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Antipituitary autoantibodies: Potential markers for autoimmune endocrine disorders

Abbreviation: APA = antipituitary antibody; ELISA = enzyme-linked immunosorbent assay

The first case of lymphocytic hypophysitis was reported in 1962.\(^1\) The findings in hypophysitis of both marked lymphocyte infiltration into the pituitary gland and concomitant Hashimoto’s thyroiditis suggested its autoimmune nature. In 1975 Bottazo et al.\(^2\) developed an assay method for detecting autoantibodies against human pituitary cells (APAs) with an indirect immunofluorescence technique. Crock et al.\(^3\) also reported a different method for the detection of APA through immunoblotting by using human pituitary cell membranes as antigens. Through the use of these technologies, the existence of APA has been established on both experimental and clinical grounds,\(^4\) although the physiologic or pathologic role of APA has not been fully characterized. Moreover, APA has been identified not only in patients with pituitary disorders but also in serum samples from patients with other endocrine disorders including insulin-dependent diabetes mellitus,\(^5\) Graves’ disease,\(^6\) and Hashimoto’s thyroiditis.\(^7\) Because potential differences in antigenicity exist between human beings and animals, human pituitary tissues are ideal sources for analysis. However, because of the limited availability of human pituitary antigens, Sugiura et al. constructed indirect immunofluorescence methods in which rat pituitary tissues\(^8\) and murine pituitary cell lines\(^9\) were used in place of human tissue. After extensive analysis by Sugiura’s group\(^8,9\) and Kobayashi’s group,\(^7\) rat pituitary tissue is now widely used for identifying APA.

In an article in this issue of the Journal, Yabe et al.\(^10\) describe the development of an ELISA system for APA that uses rat pituitary soluble antigens. Western blot analysis identified a single 22 kd protein band, a finding that supports the quality of the ELISA data. Because of its simplicity and high throughput with 96-well microplates, this assay is ideal for mass screening of APA. In fact, the article by Yabe et al. has demonstrated APA-positive serum in patients with various endocrine disorders as well as in small numbers of normal healthy individuals, which leads us to ask the following questions. (1) What is the target antigen of APA? (2) Why, where, and when is APA induced? (3) How does APA influence biologic functions?

The finding by Yabe et al.\(^10\) of APA-positive serum in a variety of patients will help researchers to characterize the biochemical nature of actual target antigens. Although APA is absorbed partially by human growth hormone, the molecular size of the rat growth hormone (24,655 daltons, based on 217 amino acids deduced from the nucleotide sequence GenBank J00739, V01239) is slightly larger than 22 kd. The positive serum samples found in the Yabe et al. study provide invaluable research materials for the isolation of target antigens, the cloning of corresponding gene(s), etc. Once target genes are identified from rat pituitary tissue, one can easily determine the expression levels of the target gene in various endocrine tissues by reverse transcription–polymerase chain reaction.\(^11\) This provides an interesting clue regarding whether APA found in non-pituitary disorders may be induced in pituitary tissue or in endocrine tissue. Moreover, human genes can be identified by low stringent hybridization techniques with rat clones.\(^12\) The resultant human sequences will allow researchers to develop a more appropriate human assay system in future.

The antigens of APA are theoretically classified into three categories: (1) pituitary cell membranes, (2) cytosolic non-secretory proteins, (3) secretory proteins (including hormones, cytokines, peptides, and enzymes). Membrane antigens and secretory proteins are exposed to the immune system during developmental stages in the fetus/newborn, where immune tolerance will be induced. Therefore the presence of APA against...
membrane proteins or secretory proteins indicates some pathologic antigen processing mechanisms or the abnormal expression of these proteins in disease states. APA against membrane antigens may recognize living pituitary cells and bind to the cell surface. Some APAs simply sit harmlessly on the surface of pituitary cells, some may activate intracellular signaling pathways to induce biologic functions, and some may activate complement/cellular immunity to damage cells. These differences are dependent on the type of immunoglobulins and the nature of antigens. APA against secretory proteins may form circulating immune complexes to induce pathologic status, although such cases seem to be quite rare. Furthermore, secretory proteins share a common structure called a signal sequence, which is responsible for secretion from the intracellular to extracellular space. Once antibodies are induced against these signal sequences, such antibodies may be cross-reactive to a wide variety of secretory proteins, which may explain the high incidence of APA in non-pituitary endocrine disorders. In contrast, cytosolic non-secretory proteins are usually away from immune recognition. If APA against non-secretory proteins is identified in patients’ serum samples, this may suggest pathologic tissue damage. To fully characterize the nature of APA in individual patients in both pituitary and non-pituitary diseases, biochemical and molecular biologic analysis of the target antigen(s) and gene(s) should be elucidated.

Although in the Yabe et al. article the authors used anti-human immunoglobulin G polyclonal antibodies alone, one of the advantages of the ELISA system is the flexibility in detecting various classes or subclasses of immunoglobulins by simply changing second anti-human antibodies. Because limited subsets of immunoglobulins can activate the complement cascade, cellular immunity, etc., subclass analysis of immunoglobulins may provide helpful information for understanding the pathophysiology of APA.

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