Antiperinuclear factor, a marker autoantibody for rheumatoid arthritis: colocalisation of the perinuclear factor and profilaggrin

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Abstract
The antiperinuclear factor, an autoantibody specific for rheumatoid arthritis, was found in 51/63 (81%) patients with rheumatoid arthritis by indirect immunofluorescence on human buccal mucosa cells. The sensitivity of the antiperinuclear factor test was increased by pretreating the buccal mucosa cells with 0-5% Triton-X100. The specificity of the test for rheumatoid arthritis as compared with control serum samples was maintained.

The localisation of the perinuclear factor in the keratohyalin granules of the buccal mucosa cells was verified by immunoelectron microscopy. The perinuclear factor was found to be an insoluble protein whose antigenicity was sensitive to various fixation procedures. In serum samples from patients with rheumatoid arthritis there was a positive correlation between the presence of antiperinuclear factor and the presence of the so-called antikeratin antibodies as detected by immunofluorescence on unfixed rat oesophageal cryostat sections. No relation was found between the presence of the perinuclear factor and either the rheumatoid factor, Epstein-Barr virus components, or any cytokeratin. By double immunofluorescence an exact colocalisation of the perinuclear factor and profilaggrin was found. Although the precise biochemical identity of the perinuclear factor remains unclear, our results suggest that it is a protein only present in the fully differentiated squamous epithelial cell layer.

Serum samples from patients with connective tissue diseases often contain antibodies against cellular components.1 In some cases the presence of these autoantibodies is specifically associated with a certain syndrome or disease, and therefore useful as diagnostic marker. Good examples of such markers are anti-Sm antibodies in systemic lupus erythematosus and anti-Jo-1 in patients with polymyositis.1 Serological support for the diagnosis of rheumatoid arthritis is not well established and mainly based on the presence of rheumatoid factors. Rheumatoid factor is not present in all patients with rheumatoid arthritis, however, and can also be found in serum samples from patients with other (autoimmune) diseases and even in healthy subjects.2 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.

All three specificities are detectable by indirect immunofluorescence. Antibodies against intermediate filaments in rheumatoid serum samples have specificity for the intermediate filament proteins vimentin or cytokeratin, or both, in cultured cells and are mostly of the IgM class.2-3 Antibodies against vimentin have been found in 54-80% of patients with rheumatoid arthritis,4-6-8 and antibodies against cytokeratin in 31-40% of such patients.4-8 Antibodies against intermediate filaments, however, are not particularly specific for rheumatoid arthritis and are often present in other diseases also.4 6-9 10 and have even been found in 14-63% of healthy subjects.4-11

Antibodies against keratin, detectable by immunofluorescence on rat oesophagus tissue, and first described in 1979 by Young and coworkers,12 are found in 36-69% of rheumatoid serum samples and in only 0-4-2% of healthy control serum samples.4 6-12 18 These antibodies are predominantly of the IgG class and can also be found in synovial fluid of patients with rheumatoid arthritis.14

The antiperinuclear factor was originally described by Nienhuis and collaborators,19 who demonstrated 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 samples19-32 and synovial fluids from patients with rheumatoid arthritis.19 Westgeest et al found that antiperinuclear factor titres correlate with the severity of disease,21 and this is most clearly seen in patients without the rheumatoid factor. An increased prevalence of antiperinuclear factor has been described in certain other syndromes.22-25 31

The major drawback of the antiperinuclear factor immunofluorescence test is the nature of the substrate. Most authors report that only a small percentage of buccal mucosa cell donors have antigen present in a satisfactory amount.16 22 26-28

In this study we modified and improved the sensitivity of the antiperinuclear factor test and reassessed its specificity. Additionally, new data on the localisation and characterisation of the perinuclear factor are presented, and the pos-
sible relation between perinuclear factor and other antibody specificities was investigated.

**Materials and methods**

**PATIENTS SERUM SAMPLES**

Most serum samples were obtained from patients seen at the department of rheumatology and internal medicine of the Academic Hospital, Nijmegen. Diagnoses were reached as described by de Rooij et al. Serum samples from patients with Lyme's disease were kindly provided by J Hardin (Yale University, New Haven, USA). All samples were stored at −70°C until required for analysis.

