Antiperinuclear Factor In Various Rheumatologic Disorders

Thesis

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By

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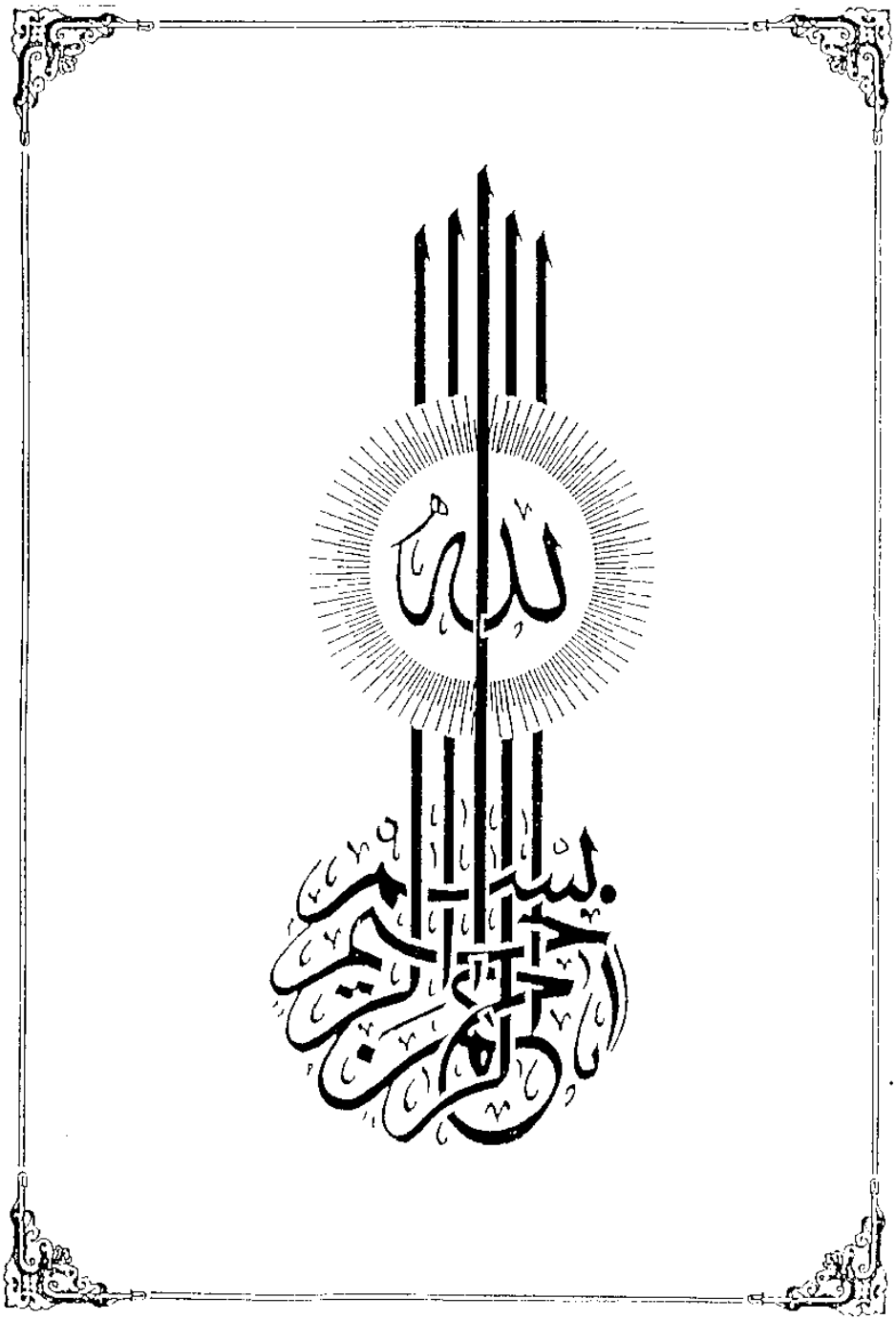

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The candidate
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List of Abbreviations

