Pemphigus and pemphigoid:
Some current concepts

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Differential diagnosis in the group of chronic vesicobullous dermatoses, particularly in early atypical borderline cases, remains one of the most difficult problems of dermatology today, in spite of advances which have been made in histopathology, cytology, electronmicroscopy and immunology.

In the present state of our knowledge it is impossible to classify and discuss such bullous diseases as pemphigus, pemphigoid, dermatitis herpetiformis and erythema multiforme on an etiological basis. Until very recently their classification was based on morphologic criteria histologically determined.

Pemphigus and bullous pemphigoid, two of the chronic bullous skin diseases of man, have been the subject of numerous investigations. Comprehensive reviews summarizing these investigations have appeared recently.[1, 2] It is interesting to trace the background of the development which has led to much of our present knowledge of these two formidable skin diseases whose severe and prolonged disability gives them importance in clinical dermatology.

Auspitz, in 1881, coined the term "acantholysis", and claimed it to be the initial process in pemphigus. In 1884, Duhring separated dermatitis herpetiformis from pemphigus.

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In 1943, Civatte[3] gave a histological description of the process of acantholysis which is basic to our understanding of the bullous diseases. He also observed that acantholysis was absent in dermatitis herpetiformis and erythema multiforme. In 1949, Tzanck and Aron-Brunetire[4] demonstrated that scrapings from the floor of pemphigus blisters showed characteristic acantholytic cells (large round epidermal cells which had no intercellular bridges remaining). Their cytologic test was based on Civatte's finding of degenerated epidermal cells in the bullae of pemphigus. In 1953, Lever[5] distinguished bullous pemphigoid from the other blistering diseases by showing that the bullae of pemphigoid were subepidermal and that acantholysis was absent. In 1959, steroids were introduced for the treatment of these two bullous diseases, with a significant reduction in their mortality and morbidity. However, because they may be uncontrollable or as a consequence of the effects on them of steroid therapy, these diseases remain a challenge to the physician.

In considering pemphigus and pemphigoid, there are certain findings which have been well established and others about which uncertainty remains. We do know that there is some peculiarity of the pemphigus patient's skin whereby his epidermal cells lose their cohesiveness and come apart, with the consequent production of blisters and bullae, a phenomenon which has been called acantholysis. Similarly, in pemphigoid, some unknown factor(s) leads to a disturbance of dermal-epidermal adhesion and separation at this junction but with no evidence of acantholysis. We are still not certain of the exact site or the sequence of events of the first changes leading to acantholysis. We certainly do not know if blister formation in pemphigus and pemphigoid is an immunologic phenomenon, i.e. whether it is a manifestation of autosensitization or, as it is more popularly known today, autoimmunity.

Electron-microscopic studies of normal and diseased skin

Electron-microscopic studies of the epidermis by Wilgram, Caulfield and Lever,[6] Braun-Falco and Vogell[7, 8] and Hashimoto and Lever[9] and others have contributed greatly to our understanding of the basic anatomy of the skin, as well as bulla formation in pemphigus and pemphigoid.

Presently available knowledge suggests that the prickle cells are held together by various means including (a) the desmosome-tonofilament complexes which are the structures making up the intercellular bridges seen by light microscopy; (b) an intercellular cement substance and (c) interlocking villous projections of the cell membrane.

Initially it was thought that the prickle cells, or keratinocytes, were firmly and permanently bound to their neighbours and moved outward as a unit. Recent work by

Mishima and Pinkus,[10] Epstein, Conant and Krasnobrod[11] and Braun-Falco and Vogell[7, 8] suggests that the desmosome-tonofilament complexes are dynamic units and not static permanent structures. From such studies it seems certain that the prickle cells can move through the epidermis independently after separation of their desmosomal connections.

Little is known about the dimensions, structure and physical properties of the intercellular space. Studies suggest that it is not just an empty series of canals but that it represents a continuous system of spaces surrounding and separating the epidermal cells.

Experimental work by Mercer, Jahn and Maibach[12] suggests that a polysaccharide-containing layer covers the cell surfaces and is closely involved with cell-to-cell cohesion. It is sparse in the basal-cell layer and is present in greater amount in the upper layers. It coats all surfaces of the interlocking pseudo-villi. Further knowledge of the biological significance of this cell-surface sugar coat, the mechanisms of its synthesis and its relation to intercellular adhesion is needed. However, it seems certain that the intercellular bridges are not the only means of cellular adhesion which keep keratinocytes attached to each other.

The natural adherence of the epidermis to the dermis is quite firm. The junction is not flat but is irregular in a wavelike pattern with a tight interdigitation of epidermal rete ridges and dermal papillae. It is important to remember that the basement lamina which forms the upper limit of the dermis is not a static persistent structure but alters as needed for cell growth. The ultrastructure, as well as the physiology of the dermal-epidermal junction, requires further study.