**MONOCLONAL AND POLYCLONAL ANTIBODIES**

The following monoclonal (m) and polyclonal (p) antibodies were kindly provided by Dr F Ramaekers: RCK102 (m) recognizing cytokeratin (ck) 5 and 8; RCK105 (m) recognizing ck 7; RGE53 (m) recognizing ck 18; RKSE60 (m) recognizing ck 10; RV202 (m) recognizing vimentin. 6B10 (m) recognizing ck 4, and 2D7 (m) recognizing ck 13 were provided by Dr G van Muijen. Monoclonal antibody 41CC4 directed against laminin A, B, and C was a kind gift of Dr J Warren. Anti-EBV-VCA (m) (Epstein-Barr virus virial capsid antigen), anti-EBV-EA(D)/m (Epstein-Barr virus early antigen diffuse component), and anti-P62 (p) (human P62 cross reacting with Epstein-Barr nuclear antigen-1) were kindly provided by Dr P J W Venables (London). AKH1, a monoclonal antibody against human profilaggrin (>400 kD) and filaggrin (37 kD), and AKH2, a monoclonal antibody reacting with an unidentified component of keratohyalin granules were kindly provided by Dr B Dale. Polyclonal serum anti-54 kD, recognizing a histidine-rich protein of rat granular cells was kindly provided by Dr K Fukuyama and monoclonal 3F6-6 (anti-rat filaggrin) was a gift of Dr I A Bernstein. Other antibodies were purchased from ICN Immuno Biologicals (monoclonal AE2 reacting with ck 1, 2, 10, 11, and with filaggrin), Dakopatts (A575, rabbit serum against human epidermal cytokeratin), and Biomakor (K8.12, monoclonal against ck 13 and 16). Monoclonal antibodies against human snRNPs proteins were obtained from Drs S O Hoch (2-73), J Steitz (Y12), and W Habets (9A9 and 4G3). D106 is a human antibody directed against topoisomerase I obtained from a patient with scleroderma.

**ANTIGENIC SUBSTRATE FOR THE ANTIPERINUCLEAR FACTOR**

Epithelial cells from human buccal mucosa from a positive donor were scraped from the inside of the cheek with a piece of foam plastic and brought into suspension by rinsing the foam with phosphate buffered saline (PBS), pH 7.4. The cell suspension was washed twice with PBS, once with 0.5% Triton-X100 in PBS, and again with PBS (five minutes' centrifugation at 800 g). A cell suspension was made in PBS and the cells were either spun down on microscopic slides with a cytopsin centrifuge or were transferred dropwise to microscopical slides and after air drying immediately used as substrate in the immunofluorescence test (100–400 cells per slide). Slides could be stored for up to two weeks at −70°C.

**DETECTION OF ANTIPERINUCLEAR FACTOR BY IMMUNOFLUORESCENCE**

The slides with buccal mucosa cells were incubated with the serum (diluted 1:5 with PBS, cleared by five minutes' centrifugation at 13,000 g) for 90 minutes in a 100% humid atmosphere. The preparations were washed in PBS (3×10 minutes) and incubated for 30 minutes with purified fluorescein isothiocyanate labelled rabbit antibody against human IgM, IgG, and IgA (Dakopatts F200, dilution 1:100). After extensive washing with PBS (3×10 minutes) the preparations were mounted in a glycerol/PBS (1:1) solution with ethidium bromide (0.5 μg/ml) as a nuclear counterstain. The slides were read under an Olympus fluorescence microscope.

**DETECTION OF ANTikeratin ANTIBODIES BY IMMUNOFLUORESCENCE**

Detection of antikeratin antibodies was performed essentially as described by Young and coworkers, 12 with a few modifications. Rat oesophagus cryostat sections were incubated for five minutes in 0.5% Triton-X100 in PBS, and then washed for five minutes in PBS before incubation with the serum. Serum samples were tested in a dilution of 1:10, and fluorescein isothiocyanate labelled rabbit antibody against human IgG, IgM, and IgA (Dakopatts F200; dilution 1:100) was used as conjugate.