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APF	Antiperinuclear factor
ARA	American rheumatism association
EBV	Epstein. Barr virus
IFT	Immunofluorescence test
JRA	Juvenile rheumatoid arthritis
PBS	Phosphate buffered saline
PMR	Polymyalgia rheumatica
PG-PSP	Peptidoglycan - Polysaccharide polymers
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RW	Rose Waaler
SLE	Systemic lupus erythematosus

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INTRODUCTION

AND

AIM OF WORK

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Introduction :

Serum samples from patients with connective tissue diseases often contain antibodies against cellular components. In some cases the presence of these auto antibodies is specifically associated with a certain syndrome or disease, and therefore useful as diagnostic marker. *[Tan E.M., 1989]*

Serological support for the diagnosis of rheumatoid arthritis is not well established and mainly based on the presence of rheumatoid factor. Rheumatoid factor is not present in all patients with rheumatoid arthritis, however, and can also be found in serum samples from patients with other (auto. immune) diseases and even in healthy subjects. *[Waller et al.; 1964]* Therefore the availability of another specific serological marker for rheumatoid arthritis would be useful.

Three other antibody specificities have been described as being specific for rheumatoid arthritis, all three directed against different components of epithelial cells - namely, antibodies against intermediate filaments in cultured cells, antibodies against a keratin like component in rat oesophageal epithelium, and the antiperinuclear factor, consisting of antibodies directed against a component in human buccal mucosa cells. *[Kataaha P.K., et al., 1985]*

The antiperinuclear factor (APF) was originally described by *Nienhuis and collaborators [1964]* who demonestrated its high specificity for rheumatoid arthritis. The antibodies, mostly of the IgG type, are directed against a protein in the 0.5 - 4 µM spherical shaped keratohyalin granules in the cytoplasm of buccal mucosa cells and are found in 48 - 86% of serum samples and synovial fluids from patients with rheumatoid arthritis *[Nienhuis R.L.E., et al., 1964]*. Many reports have suggested the use of the antiperinuclear factor assay as a diagnostic test for rheumatoid arthritis. This was based on observations of a high prevalence of antiperinuclear factor in both seropositive and seronegative rheumatoid arthritis patients and the infrequent occurrence of antiperinuclear factor in other groups.

Aim of the work :

To assess the diagnostic significance of the antiperinuclear factor for rheumatoid arthritis.

REVIEW OF LITERATURES

The perinuclear antigen

* Nature, Ultrastructure :

The chemical nature of the antigen was investigated by testing the effect of several chemical and enzymatic treatments on it. After these treatments a normal antiperinuclear factor immuno-fluorescence test was performed. The results indicate that the perinuclear factor is an insoluble protein, sensitive to freezing and thawing. Fixation of the cells with methanol or acetone partly destroys antigenicity. Treatment of the cells with Triton-X 100 not only retains the perinuclear factor but intensifies the immunofluorescence staining. *[Smit J., et al., 1980]*

Light microscopic techniques have shown that the perinuclear factor is located in the keratohyalin granules. Nothing is known about the intragranular distribution of the antigen, however. Using an immunoelectron microscopical approach established that the perinuclear factor is localized in the keratohyalin granules of buccal mucosa cells. The whole body of the poorly structured granules seemed to contain the antigen, supporting the idea that these granules are an amorphous mixture of densely packed proteins. Only treatment with proteolytic enzymes seems able to untangle and degrade this mass, whereas denaturing by fixatives renders it even more inaccessible for antibodies. Until now not much was known about the biochemical composition of the keratohyalin

granules in buccal mucosa cells. The protein (pro) filaggrin in these granules was identified and it was found to be co-localised with the perinuclear factor. *[Hoet RMR., et al., 1991]*

