Failure of Specific Human Tissue Substrates to Increase the Sensitivity of Indirect Immunofluorescence Testing in Cicatricial Pemphigoid

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Powell FC, Connolly SM, Rogers RS III, Schroeter AL. Failure of specific human tissue substrates to increase the sensitivity of indirect immunofluorescence testing in cicatricial pemphigoid. Acta Derm Venereol (Stockh) 1984; 64: 540–543.

Indirect immunofluorescence testing was carried out on sera from 14 patients with cicatricial pemphigoid with use of both autologous and homologous human tissue substrates. The

results were largely negative. The sensitivity of detection of circulating antibody is not increased with these substrates over that with animal tissue substrates. *Key words: Cicatricial pemphigoid; Negative indirect immunofluorescence; Homologous substrates.*
(Received March 6, 1984.)

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Histologic examination of involved skin in cicatricial pemphigoid shows dermoepidermal separation, which electron-microscopic studies have demonstrated to be above the basal lamina in the lamina lucida (1). Direct immunofluorescence testing reveals deposits of immunoglobulins and complement in a linear pattern along the basement membrane zone (2), and immunoelectron microscopy has also localized these deposits above the basal lamina (3). All these changes are similar to those seen in bullous pemphigoid, and it has been suggested that cicatricial pemphigoid is a scarring variant of bullous pemphigoid with a predilection for mucosal surfaces (4). However, unlike those in bullous pemphigoid, results of indirect immunofluorescence testing usually are negtive in patients with cicatricial pemphigoid (4, 5).

Some reports suggest, however, that use of human mucosal substrates increases the ability to detect circulating immunoglobulins in cicatricial pemphigoid (6, 7). To determine the reproducibility of these previous positive results, we used indirect immunofluorescence techniques on selected human tissue substrates to study sera from patients with cicatricial pemphigoid.

MATERIALS AND METHODS

Sera were obtained from 14 patients with cicatricial pemphigoid for substrate testing. All 14 (5 male, 9 female) ahd typical clinical findings of cicatricial pemphigoid. Twelve patients had lesions of the conjunctiva, 11 had oral involvement, 4 had genital lesions, and 2 had additional cutaneous lesions. The ages of the patients at onset of disease ranged from 48 to 75 years, with a mean of 66 years. Ten patients had histologic and immunofluorescent confirmation, consisting of subepidermal bulla and linear deposition of immune reactants at the basement membrane zone. Three of the patients who underwent biopsy had nonspecific histologic changes, but results of direct immunofluorescence in these patients were positive, showing linear deposition of immunoglobulin at the basement membrane zone.

Normal human conjunctiva, oral mucosa, vagina, and skin acquired during surgical procedures and cynomolgus monkey esophagus were used as substrates. All tissues were immediately snap-frozen in liquid nitrogen, and 4-μm sections were cut on a cryostat and air-fixed on glass slides. Frozen sections were then stained for indirect immunofluorescence by established techniques. Briefly, sera were incubated on each substrate for 30 minutes in a moist chamber. They were then incubated with fluorescein-conjugated goat anti-human IgG (Cappel Laboratories; concentration, 16 unitage diluted 1 : 10). One patient in addition had screening done on a sample of her own (autologous) conjunctiva, and another had it done on autologous skin. Herpes gestationis factor was tested for by complement amplification on each patient's sera on each substrate with use of established techniques. Fresh normal human complement was used in this assay, which involved the additional step of incubation of the substrate with fresh normal human complement for 30 minutes after the serum incubation for indirect immunofluorescence; this was followed by incubation with fluorescein-conjugated goat anti-human C3 (Cappel Laboratories; concentration, 8 unitage diluted 1 : 6). All sera were screened at dilutions of 1 : 10 and 1 : 20 (indirect immunofluorescence) and 1 : 20 (complement amplification) in veronal buffer. Serum from a patient with bullous pemphigoid was used as a positive control for the indirect assay, and serum from a patient with herpes gestationis was used as a positive control for the complement amplification assay. Sera from two patients with nonbullous skin diseases served as negative controls. Sections were examined within 24 hours with a Leitz Ortholux II fluorescence microscope at a magnification of ×500 with use of water-immersion lenses. Fluorescence was graded from 0 (negative) to +4 (intensely bright fluorescence).

RESULTS

Sera from three patients showed faint positive linear staining of the basement membrane zone. One patient had weakly positive linear staining (strength, +1) of the basement membrane zone on vaginal tissue, and two other patients had weakly positive (+1) linear segmental staining on monkey esophagus. In all three, the serum dilution was 1:10. In two patients, the positive finding was obtained with routine indirect immunofluorescence and in another, with the herpes gestationis assay. None of our patients had positive findings when oral mucosa, skin, or conjunctiva was used as substrate, and the two patients in whom tests were done on autologous tissues also had negative results.