**RHEUMATOID FACTOR**

Serum samples were tested for rheumatoid factor by the medical microbiology department of the Academic Hospital, Nijmegen, using the Waaler-Rose and latex fixation tests. These agglutination assays were performed as a microtitre modification. All patients scored as rheumatoid factor negative had a maximum titre of only 1/32 (Waaler-Rose test) or 1/40 (latex fixation test).

**PREPARATION OF CELL EXTRACTS**

Extracts of human foreskin epidermis were prepared as described by Fleckman et al for human epidermis, 46 and finally dissolved in S buffer (8 M urea, 50 mM TRIS, HCl (7.6), 100 mM dithiothreitol, 0.13 M 2-mercaptoethanol, 100 μg/ml phenylmethylsulphonyl fluoride, 100 μg/ml aprotinin). After centrifugation (10 minutes, 13,000 g) 1/4 volume of 4× sodium dodecyl sulphate sample buffer 47 was added. Buccal mucosa cells were first treated with 0.5% Triton-X100 in PBS and after centrifugation (10 minutes at 800 g) dissolved in S buffer. Finally, 1/4 volume 4× sodium dodecyl sulphate sample buffer 47 was added.
GEL ELECTROPHORESIS AND IMMUNOBLOTTING
Sodium dodecyl sulphate/polyacrylamide gel electrophoresis, transfer of proteins from polyacrylamide gels onto nitrocellulose sheets, and detection of the antigens on the blots were performed as described.50 51

IMMUNOELECTRON MICROSCOPY
Buccal mucosa cells were spun down on microscopic slides coated with a plastic sheet (Melinex, ICI, Herts, UK) and immediately fixed in sodium cacodylate buffered 1% paraformaldehyde for one minute at 4°C. After rinsing the cells for 10 minutes in PBS the endogenous peroxidase activity was blocked with 0-9% H2O2 in PBS for five minutes, washed for 10 minutes with PBS, preincubated for 20 minutes with 10% normal rabbit serum, immediately followed by one hour’s incubation with a rheumatoid serum sample containing antiperinuclear factor (diluted 1:50). Extensive washing with PBS (3×5 minutes) was followed by 30 minutes’ incubation with peroxidase conjugated rabbit antihuman IgG (Dakopatts, diluted 1:80). After three additional five minute washing steps with PBS the cells were stained for five minutes with 0-65% imidazole in PBS with 0-0015% H2O2. The staining was quenched in water and the cells were postfixed for 10 minutes in 2-5% glutaraldehyde in 0-1 M sodium cacodylate buffer. After rinsing for 10 minutes in 0-1 M sodium cacodylate buffer the cells were treated for 30 minutes with 1% OsO4 in Palade buffer (70 mM sodium acetate, 70 mM sodium-veronal, pH 7-4), washed for 2×10 minutes in Palade buffer, and after dehydration with successively ethanol 70%, 90%, 100% and propylene oxide, embedded in Epon 812. Thereafter a ‘pop-off’ method was used essentially as described by Ruiter et al.32 Semi-thin sections were light microscopically examined for the presence of immunostaining. Ultrathin sections were made from areas selected, not contrasted, and examined and photographed on a Philips 300 electron microscope at 40 kV.

Results
THE ANTIPERINUCLEAR FACTOR TEST AND ITS RELIABILITY
For detection of the antiperinuclear factor most investigators19–32 have used unfixed buccal mucosa cells because fixatives have a negative effect on the antigenicity of the perinuclear factor. We modified the antiperinuclear factor test by pretreating the cells with 0-5% Triton X-100 in PBS. This pretreatment increased the percentage of rheumatoid patients with the antiperinuclear factor from 73% (46/63) to 81% (51/63), whereas the percentage of healthy controls remained unchanged (6% (3/51) in both cases). In our hands the modified test was not only more sensitive but also more reproducible. Figure 1 shows a typical reaction of a buccal mucosa cell with a serum sample positive for antiperinuclear factor. Table 1 summarises the percentages of serum samples positive for antiperinuclear factor from patients with rheumatoid arthritis and healthy subjects (controls) described in other reports and gives our own data obtained with the modified antiperinuclear factor test. The sensitivity of antiperinuclear factor for rheumatoid arthritis was 81%. Investigation of the specificity of the antiperinuclear factor (table 2) showed that the prevalence of antiperinuclear factor is also somewhat higher in serum samples from patients with systemic lupus erythematosus (21%), systemic sclerosis (26%), and Sjögren’s syndrome (29%).

