, several investigators have demonstrated that expression of laminin 5 is associated with increase in tumor cell migration (21, 22), enhanced invasiveness of the tumor (21, 23) and poor prognosis (18). Most of laminin 5 antigens have been located in the cytoplasm of the invading epithelial cells. (24) Increased expression of laminin 5 has been reported in cervical, (25) colorectal, (26) pancreatic, (27) and oral cancer (28), but decreased expression has been reported in prostate cancer (29).

Antibodies to laminin 5 are detected in the sera of patients with many cancers. Some of these include gastric, lung and endometrial cancers (30–32). These patients develop a subset of MMP, referred to as anti-epiligrin cicatricial pemphigoid. Patients with AECP who have anti laminin 5 antibodies have an increased incidence of cancer compared with a reference population from the NCI SEER Registry, with a relative risk as high as 15 (4).

Recent reports suggest that α6-integrin subunit plays a role in tumor cell survival, proliferation, invasion, and prognosis (5). Increased expression of α6-integrin subunit appears to be present at the periphery of the breast, colon, kidney, parotid, salivary gland, prostate, and esophageal tumors (6–8). The over expression of α6 integrin subunit has been associated with local tumor invasion in esophageal, prostate, and breast cancers (7–9). The over expression of α6 integrin subunit in breast cancer may be a negative prognosticator because it is associated with local tumor invasion and increased risk for metastasis (9, 10). Using immnohistochemical techniques Thorup and coinvestigators have demonstrated a batchy loss of α6β4 and laminin 5 expression at the invasive margins of squamose cell carcinomas of the oral cavity (33). Interestingly, decreased expression of α6-integrin subunit was also associated with tumor invasion and metastasis in salivary gland carcinoma (34). Hence the direct role of α6-integrin in tumor biology may be complex and is not fully understood.

Several investigators have studied the effects of antibodies to α6-integrin subunit on cancers. When melanoma cells were injected into the mouse-tail vein, their potential to metastasize is inhibited by anti- α6 antibodies (35). The effect of antibodies to α6 integrin subunit on tumor invasiveness and metastasis has been studied *in vitro*. Antibodies to α6-integrin subunit appear to inhibit the invasion of intestinal-type carcinoma cells through the basement membrane (11) and inhibit the hematogenous spread of colon cancer cells (36). When fibrosarcoma and osteosarcoma cells were interacted with artificially created basement membranes, the tumors treated with anti α6-integrin subunit antibody permeated the basement membrane statistically less than tumors that were not treated with anti-α6-anitbodies (37, 38). We note that the effects of α6 integrin or anti-α6-antibodies have not been specifically studied in the three types of cancer we report in OP patients. Hence, although preliminary and early, it would appear that increased expression of α6-integrin subunit is associated with tumor invasion, migration, and metastasis. However, antibodies to α6-integrin subunit, in animal models, appear to have the reverse effect and provide the host protection from cancer.

The authors recognize that this epidemiologic study demonstrates that the presence of antibodies to α6-integrin subunit is associated with reduced risk for cancer. However, it is also acknowledged that the inability to assess environmental, genetic and a variety of other factors that predispose to developing malignancy, a heterogeneous population with wide age range and the long duration of sample collection are some of the limitations for this study. The authors also realize that not all OP patients may have autoantibodies to α6-integrin subunit (39). Another limitation is that it is a retrospective study. In the view of some investigators, 72 patients with OP may not be a large sample size and could be viewed as a limitation of the study. A prospective study would require a large cohort of

patients that could only be obtained from a multicenter or perhaps multinational study. However, it needs to be emphasized that OP is an extremely rare disease and there are a limited number of investigators studying it. Hence, an investigator studying OP will experience difficulty in obtaining large number of patients in a short time span.

In summary, our data suggest that patients with OP with antibodies to α6 integrin subunit may have a reduced risk for developing cancer when compared with age-matched men and women. It is important to note, however, that we were not able to control for other differences between our population and the reference population, and these differences could influence these observations. Nonetheless, we believe that the identification of patients with OP and separating them from patients with generalized AECP are important, from a prognostic perspective. Furthermore, determining the presence of antibodies to α6 integrin subunit is valuable in these patients. Oral involvement is common in both MMP and AECP. If serological studies are carried out early, then patients can be treated and monitored accordingly. The observations in this report have clinical significance and implication. The preliminary data strongly recommend that a multicenter, perhaps multinational study should be undertaken. This preliminary study suggests that a better understanding of the relationship between integrins, the basement membrane, and the biology of cancers need to be investigated further to better identify their interaction in normal human oral health and disease.

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## Acknowledgments

This study was supported by NIH grant no R0l-EY014228 to Dr A. R. Ahmed. The authors are grateful to Christopher Vaillancourt and Olga Lyczmanenko for the preparation of the manuscript.