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### A practical technique for differentiation of subepidermal bullous diseases: localization of in vivo-bound IgG by laser scanning confocal microscopy

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#### ABSTRACT

**OBJECTIVE:** To develop a practical technique to distinguish autoimmune subepidermal bullous diseases.

**DESIGN:** A prospective study.

**SETTING:** Academic referral center—the Department of Dermatology, Medical University of Warsaw. Patients Forty-two patients fulfilling clinical, immunological, and/or immunoelectron microscopic criteria for bullous pemphigoid (n = 31), mucous membrane pemphigoid (n = 6), or epidermolysis bullosa acquisita (n = 5), diagnosed as having disease and treated from January 1, 1997, to December 31, 2002.

**MAIN OUTCOME MEASURES:** We applied laser scanning confocal microscopy to determine the localization of in vivo-bound IgG at the basement membrane zone in biopsy specimens taken from patients' skin to compare the localization of basement membrane zone markers: antibody against beta4 integrin, antibody against laminin 5, and antibody against type IV collagen. In vivo-bound IgG was visualized by labeling with fluorescein isothiocyanate-conjugated anti-human IgG antibody, whereas basement membrane zone markers were labeled with anti-mouse Cy5-conjugated antibodies.

**RESULTS:** In patients with bullous pemphigoid, in vivo-bound IgG was localized on the epidermal side of laminin 5 and co-localized with beta4 integrin. In patients with mucous membrane pemphigoid, IgG was in vivo bound to the dermal-epidermal junction between localization of laminin 5 and type IV collagen. In patients with epidermolysis bullosa acquisita, in vivo-bound IgG was present on the dermal side of type IV collagen.

**CONCLUSIONS:** Laser scanning confocal microscopy allows precise localization of in vivo-bound IgG in patients' skin and, thus, it is a rapid method for the differentiation of mucous membrane pemphigoid from bullous pemphigoid and epidermolysis bullosa acquisita. This tool is suitable for the routine diagnosis of individual patients and for retrospective studies. This method is of special value in those patients in whom circulating autoantibodies are not detectable.

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