Table 1  Percentages of serum samples positive for antiperinuclear factor in patients with rheumatoid arthritis and healthy controls summarised from published reports

<table>
<thead>
<tr>
<th>First author</th>
<th>Year/ref</th>
<th>Rheumatoid serum samples (n)</th>
<th>APF*(+) (%)</th>
<th>Control serum samples (n)</th>
<th>APF*(+) (%)</th>
<th>Serum dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nienhuis</td>
<td>1964/19</td>
<td>105</td>
<td>49</td>
<td>431</td>
<td>&lt;1</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Marmont</td>
<td>1967/29</td>
<td>100</td>
<td>51</td>
<td>58</td>
<td>0</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Smit</td>
<td>1979/22</td>
<td>103</td>
<td>78</td>
<td>111</td>
<td>4</td>
<td>1:5</td>
</tr>
<tr>
<td>Johanson</td>
<td>1980/20</td>
<td>97</td>
<td>79</td>
<td>96</td>
<td>4</td>
<td>1:5</td>
</tr>
<tr>
<td>Youinou</td>
<td>1981/16</td>
<td>102</td>
<td>81 (92)†</td>
<td>60</td>
<td>14 (20)†</td>
<td>1:5</td>
</tr>
<tr>
<td>Katsaka</td>
<td>1983/26</td>
<td>178</td>
<td>68</td>
<td>67/76‡</td>
<td>7/12‡</td>
<td>1:5</td>
</tr>
<tr>
<td>Westgreen</td>
<td>1984/4</td>
<td>72</td>
<td>74</td>
<td>93</td>
<td>3</td>
<td>1:5</td>
</tr>
<tr>
<td>Janssens</td>
<td>1987/21</td>
<td>132</td>
<td>52</td>
<td>54</td>
<td>2</td>
<td>Undiluted</td>
</tr>
<tr>
<td>Our own results</td>
<td>This paper</td>
<td>127</td>
<td>86</td>
<td>262§</td>
<td>45</td>
<td>1:5</td>
</tr>
</tbody>
</table>

*APF=antiperinuclear factor.
†The value in parentheses also include weak positive serum samples.
‡Aged <60/>60 years.
§Other autoimmune diseases.

Figure 1  Immunofluorescent staining of a buccal mucosa cell with an antiperinuclear factor positive serum sample from a patient with rheumatoid arthritis. Immunofluorescence was carried out as described in ‘Materials and methods’. The nucleus (N) was counterstained with 0-5 μg/ml ethidium bromide.

Marked autoantibody for rheumatoid arthritis

1964/19 105 49 431 <1 Undiluted
1967/29 100 51 58 0 Undiluted
1979/22 103 78 111 4 1:5
1980/20 97 79 96 4 1:5
1981/16 102 81 (92)† 60 14 (20)† 1:5
1983/26 178 68 67/76‡ 7/12‡ 1:5
1984/4 72 74 93 3 1:5
1987/21 132 52 54 2 Undiluted
1988/28 127 86 262§ 45 1:5

*APF=antiperinuclear factor.
†The value in parentheses also include weak positive serum samples.
‡Aged <60/>60 years.
§Other autoimmune diseases.
In contrast with other studies we found that at least 70% (35/50) of normal healthy subjects (aged 18–35 years) 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 (data not shown). 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.

