CLINICAL SIGNIFICANCE OF ANTIPERINUCLEAR FACTOR AND ANTIKERATIN ANTIBODY FOR RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid Factor (RF) is the only serologic marker used in the diagnosis of rheumatoid arthritis (RA). Since it is present in a number of diseases other than RA and found negative in some patients with RA, diagnostic utility of RF has some limitations. Several more specific autoantibodies detected in the sera of RA patients are not routinely tested. Antiperinuclear factor (APF) and antikeratin antibodies (AKA) are two of these antibodies. In previously reported series, the APF was present in 49% and 87% of RA patients with a specificity between 73% and 99%. AKA was detected positive in 36% to 59% of the RA patients, and its specificity was 88% to 99. In this article, we have reviewed characteristics, significance in the pathogenesis and the diagnostic use of these two antibodies in RA.

Key Words: Rheumatoid Arthritis, Autoantibodies, Antiperinuclear Factor, Antikeratin antibody.

INTRODUCTION

Rheumatoid Arthritis (RA) is a systemic autoimmune disease of unknown etiology. The disease is characterized by chronic inflammation of the joints eventually resulting in joint deformation that can lead to severe disability. RA is the most common of the inflammatory joint diseases, affecting about 1 % of the world population (1). The diagnosis of RA depends primarily on clinical manifestations of the disease, with only limited serological support. The only serological test routinely used is to determine the presence of rheumatoid factors (RF) in the serum. RF can be detected in up to 70-90 % of RA patients, but is also detected in relatively high percentages in other autoimmune and infectious diseases, and in healthy individuals (2). Antibodies of a more specific nature have also been found in sera of RA patients (3). Two of the such antibodies are the antiperinuclear factor (APF) and antikeratin antibody (AKA). In this article, we have reviewed characteristics, significance in the pathogenesis and the clinical utility of these two antibodies in RA.

WHAT IS APF?

APF, first described in the mid-1960s by Nienhuis and Mandema (4), is an autoantibody that recognizes cytoplasmic granules in human buccal mucosa cells. Antibody staining of cells by indirect immunofluorescence (IIF) produces characteristic pattern denoted by the presence of several brightly fluorescent, round, homogeneous-appearing granules, 0.5-4.0 µm in diameter, located in the cytoplasm around the
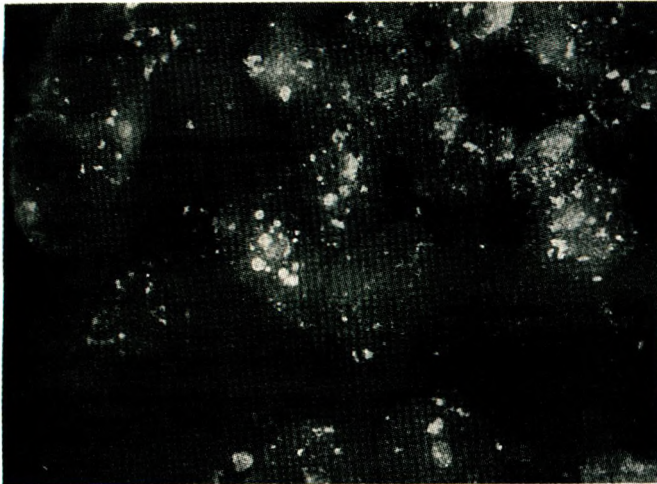
Fig. 1.: Perinuclear staining of kerohyaline granules with IIF (4).

nucleus (5) (Fig. 1). These APF factor antibodies, mostly of the IgG type, are reported to be present in 49-87 % of RA patients with a specificity between 73 and 99 % (6).

Human oral mucosa is mostly lined by nonkeratinized squamous epithelium of "lining mucosa" (7). The palate, gingiva, and dorsum of the tongue are covered by keratinized epithelium of the "masticatory mucosa". In keratinizing epithelium, the cells form a stratum granulosum. Whereas, the nonkeratinized squamous epithelium lacks the stratum granulosum and stratum corneum; the surface cells retain their nucleus, and the cytoplasm does not contain keratin filaments. The cells of stratum granulosum have numerous intracytoplasmic granules, called keratohyaline granules. The APF antibody binds to cytoplasmic granules in human buccal mucosa cells, which have presumptively been termed "keratohyaline granules" based on their rough histologic resemblance to the keratohyaline bodies in the stratum granulosum of human epidermis. APF-reactive granules bear little resemblance to the 3 oral mucosal cell granules previously described (5).

