

Demonstration of Antibodies to Human b4 Integrin in the Sera of Ocular Cicatricial Pemphigoid Patients Using Indirect Immunofluorescence Assay

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Our research team has been studying the immunology of the autoimmune disease Cicatricial Pemphigoid for a decade. The underlying genetic susceptibility to the disease has been identified, and the target autoantigen has been identified. We now show that patients with active (but not those with inactive) Ocular Cicatricial Pemphigoid (OCP) have present in their serum an autoantibody which recognizes the cytoplasmic domain of human b 4 integrin. We did this by using normal human conjunctiva substrate in an indirect immunofluorescence assay. Serum from patients with active OCP, from patients with inactive OCP, from normal individuals, and monoclonal antibodies directed against human b 4, a 6, a 5, and b 1 integrins were employed in the experiments. Experiments in which binding to the epithelial basement membrane zone (BMZ) was detected were followed by blocking experiments after absorbing the sections with different reagents and then reprobing them in the immunofluorescence assay.

Nine of ten OCP sera, as well as monoclonal antibodies directed against human b 4 and against a 6 integrins, showed a homogenous smooth linear binding to the human conjunctival epithelial basement membrane zone, in a binding pattern identical to that seen when one probes inflamed conjunctiva of a patients with pemphigoid in the direct immunofluorescence immunopathologic assay. Normal human serum, serum from patients with inactive OCP, and monoclonal antibodies directed against b 1 and a 5 integrin did not show any binding. If conjunctival sections were first repeatedly "absorbed" with OCP's active sera, and subsequently probed with antibody directed against b 4 integrin, a marked reduction in binding of antibody directed against b 4 at the BMZ was observed. If sections were repeatedly absorbed with antibodies to b 4 integrin first, and subsequently probed with OCP sera, binding with the OCP sera was completely eliminated. This effect was not observed with normal human sera. These results indicate that selective deposition of antibodies directed against b 4 integrin is an important early event in the pathogenesis of Ocular Cicatricial Pemphigoid.