**POSSIBLE RELATION BETWEEN ANTIPERINUCLEAR FACTOR, RHEUMATOID FACTOR, AND ANTIKERATIN ANTIBODIES**

It has been suggested that the antikeratin factor might be a kind of rheumatoid factor. This possibility was tested by determining the correlation between rheumatoid factor and antiperinuclear factor (table 3). Although the percentage of serum samples positive for rheumatoid factor among patients with rheumatoid arthritis (83%) was comparable with the percentage positive for antikeratin factor (81%), there was no complete overlap. More than 11% of the serum samples without antikeratin factor had the rheumatoid factor, whereas more than 6% without rheumatoid factor contained the antikeratin factor. So it seems unlikely that the antikeratin factor is some kind of rheumatoid factor.

<table>
<thead>
<tr>
<th>Disease*</th>
<th>Number of serum samples</th>
<th>APF*(+) serum samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>63</td>
<td>81</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>33</td>
<td>21</td>
</tr>
<tr>
<td>Mixed connective tissue disease</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Undifferentiated connective tissue disease</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>34</td>
<td>29</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>42</td>
<td>26</td>
</tr>
<tr>
<td>Lyme's disease</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>51</td>
<td>6</td>
</tr>
</tbody>
</table>

*Diagnoses were reached according to published criteria.33

**LOCALISATION AND BIOCHEMICAL NATURE OF THE PERINUCLEAR FACTOR**

Light microscopic techniques have shown that the perinuclear factor is located in the keratohyalin granules.30 Nothing is known about the intragranular distribution of the antigen, however. Using an immunoelectron microscopic approach we established that the antigen is present throughout the keratohyalin granule (fig 2). 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 antikeratin factor immunofluorescence test was performed. The results (table 5) 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-X100 not only retains the perinuclear factor but intensifies the immunofluorescence staining. Table 6 shows the results of experiments with various specific antibodies that were tested on

**Table 4 Correlation between the so called antikeratin antibodies and the antiperinuclear factor in 63 patients with rheumatoid arthritis. Results are given as number of patients (percentage of the 46 patients)**

<table>
<thead>
<tr>
<th>Antikeratin antibodies (No (%))</th>
<th>Antiperinuclear factor (No (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>25 (54)</td>
</tr>
<tr>
<td>+/-</td>
<td>12 (26)</td>
</tr>
<tr>
<td>–</td>
<td>9 (20)</td>
</tr>
</tbody>
</table>

Correlation between antikeratin antibodies (+ v +/- and – results) was calculated with the $x^2$ test with Yates's correction. $x^2$=6.11, 0.025 $>$p>0.01.
buccal mucosa cells and rat oesophagus cryostat sections. Rat oesophagus epithelial layer gave a positive immunofluorescence staining with monoclonal antibodies 6B10, 2D7, and K8.12, which all recognise cytokeratins 4 and 13 known to be specifically present in squamous epithelium. These monoclonal antibodies (6B10, 2D7, K8.12) also gave a specific immunostaining with a rat oesophagus extract on western blot, whereas we could not identify a specific immunostaining with serum samples with and without antikeratin antibodies (data not shown).

The keratohyalin granules of the buccal mucosa cells did not react with polyclonal and monoclonal antibodies against intermediate filaments (cytokeratins, lamin, vimentin), Epstein-Barr virus proteins, and U snRNPs. In fact, only polyclonal and monoclonal antibodies against (pro)filaggrin gave a positive staining of the keratohyalin granules. A monoclonal antibody against human profilaggrin AKH1 was then used in a double immunofluorescence test with a number of serum samples strongly positive for antiperinuclear factor, and the results (figs 3A and B) show that in every cell an exact colocalisation of the perinuclear factor and profilaggrin could be seen. This exact colocalisation of profilaggrin with the perinuclear factor suggests that these two proteins may be identical. This possibility was tested in several ways.

Firstly, competition of the binding of AKH1 (antifilaggrin) by increasing concentrations of antiperinuclear factor antibody did not abolish the binding of AKH1 to the keratohyalin granules. Therefore another epitope of filaggrin or another antigen is recognised.

Secondly, when extracts of buccal mucosa cells on a western blot were probed with AKH1 (fig 4, lane 1) a band of filaggrin (37 kD) appeared. (Profilaggrin is an unstable protein and easily degrades to filaggrin.) Most of the serum samples containing antiperinuclear factor, however, stained the filaggrin band only weakly.

