An Autoantibody Reactive With Nuclei of Polymorphonuclear Neutrophils: A Cell Differentiation Marker

By Senga Whittingham, George Morstyn, John W. Wilson, and Mathew A. Vadas

An autoantibody that reacted with nuclei of polymorphonuclear neutrophils (PMN) was detected at titers of greater than 10 in sera of 25 of 50 patients with rheumatoid arthritis and 36 of 50 with autoimmune chronic active hepatitis but in none of 160 controls comprising 24 patients with alcoholic cirrhosis, 36 with multiple myeloma, and 100 healthy subjects. Through the use of enriched populations of hemopoietic cells, this antibody was shown to be cell-specific, reacting only with the nucleus of the mature neutrophil. It was unreactive with nuclei of progenitor cells in the myeloid series and with nuclei of eosinophils, monocytes, lymphocytes, and thrombocytes. It reacted with a determinant that appeared to be a differentiation antigen. This cell-specific autoantibody may prove to be of value in analytical studies of granulocyte maturation.

HIGH TITER antinuclear antibodies occur mainly in systemic lupus erythematosus (SLE) and autoimmune diseases associated with SLE. In these diseases many antigenic determinants in the nucleus have been implicated and these appear to be common to all nucleated cells. An exception to these broadly reactive antinuclear antibodies are the granulocyte-reactive antibodies described in patients with rheumatoid arthritis and Felty's syndrome. The reactivity of these antibodies with the nuclei of leukocytes has not been previously conclusively defined. The initial description suggested that these antibodies reacted not only with polymorphonuclear cells in the peripheral blood but also with myeloblasts and myelocytes derived from patients with myeloid leukemia. In other studies it has been claimed that these antibodies reacted with monocytes and eosinophils but not with the nuclei of lymphocytes. All studies agree that these antibodies do not appear to react with the nuclei of other cell types such as thyroid cells.

In the present study we describe an antibody that reacts selectively with the nucleus of the polymorphonuclear neutrophil (PMN), is nonreactive with nuclei of eosinophils, monocytes, and progenitor cells of the PMN, and is present in high titer in patients with rheumatoid arthritis and autoimmune chronic active hepatitis. The antigenic determinant for this PMN-specific antinuclear antibody appears to be a differentiation antigen in the myeloid cell series. It has been possible to identify definitively the cellular distribution of this antigen by the use of recently developed cell separation techniques.

MATERIALS AND METHODS

Sera

All samples were collected according to the procedure laid down and approved by the Ethics Subcommittee of The Walter and Eliza Hall Institute, in accord with an assurance filed with and approved by the Department of Health, Education and Welfare, U.S.A. Sera from 50 patients with classical rheumatoid arthritis and 50 with autoimmune chronic active hepatitis were titrated in tenfold dilutions and these dilutions were tested by immunofluorescence against cyto centrifuge preparations of alcohol-fixed human buffy coat cells. High titers of antinuclear antibodies reactive with granulocytes had been observed previously in patients with these diseases. These sera with titers >100 against the nuclei of PMN were tested for the specificity of this reaction. Control sera from 24 patients with alcoholic cirrhosis, 36 patients with multiple myeloma, and 100 healthy subjects matched for age and sex with the test groups were also tested to confirm the disease specificity of the antibody. In addition, γ-globulin from normal human serum separated either as Cohn fraction II (Commonwealth Serum Laboratories, Melbourne) or by means of a protein-A-Sepharose CL-4B (Pharmacia, Sweden) column were heat-aggregated according to the methods of Dickler and Kunkel. These were used to exclude nonspecific binding of complexed γ-globulin to cells.

Cell Suspensions

Suspensions of PMN, eosinophils, lymphocytes, and monocytes were prepared to 48%–99% purity from samples of human peripheral blood. PMN and eosinophils were separated from two samples of blood with raised counts of eosinophils and two samples with normal counts. Lymphocytes and monocytes were separated from three samples with normal counts. Cell enrichment was achieved by centrifugation of the buffy coat suspension of cells through discontinuous gradients of metrizamide (Nyegaard, Oslo) in Tyrode's solution with 0.1% gelatin. Thymocytes obtained from thymuses of children requiring partial resection of the thymus during cardiac surgery were prepared as single cell suspensions. Enriched populations of blast cells, promyelocytes and myelocytes were obtained from the marrow of three hematologically normal patients requiring rib resection. Cells were flushed from the marrow and centrifuged through Ficoll-Paque (Pharmacia, Sweden) of density 1.077 g/cu cm. Cells at the interface were further purified with the aid of a fluorescence activated cell sorter (Becton Dickinson, USA) using

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three parameters for sorting, low and wide angle light scatter, and fluorescence of cells labeled with fluorescein-conjugated fucose binding lectin from *Lotus tetragonolobus*. Buffy coat suspensions were separated from two patients with chronic myeloid leukemia.

Suspensions of these cells were adjusted to approximately 10⁷/ml and cytocentrifuged onto microscope slides that had been specially cleaned by soaking in hot detergent and washing several times in tap water and distilled water. The cell suspensions were fixed in absolute alcohol at 4°C for 5 min and stored in sealed boxes at −20°C until tested by immunofluorescence.