The location of primary defect leading to acantholysis in pemphigus remains controversial. However, it seems likely, as Hashimoto and Lever[9] and Wolff, Tappeiner and Schreiner[13] have shown, that the primary factor is damage or dissolution of the intercellular cement substance. Tonofilament retraction and dissolution of the desmosomes with eventual lysis of the affected prickle cells are probably secondary phenomena. While acantholysis is not absolutely diagnostic of pemphigus since it also occurs in familial benign chronic pemphigus, Darier's disease and several viral diseases, it is a fundamental part of the histological picture of pemphigus.

Histologically, the two diseases pemphigus and pemphigoid are quite different. In pemphigus the earliest changes are an intercellular edema within and above the basal cell layer. The attachment between the basal cells and the intact basal lamina, mediated mainly by the half-desmosomes (junction granules) and the anchoring filaments, is extremely resistant to the acantholytic process. It produces the so-called tombstone row of basal cells, where their lateral attachments have disappeared, and leads to the characteristic intra-epidermal bulla formation, usually suprabasilar and showing this phenomenon of acantholysis. In pemphigoid the basic finding is a dermal-epidermal separation, with the formation of subepidermal blisters which occupy the intermembranous space between the basal cell plasma membrane and the basement lamina.

Autoimmunity, pemphigus and pemphigoid

Various immunologic studies of pemphigus and pemphigoid suggest that their pathogenesis may indeed be due to immunologic phenomena. It is not clear how the patient's immune response might be directed towards his own tissue. It is possible that epithelial components may be altered under the influence of extraneous stimuli and thereby become antigenic; or the immune response may be directed towards a different antigen but one which cross-reacts with the skin. Such possibilities continue to intrigue investigators. Again, it is interesting to retrace the path leading to these observations.

Fluorescent antibody technique

Coons, in 1941, showed that antibody, i.e. gamma globulin molecules, could be chemically combined or conjugated with simple chemical compounds such as a fluorescent dye and yet retain its immunological reactivity, while acquiring a conspicuous label. Using the immunofluorescent technique devised by Coons and Kaplan,[14] Beutner and Jordon[15] provided the earliest evidence for this autoimmune concept, with the demonstration of antiepithelial autoantibodies in the sera of patients with these two diseases. I will briefly describe this technique and discuss its use.

The basic theory of the fluorescent antibody technique is simple and obvious. Antigenic material present in the tissue (e.g. in cell, at cell margin, at basement zone) will react specifically with its related antibody. This immunological reaction results in the deposit of minute amounts of specific antibody over those areas of a tissue section where the antigen is present. When the antibody molecules have previously been chemically marked with fluorescein the microdeposit of fluorescent antibody is visible as a brilliant yellow-green light under the fluorescence microscope.

Indirect immunofluorescent test

In the indirect immunofluorescent test, the serum antibody reacting with the skin is itself not labelled, and the fluorescent marker is carried by a second antibody especially prepared to have specificity for aspects of the first immunoglobulin which is already specifically fixed to antigen(s) in the skin. The indirect immunofluorescent test is more sensitive and results in a brighter staining than the direct test, because it is capable of attaching more label per antigenic site. For testing, sera are serially diluted with isotonic saline, starting with a 1:10 dilution. The antibody titre is considered the highest dilution of a serum which gives a positive immunofluorescent test. A constant dilution of labelled anti-human gamma globulin is used. This fluorescein-labelled antibody is commercially available. It is usually prepared by injecting suitable animals with purified human gamma globulin. After an interval the hyperimmune blood is collected. The serum is extracted and chemically combined with a fluorescent dye. Skin or mucous membrane from humans or laboratory animals is used as the assay sub-

strate. The technique of preparing the sections is as follows. Tissues are quick-frozen in liquid nitrogen and used without chemical fixation which would injure the immunological reactivity of the antigenic material. Four micra sections are cut in a cryostat and placed on a glass slide. The tissue section is first washed thoroughly and then overlaid with one or two drops of a dilution of untreated serum and incubated at room temperature in a moist chamber for 30 minutes. Excess serum is removed by washing with phosphate-buffered saline. The rinsed tissue section is then covered with one or two drops of fluorescein-isothiocyanate-tagged goat antihuman gamma globulin and again incubated and washed. The section is then examined microscopically for fluorescence.

Direct immunofluorescent test

In contradistinction to the above, in the direct immunofluorescent test the antibody which reacts with the tissue component(s) directly is itself labelled with fluorescein. This antibody may be derived from the patient or other sources. The substrate is the patient's skin to which his own antibodies are presumably attached. Antibody demonstration is accomplished by overlaying the patient's skin section with one or two drops of a dilution of labelled antiserum directed against the attached patient's antibody. The crucial differential point between the two tests is the site of the label. In the direct test the label is on antibody. In the indirect test the label is carried on the anti-antibody molecule.