Table 6 Reactivity of buccal mucosa cells and rat oesophagus sections with various monoclonal and polyclonal antibodies

<table>
<thead>
<tr>
<th>Antibody code</th>
<th>Antigen</th>
<th>APF*</th>
<th>AKA*</th>
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<tbody>
<tr>
<td>α-EBV-VCA (m)*</td>
<td>EBV-VCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-EBV-D (m)</td>
<td>EBV-RA (D)</td>
<td></td>
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<tr>
<td>α-P62 (p)*</td>
<td>P62 (human)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCK102 (m)</td>
<td>CK* 5-8</td>
<td></td>
<td></td>
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<tr>
<td>A572 (p)</td>
<td>Epidermal CK</td>
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<td></td>
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<tr>
<td>RGE53 (m)</td>
<td>CK 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RKS60 (m)</td>
<td>CK 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6B10 (m)</td>
<td>CK 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2D7 (m)</td>
<td>CK 13</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>RCK105 (m)</td>
<td>CK 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K8.12 (m)</td>
<td>CK 13+16</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>RV202 (m)</td>
<td>Vimentin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41CC4 (m)</td>
<td>Lamin A, B, C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9A9 (m)</td>
<td>A/B (U snRNPs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4G3 (m)</td>
<td>B* (U snRNPs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y12 (m)</td>
<td>Sm (U snRNPs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,73 (m)</td>
<td>70 kD (U snRNPs)</td>
<td></td>
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<tr>
<td>D106 (p)</td>
<td>Topoisomerase 1</td>
<td></td>
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</tr>
<tr>
<td>AE2 (m)</td>
<td>Human filaggrin</td>
<td></td>
<td></td>
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<tr>
<td>AKH1 (m)</td>
<td>Human filaggrin</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>AKH2 (m)</td>
<td>KHG* epidermis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5F6-6 (m)</td>
<td>Rat filaggrin</td>
<td></td>
<td></td>
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<tr>
<td>α-54 KD (p)</td>
<td>Rat filaggrin</td>
<td>++</td>
<td></td>
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</tbody>
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*APF=antiperinuclear factor; AKA=antikeratin antibodies; m=monoclonal antibody; p=polyclonal antibody; ck=cytokeratin; KHG=keratohyalin granules.
or not at all. In fact only one human serum sample (fig 4, lane 8) out of 30 tested reacted strongly with filaggrin. We did not find other protein band(s) on a western blot that reacted reproducibly with antiperinuclear factor antibody.

Thirdly, in immunofluorescence studies antibodies against (pro)filaggrin react with their antigen in keratohyalin granules of human foreskin keratinocytes, whereas antiperinuclear factor did not. Protein blots containing foreskin keratinocyte extract also did not react with serum samples containing antiperinuclear factor (data not shown). Apparently keratohyalin granules of keratinised epithelium (human foreskin) contain (pro)filaggrin but not the perinuclear factor. We conclude that the perinuclear factor is not identical with (pro)filaggrin, though in buccal mucosa cells both proteins have the same localisation.

Discussion

The antiperinuclear factor has been described as a specific serological marker for rheumatoid arthritis. In this study the original test19 was modified by pretreating the cells with Triton-X100, which resulted in an increased sensitivity (73% v 81%) while maintaining a high specificity compared with healthy controls (table 1). The main reason for the increased sensitivity is the fact that the detergent treatment increases the permeability of the cell membranes for the antibody while at the same time the cytoplasmic background staining is decreased because all soluble components of the cells (proteins, most ribosomes, tRNA) and residual mouth flora are removed.