Ultrastructural criteria indicate that the APF-reactive cytoplasmic granules resemble aggregates of rough endoplasmic reticulum containing lipid droplets and vesicles, which suggests that granule formation during cell maturation occurs as a result of segregation or exclusion of cytoplasmic elements by space occupying tonofilaments. The presumptive APF antigen may represent an altered or unique cellular protein (e.g., cell - surface receptor) that is synthesized in the rough endoplasmic reticulum but is found only in certain individuals or only in certain cells. Alternatively, the antigen could be a soluble product that is present in every cell but is not easily recognized by immunofluorescence until clumping of the rough endoplasmic reticulum occurs. This clumping process may also be unique to certain individuals and could potentially change either the concentration or configuration of the perinuclear antigen, thereby facilitating its detection in buccal cells. *[Frederick B. vivino et al., 1990]*

Prevalence of antigen in different cells and individuals :

At present, further use of APF assay on routine basis remains unfeasible for several reasons. An abundant source of perinuclear granule - bearing cells is not always readily available, and the "quality" of donors may vary. *Sondag - Tschroots I., et al [1979]* observed that only 10% of potential human donors have the perinuclear antigen in amounts sufficient for assay purposes. Similarly, *Johnson, et al [1981]* reported that human buccal mucosal cells from only 3 of 27 potential normal donors showed stained inclusions when treated with APF - positive serum. *Janssens X, et al., [1988]* found only 2 satisfactory donors among 18 individuals tested. The significance of this difference among individuals and its relationship to connective tissue disease has not been explored further.

On a study done by *Youinou - P et al., [1990]*, to investigate the possible factors determining the expression of appropriate antigens by the cells. They failed to find any relationship of the expression of perinuclear antigens to donor's smoking habits, the degree of contamination with saprophytic bacteria, the presence or absence of blood group substances in saliva, or the titres of serum antibodies to Epstein - Barr virus. Family studies were also performed to further elucidate a genetic predisposition to the expression of the APF antigen.

Since fewer than 25% of cells from a single scraping appear to contain the perinuclear granules, large numbers of cells must be screened to avoid false - negative interpretation. This presents a

problem, since many cells tend to wash off the coverslips during the assay. The success is limited in using various fixatives and cell adhesives to alleviate this problem. *[Frederick B. et al., 1990]*

Improvement and standardization of current assay techniques are necessary before regular use of APF determination for diagnostic purposes can be considered.

On a study done by *Hoet RMA. et al., 1991;* the test was modified by pretreating the buccal mucosa cells with 0.5% Triton - X 100. It was found that at least 70% (35/50) of normal healthy subjects have the perinuclear factor in their buccal mucosa cells and therefore are potential positive donors. To test possible qualitative differences in antigen composition between different positive donors 10 of them were screened with 10 positive rheumatoid serum samples. Each of the 10 donors reacted with all positive serum samples. The percentage of cells that were stained varied between donors, depending on the titre of the serum, indicating that there are only quantitative differences in the amount of antigen among the different donors.

The perinuclear antigen in cultured and animal cells :

On a study done by *Hoet - RM, et al [1991]* to obtain a more reliable antigen source and to gain more insight into the origin and nature of the perinuclear factor they attempted to culture perinuclear factor - containing buccal mucosa cells. They had successful culturing of such cells, which however, did not contain keratohyalin granules nor the perinuclear factor. They were able to induce keratohyalin granules in both cultured primary buccal mucosa cells and squamous carcinoma cell line of the cheek. These induced keratohyalin granules do contain the protein profilaggrin, which in vivo, in fresh buccal mucosal cells, co-localizes with the perinuclear factor. However, they were not able to demonstrate the presence of the perinuclear factor, not even after induction of terminal differentiation of the cultured cells nor after E.P virus infection. So, they suggested that the PF, in contrast to profilaggrin, is not an integral component of buccal mucosa cells.

It was found that human vaginal epithelial cells and cryostat sections of human and rabbit buccal and oesophageal mucosa incubated with APF +ve sera showed the same fluorescent granules in the IFT as human buccal mucosa cells. Cryostat sections of rabbit buccal and oesophageal mucosa were tested as an alternative substrate in the APF test. Since the specificity as well as the sensitivity decreased when these sections were used as a substrate, they are not suitable for diagnostic purposes. *[Smit JW, et al., 1980]*