

Precipitation of Epithelia Antigen(s) with Pemphigus-Like Autoantibodies of Rabbit and Human Pemphigus Autoantibodies

Immunofluorescent (IF) studies have revealed that the sera of patients with pemphigus vulgaris and its variants contain autoantibodies to an intercellular (IC) substance of normal stratified squamous epithelium^{1,2}.

Suitable means have been developed³⁻⁵ for the preparation of aqueous and ethanolic extracts of human, rhesus monkey and bovine esophageal mucosa which contain antigen(s) possessing tissue and serologic specificity for autoantibodies to the IC substance. Heteroimmunization with ethanol soluble extracts of bovine esophageal mucosa has resulted in the stimulation of pemphigus-like antibodies in rabbits; i.e. IF staining patterns indistinguishable from those seen with pemphigus autoantibodies. These antibodies were shown to be autoantibodies, by the demonstration of positive in vitro reactions with the antibody-producer's own tissue⁴⁻⁷.

In the present paper an attempt was undertaken to study the possible relationship of pemphigus-like autoantibodies of rabbit to human pemphigus autoantibodies by gel-precipitation. It was felt that such a study might provide additional information for further investigations on the possible role of the immunologic phenomenon in the etiology of pemphigus.

Antigen(s) and antisera were prepared and obtained following the procedures previously described⁷. In addition to ethanolic extracts of bovine esophageal mucosa (BEM-F5), comparable extracts of gastric mucosa, kidney, liver and spleen were prepared. Double diffusion precipitation in gel was performed according to the method of OUCHTERLONY⁸.

The ethanol soluble fraction of BEM-F5 yields precipitation reactions with rabbit anti-BEM-F5 sera (pemphigus-like antibodies) and with human pemphigus autoantibodies. As shown in the Figure, bands of precipitate between the center well (BEM-F5) and wells 1, 3 and 5 containing sera of patients with pemphigus (Brazilian pemphigus foliaceus), appeared to coalesce with the bands formed between wells 2 and 4, containing rabbit serum 2950 (from heteroimmunization with BEM-F5), forming a precipitin pattern suggestive of a reaction of identity and a common specificity for these antibodies. The negative reaction of normal serum (well 6) may be seen in contrast to the precipitates formed by the pemphigus sera.

The tissue and serologic specificity of this reaction was also analysed by gel-precipitation. For evaluation of anti-

genic specificity, a variety of tissue extracts of bovine origin were tested with a standard pemphigus-like antiserum (2950) and with a standard pemphigus serum (Vie.). No precipitation occurred in tests with gastric mucosa, kidney, liver or spleen. Evaluation of serologic specificity was carried out by a comparison of serum samples from patients with various diseases, in addition to the testing of normal human sera, with esophageal extracts. Such tests were consistently negative.

It is known that patients with pemphigus contain autoantibodies to an IC substance of stratified squamous epithelium demonstrable by IF staining. However, no study as yet has been reported, to our knowledge, employing precipitation as a method for demonstrating this immune response.

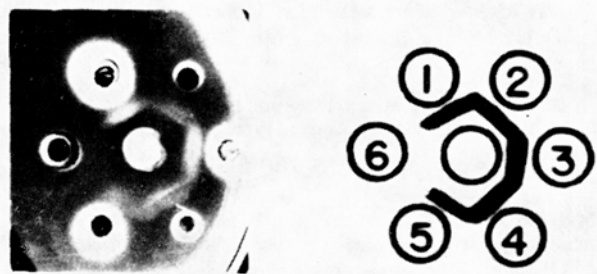
Comparison of pemphigus-like antibodies of rabbit and human pemphigus autoantibodies by gel-precipitation with an ethanol soluble fraction of bovine esophageal mucosa are suggestive of the presence of a common specificity for these antibodies.

Findings of the present report appear to be in agreement with previous IF studies suggesting that pemphigus-like antibodies of rabbit are tissue specific; and histologically and serologically indistinguishable from human pemphigus autoantibodies^{4-7,9,10}.

Zusammenfassung. Der Vergleich von Pemphigus-ähnlichen Kaninchenantikörpern und menschlichen Pemphigus-Autoantikörpern mittels Gelpräzipitation mit einer äthanollöslichen Fraktion aus der Mucosa des Rinder-Ösophagus weist auf eine gemeinsame Spezifität dieser Antikörper hin. Die Resultate entsprechen früheren Immunofluoreszenz-Untersuchungen.

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Photograph and diagram of double diffusion gel-precipitation pattern after 4 days incubation. Center well, ethanol soluble extract of bovine esophageal mucosa (BEM-F5). Peripheral wells: 1, 3 and 5, sera of patients with pemphigus; 2 and 4, rabbit anti-BEM F5 serum (2950), and 6, normal rabbit serum. (1% Difco Noble agar, wells: 2.5 mm diameter; 8.0 mm from center, and separated center-center 7.5 mm).

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