With this modified technique we established that the perinuclear factor is present in buccal mucosa cells of most people and that finding a 'good' donor for buccal mucosa cells is probably not a major problem. We also noted that the antiperinuclear factor can be found in somewhat higher prevalences in serum samples from patients with other autoimmune diseases (table 2). Although the possibility that these patients may be in the process of developing rheumatoid arthritis (most marker autoantibodies have a prognostic character4) cannot be completely excluded, other studies have also indicated higher prevalences of antiperinuclear factor in the serum samples of patients with systemic lupus erythematosus, systemic sclerosis, and Sjögren's syndrome than in those of healthy controls.22 23 30 31 Apparently the presence of antiperinuclear factor is not restricted to rheumatoid arthritis only.

Using an immunoelectron microscopical technique we confirmed that the perinuclear factor is localised in the keratohyalin granules of buccal mucosa cells (fig 2). The whole body of the poorly structured granule 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 (table 5). Until now not much was known about the biochemical composition of the keratohyalin granules in buccal mucosa cells. We identified the protein (pro)filaggrin in these granules and found it to be colocalised with the perinuclear factor. Several experiments emphasised that the perinuclear factor is not identical with (pro)filaggrin. The exact biochemical nature of the perinuclear factor remains a mystery, therefore.

It probably is one antigen as all 10 serum samples containing antiperinuclear factor tested react with the antigen in granules of various donors. It is unlikely that the antiperinuclear factor is identical with a rheumatoid factor subclass22 because 11% of our rheumatoid factor positive serum samples contained no antiperinuclear factor and 6% of the serum samples without rheumatoid factor did contain antiperinuclear factor (table 3). Other authors have also found serum samples without rheumatoid factor and with antiperinuclear factor in 4 to 18% of subjects.16 19 21 22 26 28 29

Westgeest et al found the antiperinuclear factor in 51% of patients with a recent Epstein-Barr virus infection,25 suggesting a relation between antiperinuclear factor and Epstein-Barr virus. We used several antibodies directed against Epstein-Barr virus proteins (VCA, EA(D), P-62), but none of them reacted with

![Figure 4](http://ard.bmj.com/Ann Rheum Dis: first published as 10.1136/ard.50.9.611 on 1 September 1991. Downloaded from http://ard.bmj.com/ on March 25, 2022 by guest. Protected by copyright.)
the keratohyalin granules in the buccal mucosa cells (table 6). A more likely possibility in our view is that the antiperinuclear factor is related to or identical with the antikeratin antibody-antigen. Both antigens for antiperinuclear factor and antikeratin antibodies are located in squamous epithelia. Not only is there a very good correlation between the occurrences of antikeratin antibodies and antiperinuclear factor (table 4) but all our serum samples containing antikeratin antibodies contained antiperinuclear factor antibody as well. The reverse is not true because the antiperinuclear factor test is more sensitive than the test for antikeratin antibodies. A certain overlap between antikeratin antibodies and antiperinuclear factor has also been reported by others. 6 25 35 We tested monoclonal and polyclonal antibodies against different keratins on buccal mucosa cells but could not find a specific reaction with the keratohyalin granules (table 6). The same antikeratin antibodies were also tested against esophageus sections, which resulted in a positive staining of the epithelial layer for cytokeratins 4 and 13 (monoclonal antibodies 6B10, 2D7, and K8.12), which are known to be present in oesophageus squamous epithelium. Although identical immunofluorescence staining was obtained with the rheumatoid serum samples containing antikeratin antibodies, subsequent two dimensional immunoblotting of a rat oesophageus extract with these samples did not show specific positive reactions with cytokeratin 4 or 13, whereas the monoclonal antibodies (6B10, 2D7, and K8.12) did react. It therefore is questionable whether antikeratin antibodies recognise a cytokeratin as the antigen. It is more likely that the antikeratin antibody-antigen is a protein associated with the cytokeratins in this type of epithelial cell. The name ‘antikeratin antibodies’, therefore, seems a most unfortunate choice and it would be better to refer to them as antirat oesophageus antibodies.

The association of the perinuclear factor with (pro)filaggrin, a protein which up to now has only been found in differentiated keratinocytes, 53 suggests a differentiation-specific expression of the perinuclear factor. Our future research, therefore, will be directed towards establishing an in vitro culturing system for buccal mucosa keratinocytes to study the differentiation of these cells and the in vitro induction of the perinuclear factor. 55

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