DISCUSSION

Circulating antibodies have been found in the sera of isolated patients with cicatricial pemphigoid (2, 6, 8), and some investigators (6, 7) have suggested that human tissue substrates are more sensitive because the antibody to cicatricial pemphigoid may be organ- and species-specific. Laskaris & Angelopoulos (7) demonstrated antibodies at low titer (1:10 to 1:40) in 12 of 32 patients with cicatricial pemphigoid when normal human oral mucosa was used as substrate. Sera from these patients were negative when tested on compound animal epithelial tissue (rabbit esophagus or lip and guinea pig esophagus or lip) and normal human skin. Our largely negative results, with only weak fluorescence in three cases in a dilution of 1:10, however, do not indicate a specificity of the cicatricial pemphigoid antibody for antigenic material in particular human tissues, especially because results in two of the three cases were positive on monkey esophagus only. These findings suggest that further screening on human tissue substrates with the routine indirect immunofluorescence technique will not help reveal the cicatricial pemphigoid antibody. This conclusion is in striking contrast to that of Laskaris & Angelopoulos (7) but consistent with earlier experience (9). One possible explanation for this difference is that serum levels of the antibody may fluctuate with the activity of the disease (10), being detectable only during the most active phases, or that therapy with corticosteroids may reduce the level of the antibody so much that it is no longer identifiable. However, several of our patients had active, extending lesions at the time their sera were assessed, and many of our patients were managed without corticosteroids.

Bean (6) and Furey et al. (11) demonstrated the presence of basement membrane zone antibodies on autologous tissue substrates (skin and oral mucosa) in two patients whose sera were negative when examined on animal tissue and nonautologous human skin, esophagus, and conjunctiva. Autologous tissue was obtained from only two of our patients, but even with these autologous substrates, detectable antibody still could not be demonstrated in the patients' own sera. Similar negative findings have been reported by other authors (12). Our experience suggests that homologous or autologous human tissue substrates do not offer increased sensitivity for detection of circulating antibodies in cicatricial pemphigoid.

REFERENCES

1. Caputo R, Bellone AG, Crosti C. Pathogenesis of the blister in cicatricial pemphigoid and in bullous pemphigoid: A comparative ultrastructural study. Arch Dermatol Forsch 1973; 247: 181–192.
2. Rogers RS III, Perry HO, Bean SF, Jordon RE. Immunopathology of cicatricial pemphigoid: Studies of complement deposition. J Invest Dermatol 1977; 68: 39–43.
3. Nieboer C, Boorsma DM, Woerdeman MJ. Immunoelectron microscopic findings in cicatricial

pemphigoid: Their significance in relation to epidermolysis bullosa acquisita. Br J Dermatol 1982; 106: 419–421.
4. Bean SF, Waisman M, Michel B, Thomas CI, Knox JM, Levine M. Cicatricial pemphigoid: Immunofluorescent studies. Arch Dermatol 1972; 106: 195–199.
5. Griffith MR, Fukuyama K, Tuffanelli D, Silverman S Jr. Immunofluorescent studies in mucous membrane pemphigoid. Arch Dermatol 1974; 109: 195–199.
6. Bean SF. Cicatricial pemphigoid: Immunofluorescent studies. Arch Dermatol 1974; 10: 552–555.
7. Laskaris G, Angelopoulos A. Cicatricial pemphigoid: Direct and indirect immunofluorescent studies. Oral Surg 1981; 51: 48–54.
8. Dantzig P. Circulating antibodies in cicatricial pemphigoid. Arch Dermatol 1973; 108: 264–266.
9. Waltman SR, Yarian D. Circulating autoantibodies in ocular pemphigoid. Am J Ophthalmol 1974; 77: 891–894.
10. Dabelsteen E, Ullman S, Thomsen K, Rygaard J. Demonstration of basement membrane autoantibodies in patients with benign mucous membrane pemphigoid. Acta Derm Venereol (Stockh) 1974; 54: 189–192.
11. Furey N, West C, Andrews T, Paul PD, Bean SF. Immunofluorescent studies of ocular cicatricial pemphigoid. Am J Ophthalmol 1975; 80: 825–831.
12. Heydenreich G, From E, Diederichsen H. Some unusual findings obtained by immunofluorescence method in bullous pemphigoid and benign mucous membrane pemphigoid. Acta Derm Venereol (Stockh) 1972; 52: 201–204.