The keratohyaline granules in buccal mucosa cells are periodic acid-Schiff-positive (PAS) and methylene blue-positive, and stain basophilic (more intensely than nuclei) with hematoxylin and eosin (HAE) and Papanicolaou stains (5,8). With oil red O, the granules stain bluish-green like nuclei, rather than the red that indicates lipids, and do not stain with Sudan Black B

(granular leukocytes), Feulgen's (DNA), methyl green-pyronin (DNA and RNA), or acid phosphatase. At electron microscopy, at higher magnification, the granules appear round or oval, with slightly irregular borders, and are electron dense and inhomogeneous. They measured 0.2-3 μm in diameter and frequently contained interdigitating membrane-like structures that resemble aggregates of rough endoplasmic reticulum. The staining characteristics of the granules most closely resemble those of nuclei. DNA staining by Hoechst bisbenzimide, methyl green-pyronin, and Feulgen's is not detected, thus making a protein, glycoprotein (PAS-positive), or ribonucleoprotein (Bernard stain positive) the most likely candidates for the antigen (5,8).

WHAT IS AKA?

AKA is an autoantibody against the stratum corneum of rat esophagus. The occurrence of AKA in RA patients was first described by Young et al. in 1979 (9). Positive reactions in RA sera has ranged from 36 % to 59 % with a specificity of 88 % to 99 % (10, 11). AKA has been detected occasionally in systemic rheumatic diseases, and seldom in the sera of healthy subjects (0% to 3%) (10, 11).

THE NATURE OF ANTIGEN FOR APF AND AKA

Colocalization of APF and profilaggrin in human oral mucosa cells was first detected by Hoet et al. In their study, the APF and a monoclonal

antibodies to human filaggrin produced an identical staining pattern by IIF (12). Subsequent studies confirmed profilaggrin or one of its variants as the target of APF in human oral (and vaginal) cells (13-16). Simon et al. (17) gathered convincing evidence that human filaggrin is the target recognized by antistratum corneum antibody (AKA) by using Western blot testing. They purified a 40 kD antigen from human epidermis proteins, which reacted with antistratum corneum antibodies and with a monoclonal antibody to human filaggrin (AKHI). This protein was recognized by six different antifilaggrin antibodies and its amino acid composition was found to be similar to that of filaggrin. It is identified as a neutral/acidic isoform of filaggrin (16).

Filaggrin (filament-aggregating protein) is synthesized as a large insoluble heavily phosphorylated precursor protein (profilaggrin) consisting of 10-12 filaggrin repeats (16). Expression of profilaggrin is thought to occur only in the outermost layers of keratinized or semikeratinized epithelia, where it is stored in the keratohyaline granules. During the proteolytic cleavage in the differentiation phase of the cells, which releases filaggrin, profilaggrin is dephosphorylated and about 20 % of its arginine residues converted into citrulline. It was recently shown that autoantibodies reactive with synthetic peptides (same amino acid sequences deduced from known cDNA sequences of human profilaggrin) containing an unusual amino acid, citrulline, are specifically present in RA sera. By ELISA 76 % sensitivity and 96 % specificity were obtained (11).

AKA and APF might be directed to immunologically related antigens, since the antigens of both antibodies are localized in similar types of squamous epithelium and most AKA-positive RA sera are also APF positive (12). The reverse is not true because the APF test is more sensitive than the AKA test. These two antibodies might measure different epitopes of a single antigen. In a recent study by Vincent et al. (18) sensitivity, specificity of APF, AKA, and antibodies to human epidermis filaggrin (AFA) were similar (0.52 and 0.97 respectively). Despite the correlations between APF, AKA, and AF titres, the three antibody populations did not totally overlap, and 19 RA serum samples have significant APF titres and undetectable AFA. Authors concluded that the population of antifilaggrin autoantibodies constituted by the three overlapping APF, AKA, and AFA antibody families, thus recognises a spectrum of epitopes, probably all presented by the eliciting antigen, but only partially shared by the three antigens used for APF, AKA, and AFA detection (18).

THE LOSS OF TOLERANCE TO FILAGGRIN

Berthelot et al. suggest that the presence of antibodies to keratinized epithelia in RA patients may only be a consequence of the abnormal necrosis of filaggrin-containing cells, with no pathogenic significance. The filaggrin-rich cells that undergo abnormal lysis may be epithelial cells in the thymic medulla rather then cells in the semi-mucosal epithelia (16).