Results of investigation

Studies using the fluorescent antibody tests[10-19] have shown that the sera of patients with pemphigus and its variants have circulating antibodies which bind *in vitro* and *in vivo* consistently and specifically to a component in stratified squamous epithelium. This component either belongs to the intercellular cement substance or is a substance at, on, or between the surfaces of the epithelial cells. These antibodies appear to be tissue-specific in that they bind only to stratified squamous epithelia, but are not species-specific in that they react with stratified squamous epithelia from various laboratory animals, as well as human epidermis. It should be emphasized that pemphigus sera contain complement-binding but no nuclear antibodies. Pemphigoid patients' sera contain circulating antibodies which bind to an antigen(s) in the subepidermal basement zone area underlying various types of epithelium, including stratified squamous epithelium but also mucosal epithelium of the urinary tract, respiratory tract and gallbladder. Pemphigoid sera also demonstrate complement-binding antibodies. The site of antibody binding in both pemphigus and pemphigoid corresponds to the site of the first observable pathologic changes. These antiepithelial antibodies occur in all active untreated pemphigus and pemphigoid patients' sera. Usually if a patient shows no clinical activity or is under active steroid treatment there will be no serologic activity. These antiepithelial antibodies have not been found in familial benign chronic pemphigus, dermatitis herpetiformis, benign mucosal pemphigoid, erythema multiforme or in any other control sera. The above findings have been repeatedly confirmed in a large number of patients. An apparent exception is that some sera taken after severe burns show indistinguishable intercellular staining patterns to pemphigus sera.[20] The post-burn anti-skin antibodies, however, may well be directed against different antigenic determinants. This finding is undergoing further investigation.

Experimental immunological studies

Experimental studies have furthered the concept that pemphigus and pemphigoid are manifestations of autoimmunity. The finding of intercellular antibodies in all forms of pemphigus has stimulated studies on antigen isolation and animal immunization. Grob and Inderbitzin[21] and Ablin and Beutner[22] were successful in experimentally producing an antiepithelial antigen which elicited antibodies giving immunofluorescent patterns indistinguishable from those obtained with pemphigus sera, whereas tissue fractions from ileum, kidney and liver gave negative results. These experimentally produced intercellular antibodies were tissue-specific in that they reacted only with stratified squamous epithelium. They were not species-specific. Bean[23] demonstrated specific autoantibodies in the blister fluid of patients with pemphigus and pemphigoid but not in the blister fluid of other diseases.

Katz, Halprin and Inderbitzin[24] have recently demonstrated that using human skin as the test substrate in the indirect immunofluorescent test is superior to the use of animal tissues. Fresh cadaver or surgically excised human skin was found to be quite satisfactory. Human skin proved to be more sensitive than when animal substrates were used in that lower serum antibody titres were detectable. By using horizontal skin sections, i.e. cut parallel to the skin surface, rather than the conventional vertical sections, the accuracy and reliability of the test were enhanced. No false positives were found. No patients with active untreated pemphigus or pemphigoid had a negative test.

New techniques making use of electron-dense ferritin-labelled antibody and ultra-thin tissue sections have enabled researchers to use the electron microscope for ultra-structural localization of disease processes.

Summary

Immunological methods have shown that antibodies present in the sera of patients with pemphigus and pemphigoid are essentially specific for their respective diseases and yield typical immunofluorescent staining patterns. The fluorescent antibody tests demonstrate that the tissue of patients with pemphigus and pemphigoid reacts in a different way from tissue in other skin diseases. The demonstrated immunofluorescence indicates the general site of abnormal antigen-antibody reactions. However, it has yet to be shown that the altered immune response is the primary event. It may only be the result of altered tissue produced by some other process. In order better to understand the disease

process it is most crucial to elucidate the nature of the antigen which participates in the immune response.

Correlation of clinical, pathologic and cytologic studies will still be needed to arrive at a correct diagnosis in borderline cases of pemphigus and pemphigoid, bullous dermatitis herpetiformis and bullous erythema multiforme; but the increasing availability of the immunofluorescent histochemical staining tests will assist in the proper diagnosis of these troublesome diseases by allowing confirmation of clinical opinion through specific serologic laboratory methods.

The understanding of autoimmunity is a challenge to the immunologist and the clinician. Each can learn from the other, and the answers depend on their mutual progress. The relationship of autoimmunity to cutaneous disease is an area of dermatology worthy of continued extensive clinical and laboratory investigation. I hope that I have shown that pemphigus and pemphigoid can rightly join the ranks of those diseases having an immune basis.

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