Detection of Autoantibodies in Autoimmune Bullous Skin Diseases: Can We Do Better?

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Autoimmune bullous skin disorders are characterized by autoantibodies against structural components of the skin: desmosomal proteins (in pemphigus), adhesion molecules of the dermal-epidermal junction (in pemphigoid diseases), and epidermal tissue transglutaminase (in dermatitis herpetiformis [1-3]. The interaction between autoantibodies and their target protein results in blister formation due to the loss of adhesions between the epidermis and dermis layers.

The most frequent autoimmune bullous diseases are bullous pemphigoid and pemphigus vulgaris. Their diagnosis (as is true for other ABSDs) relies on a combination of clinical picture, histopathological findings and diagnostic techniques for the detection of autoantibodies in the skin and/or serum [3-6]. Those diagnostic techniques include non-specific methods such as direct and indirect immunofluorescence microscopy, as well as specific target antigen detection such as enzyme-linked immunosorbent assay and immunoblotting [3].

The gold standard diagnostic method in autoimmune bullous diseases is direct immunofluorescence microscopy of a perilesional biopsy that detects autoantibodies in skin or mucous membranes. In pemphigus, direct immunofluorescence demonstrates intercellular immunoglobulin G and C3, and in pemphigoid a linear C3 and IgG along the junction of dermis and epidermis are visible [6,7].

Indirect immunofluorescence microscopy performed on monkey esophagus can demonstrate circulating IgG autoantibodies in the serum of pemphigus patients. These antibodies target the monkey esophagus epithelium, forming an intercellular network (honeycomb pattern). In pemphigoid, linear IgG deposits can be seen at the basement membrane level by using monkey esophagus or applying the "salt split" method, in which a human dermal-epidermal junction is split by 1 M NaCl solution, revealing the level of the split within the basement membrane. These screening tests have been described as the most sensitive [8,9].

The molecular characterization of several target antigens in the pathogenesis of ABSD in the last decade has fostered the development of sensitive and specific diagnostic tools, such as ELISA or immunoblotting. These confirmatory analyses are performed using recombinant proteins or keratinocyte extracts. The former is highly sensitive and specific but is limited by the number of known antigens it can detect and by the limited number of commercial kits presently available [10-13].

Immunoblotting is considered a very useful method since a larger variety of antigen can be detected. In addition to being diagnostic, it can serve for immunoserological follow-up. With this method proteins are extracted from normal human epithelium (from a human donor). Following their extraction the patient's serum is tested for the presence of autoantibodies against skin components. The antibodies of pemphigus react with desmogleins 1 and 3, and those of pemphigoid react with BP180 and BP230 [14]. However, during the extraction, loss of antigenic polypeptide and distortion of antigen conformation and distribution may occur, leading to debatable results [15]. To overcome this technical problem two other extracts were suggested: cultured A431 epidermoid cells and human amniotic membrane extract. The former indeed overcame this technical problem; however, the facilities it requires are not accessible in most diagnostic laboratories [16]. The HAM extract is considered to contain all the proteins targeted by autoantibodies in ABSD. Furthermore, since this method does not require splitting of the skin, it avoids the loss of antigens. Another advantage is its reduced immune reactivity with intermediate filaments found in keratinocytes, which can further complicate interpretation of the results. A few studies have shown that HAM is a readily available and more useful substrate for immunoblotting analysis, with a sensitivity of 64–100% in pemphigus and 50–70% in pemphigoid [17,18].

In the current issue of *IMAJ*, Sezin et al. [19] further assessed the accuracy of immunoblotting and indirect immunofluorescence by applying HAM as a substrate in pemphigus and pemphigoid. They compared these results with those of immunoblotting using normal human skin as a

ABSD = autoimmune bullous skin disorder

ELISA = enzyme-linked immunosorbent assay

HAM = human amniotic membrane

substrate, indirect immunofluorescence performed on monkey esophagus, and ELISA. They studied sera from 66 adults with ABSD (25 pemphigus, 31 pemphigoid) and 36 healthy individuals serving as the control group. In their study immune mapping of HAM demonstrated all the antigens involved in autoimmune bullous disease, except for BP230. They found that HAM extract was inferior in detecting pemphigus autoantibodies in pemphigus patients compared to human epidermis extract for immunoblotting, monkey esophagus for indirect immunofluorescence, and ELISA for detecting DSG1 and DSG3. For pemphigoid antibodies detection in patients with pemphigoid, HAM extract was comparable to normal human skin extract and monkey esophagus in immunoblotting and indirect immunofluorescence respectively. Both were inferior to the sensitivity of ELISA. Thus, unlike previous studies, their results show that HAM extract as a substrate for diagnosing ABSD does not offer any advantage over the currently available methods. It also shows that ELISA is an accurate specific method. However, they point out a potential use of the HAM extract in more rare diseases like paraneoplastic pemphigus and subtypes of subepidermal blistering diseases.

The incidence of autoimmune disorders around the globe is rising, and even rare diseases like bullous skin diseases are encountered more frequently in the physician's clinic. With time, health providers will get to see a larger variety of cases in that spectrum and with different implications, such as atypical clinical picture, unknown prognosis, treatment dilemmas, and overlap with other diseases. It highlights the current need to find a novel, readily available and accurate test for screening autoantibodies in the diagnosis of autoimmune bullous diseases. This calls for a concentrated effort of the medical community to seek other diagnostic tools to improve our understanding of autoimmune diseases in general and bullous diseases of the skin in particular.

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Capsule

Gut immune tolerance

With the constant assault of food antigens and its billions of resident microbes, the gut is an important site of immune tolerance. By studying specific intestinal immune cell populations in genetically modified mice, Mortha and co-authors found that gut macrophages produce the cytokine interleukin-1 (IL-1) in response to signals derived from the microbiota. IL-1 acts on type 3 innate lymphoid cells in the intestine, which then produce the cytokine, colony-stimulating factor 2 (Csf2). Csf-2, in turn, induces myeloid cells (including dendritic cells and macrophages) to produce regulatory factors like retinoic acid and interleukin-10, which support the conversion and expansion of regulatory T cells, a population of cells known to be critical for maintaining immune tolerance in the gut.

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