Another hypothesis involves a low level of transcription of the profilaggrin gene in the target organs of RA, including synovial cells and chondrocytes in normal conditions. Friction significantly increases the expression of filaggrin in the semi-keratinized hamster cheek pouch epithelium, and synovial cells and chondrocytes (19). Profilaggrin is also shown expressed in the foci of epithelial metaplasia seen in Hashimoto's thyroiditis, in which APF can be detected (20).

A number of other hypotheses were proposed by Berthelot et al. to explain the loss of B-cell tolerance to filaggrin (16). (1) Antistratum corneum antibody and APF may have anti-idiotype significance. (2) There would be a cross-reaction with an endogenous or exogenous antigen, although there are few sequence homologies between the profilaggrin gene and other genes; among known homologies, the most significant for rheumatologists is that involving the primary sequence of procollagen genes. Primary sequence homology is greatest with the E2 protein of the human papillomavirus 47. (3) Close association of filaggrin with an antigen may cause an increase in antifilaggrin antibodies after internalization of the complex by antifilaggrin B-cells. (4) Filaggrin may form a complex with one or several RNAs. Association of the cytoskeleton with nontranscribed messenger RNAs for a number of viruses has been reported. (5) It is possible that none of the above mentioned

hypotheses are valid, or that all are valid to some extent; for instance, a virus encoding proteins characterized by either a resemblance to filaggrin or the ability to form complexes with filaggrin might infect filaggrin-rich cells, thereby increasing the exposure of filaggrin or filaggrin peptides to the immune system.

DIAGNOSTIC SIGNIFICANCE

No single currently available clinical or laboratory criterion is pathognomonic of RA. The presence of four of the seven criteria selected by the American College of Rheumatology (ACR) in 1987 is the most widely accepted definition of RA and RF is the only laboratory marker included among the criteria (21).

RHEUMATOID FACTOR

RF is a useful diagnostic tool. It is reported as positive in 70% to 90% of patients with RA (2). However, the specificity of this test and the circumstances under which it is ordered affect its utility. RF would be positive in up to 5% of healthy people and in some rheumatic and nonrheumatic diseases (2). While usually in low titer, the incidence of RF positivity increases from less than 5% among patients aged under 55, to as high as 25 %, in those aged over 70, without regard to joint disease (2). Further complicating the interpretation of the RF is the sizable subset of patients with RA who are RF-negative and the occasional patient with a seronegative polyarthritis (such as spondyloarthropathy or polyarticular crystal-induced disease), that is clinically indistinguishable from seronegative RA at initial presentation. The clinical usefulness of RF therefore depends largely on the clinicians' ordering behavior and ability to interpret test results in the light of the clinical context.

The RF used in clinical practice is an IgM antibody directed against IgG. It has traditionally been detected by agglutination with sheep red blood cells (SCAT or Waaler-Rose test) or latex particles attached to human IgG. Results are expressed in antibody titers as the dilution required to eliminate reactivity. More recently, radioimmunoassays, enzyme-linked immunosorbent assays, or nephelometric techniques have been utilized, with the purported advantages of quantifiable results, and improved sensitivity, specificity, and reliability (2, 22). "Hidden" IgM, RF detected by assays other than

traditional agglutination tests, or RF of an IgG, IgA, or IgE isotype is said to be present in some otherwise seronegative patients with RA (2, 22). However, assays for hidden RF's are not readily available and their clinical significance is unclear.

Several elements contribute to the diagnostic utility of a test, like the prevalence of the disease in a given population. Age, gender, and family history are among the other factors that affect the likelihood of RA for an individual. The clinician's estimated likelihood that RA is present (the pretest probability of disease) determines in large part the RF's ability to aid in diagnosis. Using assumptions of a 1% pretest probability (based on disease prevalence in the general population), a test sensitivity of 80 %, and a test specificity of 90 %, Shmerling and Delbanco estimated the positive predictive value of RF (likelihood of RA based on a positive RF) to be only 16 % (2).