**Immunofluorescence**

The procedure used for indirect immunofluorescence has been described elsewhere. The dilution of serum used to determine the specificity of reactivity against nuclei of hemopoietic cells was one tenfold dilution higher than the titer of reactivity against nuclei of other hemopoietic cells. A dilution of 1/18 of fluorescein-conjugated anti-human globulin (Wellcome, U.K.) optimal for the test was predetermined by chessboard titration. For each test, the positive control was a serum from a patient with SLE, which at a dilution of 1/100 reacted with nuclei of all cells in the preparation. Slides were examined with an incident-light Zeiss microscope with a 25× objective. Nuclei stained by immunofluorescence were identified morphologically. Confirmation of the identity of the cell with nuclear staining was sought by randomly selecting three sera from patients with rheumatoid arthritis and three sera from patients with chronic active hepatitis and performing the following tests. First, mean counts of nuclei stained by these sera in immunofluorescence preparations were compared with mean counts of PMN stained by Giemsa in preparations made from the same cell suspension. Second, buffy coat cells from the patients with leukemia were stained by immunofluorescence, the positions of the stained nuclei recorded, the coverslip removed, the preparation stained with Giemsa stain, and the identity of the cells confirmed by light microscopy.

**RESULTS**

Antinuclear antibodies to PMN in buffy coat suspensions were detected at titers greater than 10 in 25 (50%) of the patients with rheumatoid arthritis and 36 (72%) of the patients with autoimmune chronic active hepatitis, but in none of 24 patients with alcoholic hepatitis, 36 patients with multiple myeloma, and 100 healthy matched controls. Comparison of the distribution of log₁₀ titers by contingency table analysis showed that the titers were significantly greater against nuclei of PMN than against other nucleated cells in buffy coat suspensions (for rheumatoid arthritis χ² = 33.8, p < 0.001, and for autoimmune chronic active hepatitis χ² = 42.5, p < 0.001). Particularly high titers were present in patients with autoimmune chronic active hepatitis, 18 (36%) of whom had titers ≥1000, whereas only 3 (6%) of the patients with rheumatoid arthritis had titers in that range (Fig. 1).

The specificity of the autoantibody for the nucleus of the PMN is illustrated in Fig. 2, where serum at a dilution of 1/200 from a patient with chronic active hepatitis is shown to react only with the nuclei of PMN. No other cellular component of the PMN stained, and the nuclei could be readily recognized morphologically as belonging to PMN. No reaction was detected with nuclei of blast cells, promyelocytes, myelocytes, lymphocytes, thymocytes, eosinophils, and monocytes. This selective reactivity was identical in all 61 sera (25 from rheumatoid arthritis and 36 from chronic active hepatitis) tested by immunofluorescence. Mean counts of all nuclei stained by immunofluorescence were similar to mean counts of PMN identified morphologically in Giemsa-stained cytocentrifuge preparations of the same cell suspensions (Table 1). Buffy coat preparations of leukemic blood sequentially stained by immunofluorescence and Giemsa confirmed that nuclei of no cell other than the PMN was stained in the immunofluorescence preparation. No nonspecific binding of aggregates of γ-globulin to nuclei was observed, thereby excluding artefact arising from the presence of immune complexes in sera.

**DISCUSSION**

This study has for the first time definitively identified an antibody that reacts specifically with the nucleus of one particular cell in a single differentiation pathway. The PMN-specific antinuclear antibody described in this study clearly only reacts with the nucleus of the PMN and with no other cells in the granulocyte series, including myelocytes, promyelocytes, eosinophils, and band cells. This antibody is probably identical to the granulocyte-specific antibodies previously identified, although it was claimed in some studies that these reacted with the nuclei of neutrophils, eosinophils, monocytes, and leukemic myelocytes and myeloblasts.
Most antinuclear antibodies react with antigens which lack both cell and species specificity, although there are exceptions that have limited specificity. For example, the antibody to rheumatoid arthritis associated nuclear antigen (RANA)\textsuperscript{18} reacts only with determinants in Epstein–Barr virus transformed B lymphocytes.

PMN-specific antinuclear antibody in titers greater than 10 was detected in 25 of 50 patients with rheumatoid arthritis and 36 of 50 with autoimmune chronic active hepatitis, with titers over 1000 in some patients. The antibody does not appear to cause destruction of neutrophils as neutropenia is not a distinctive feature of rheumatoid arthritis or autoimmune chronic active

Table 1. Percent Counts of Cell Nuclei Stained by Immunofluorescence Compared With Percent Counts of PMN in Giemsa-Stained Preparations

<table>
<thead>
<tr>
<th>Cell Source (No. of Samples)</th>
<th>Enriched Cell</th>
<th>Percent Enrichment (± SD)</th>
<th>Mean* Percent Counts ± SD of PMN Stained by Giemsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rib marrow</td>
<td>Blasts, myelocytes, and promyelocytes</td>
<td>76 ± 25</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4)</td>
<td>PMN</td>
<td>90 ± 7</td>
<td>91 ± 7</td>
</tr>
<tr>
<td>(4)</td>
<td>Eosinophils</td>
<td>87 ± 7</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>(3)</td>
<td>Lymphocytes</td>
<td>97 ± 2</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>(3)</td>
<td>Monocytes</td>
<td>55 ± 7</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

* A total of 200 cells were counted in each preparation.
† Mean count for 6 sera.
‡ Main contaminants were lymphocytes.
hepatitis untreated by immunosuppressive drugs, although it is a feature of Felty’s syndrome. The immunopathologic significance of the antibody is not clear. However, detection of high titer PMN-specific antinuclear antibody may be a valuable guide to the identification of a subset of these diseases.

The PMN-specific differentiation antigen is unusual in that it is cell-specific, and the nucleus of the PMN is unique in that it undergoes profound morphological change during maturation. Clearly, further studies are needed to characterize this antigen, and the events that confer maturity on the neutrophil and result in the emergence of the determinant. The observation that the antigen is only present in the nucleus of the polymorphonuclear cell and is not present in the nucleus of the monocyte may be particularly important since these two cells seem to share a common precursor.

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REFERENCES

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