APF, DIAGNOSTIC USE

The main reason for disuse of APF in practice may have been the difficulty in obtaining appropriate substrate material. Human buccal mucosa cells have been used as substrate in IIF. These cells can be obtained from healthy donors by scraping the inside of their cheeks, generally with a wooden tongue depressor. Unfortunately, the suitability of particular human buccal mucosa epithelial cells is unpredictable. It has been suggested that a good donor should have at least 50% of his buccal mucosa cells stained positive for the antigen with IIF (this is the case in about 10% of population) (23). The sera are regularly scored positive when at least one buccal cell is found eliciting a conspicuous perinuclear immunofloerescein array. Some authors state a risk that any artifact could be mistaken for a stained granule, so it was suggested that at least 10% of the cells have to be identified by the test serum to be recorded as positive (5). Many authors do not titrate serum and their interpretation is all-or-nothing. Some authors suggest a titration of 1/80 or 1/100 to increase specificity (5,24). In an attempt for standardization, 5 European groups set up a consensus study on the interlaboratory variability of the APF test (25). Despite the use of different cells, conjugates and criteria for positivity, their results were comparable. The

effects of time and/or freezing on the validity of the substrate are well demonstrated: in a study by Berthelot et al. (25) when the slides were not frozen the results of the APF test dropped from three dilutions at one week for the four sera tested (normally diluted as 1/100); when the slides were frozen (either - 25 or - 80°C) the decrease in titers was less dramatic, dropping from only one dilution at one week, and three dilutions at two weeks. It was suggested thus that the test must be performed rigorously and within 24-48 hours after cell fixation.

This autoantibody has been described in 10 to 25 % of other autoimmune disorders (mainly Sjogren's syndrome, lupus, and thyroiditis) (23, 25). In a study by Berthelot et al. (26) at the 1:100 dilution, 204/251 (81%) RA, 8/63 (13%) unclassified polyarthritis and 33/690 (5%) non-RA (including 5/77 lupus, 4/50 Sjogren's syndrome, 0/10 scleroderma, 4/63 psoriatic arthritis, 3/40 vasculitis, 0/48 monoclonal gammopathies, 5/58 thyroiditis, 0/12 polymyalgia rheumatica and 2/27 ankylosing spondylitis) were found positive for the APF. The APF titer in these conditions were lower than in RA, and only rarely exceeded 1/500.

The diagnostic potential of the APF test, i.e., the relative equilibrium between sensitivity [48.6% (4) to 86.6 % (26)] and specificity [72.7 % (5) to 99 % (4)] would be beneficial in RA. High specificity would make the test suitable, especially to rule in the disease. Correlation between the presence and titer of APF and severity of disease have been inconsistent (27). The APF is present in about 40% of RF seronegative-RA patients and may be useful as an additional serological indicator. The presence of APF in seronegative RA patients is associated with more severe disease, higher American Rheumatism Association functional class, more extra-articular features per patient and more rapid radiological changes (28). APF-IgG is detectable early in the disease, generally before the four ACR criteria. In a study by Berthelot et al. (29) 45% of patients were APF positive (>1:100) before ACR criteria were fulfilled. When four criteria were present for the first time, the APF titer was already 1:100 in 72% patients for an average of 7.1 months, and RF was then positive

in only half of the cases (Rose-Waaler or latex). This was in agreement with the findings of Aho et al. (10) who showed that APF could be present in the sera of RA patients several years before the onset of the first clinical signs, and with the conclusion of Saraux et al., who reported that APF 1:80 was more useful than RF for the diagnosis of early RA (16).

AKA, DIAGNOSTIC USE
The AKA is detected by IIF staining of stratum corneum in the rat esophagus. AKA produces a linear lamellar staining of stratum corneum. Other staining patterns which can occur in this tissue but are not restricted to RA, must be excluded (30). IgG AKA gives high specificity for RA. The presence AKA is associated with raised C reactive protein, sedimentation rate, IgM RF, subcutaneous nodules and functional criteria such as Steinbrocker's index but not with age, sex, disease duration or other laboratory parameters (30). AKA would be detected in up to 1/3 of seronegative patients and precede the disease (10, 31).

CONCLUSION

Although APF and AKA have been known for years with very favorable results in RA, they have not been widely used. Considering their suggested specificity in RA, they could play a valuable role to establish the diagnosis of RA. They would also be useful in seronegative-RA patients. APF test would be cost-effective, but to find a donor and continuous supply of substrate would be regarded as a problem in clinical settings. Using several "good donor" interchangeably would help to use this test routinely (32). An AKA test kit has become available commercially in the United States of America and given Food and Drug Administration approval for research only (32). However, the sensitivity of the test is low and the stability of the antigen used in these kits has not been evaluated by long-term studies. Clearly practical utility of these antibodies requires isolation and possibly synthesis of the antigens they have targeted. Studies on citrulline containing peptides have been going on for a while and give hope in that regard (33).

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