A monoclonal anti-DNA antibody also binds to cell-surface protein(s)

(autoimmune/disease)

LAURENT JACOB*, FRANÇOIS TRON*, JEAN-FRANÇOIS BACH*, AND DANIEL LOUVARD†

*Institut National de la Sante et de la Recherche Medicale U25, Hopital Necker, 161 rue de Sèvres, 75015 Paris, France; †Institut Pasteur, Département de Biologie Moleculaire, Unité de Biologie des Membranes, 75015 Paris, France; and ‡Département de Médecine Interne, Hopital Cochin, Paris, France

Communicated by André Lwoff, February 21, 1984

ABSTRACT A murine monoclonal anti-DNA antibody (PME77) has been found to bind tightly to the plasma membrane of Raji cells. We show here that this monoclonal anti-DNA antibody reacts in a radioimmunoassay with the cell surface of a variety of mammalian cell types and that the antigenic determinant recognized by the monoclonal anti-DNA antibody at the surface of Raji cells is resistant to DNase. It belongs to polypeptides removed from the cell surface by a mild proteinase K treatment.

Most of the tissue lesions observed in murine and human systemic lupus erythematosus (SLE) are considered to be due to the presence of anti-double-stranded DNA antibodies spontaneously produced in this disease (1), but paradoxically it is extremely difficult to produce antibodies to double-stranded DNA by immunization (2).

We recently reported that a murine anti-DNA monoclonal antibody (mAb) reacted with the surface of Raji cells and that the binding was not abrogated by DNase treatment of the cells (3). We show here that (i) this mAb does indeed react with the surface of a variety of mammalian cell types and (ii) the determinant detected by this mAb at the surface of Raji cells belongs to polypeptides removed from the cell surface by a mild proteinase K treatment.

MATERIALS AND METHODS

Lymphoblastoid B-Cell Lines. The Raji lymphoblastoid cell line was obtained and maintained as described (3). The human Ramos B-cell line was derived from a B-lymphoma line. The human HSB2 T-cell line was derived from an acute lymphocytic T-cell leukemia.

Cell lines were maintained in suspension culture in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum/penicillin (100 units/ml)/streptomycin (100 µg/ml), all from GIBCO.

Human Tissue. Neuroblastoma cells were obtained from Flow Laboratories and maintained in suspension culture in Hanks’ medium (Flow Laboratories) supplemented with 10% fetal calf serum.

Preparation of Peripheral Blood Lymphocytes. Heparinized blood was obtained from healthy adult donors. Mononuclear cells were separated by centrifugation on Ficol-Paque (Pharmacia) as described (4). Non-adherent cells were washed twice in Hanks’ balanced salt solution and separated into E+ and E- subpopulations by rosetting 2 times with sheep erythrocytes. The E+ cells were liberated from the sheep erythrocytes by hypotonic lysis (5). Viability, assessed by trypan blue dye exclusion, was at least 95%.

Preparation of Human Erythrocytes. Erythrocytes were separated from mononuclear cells by centrifugation on Ficol-Paque as described (4).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

All cell types were used at a concentration of 0.5-1 x 10^7 cells per ml.

Murine mAb. The hybridoma PME77 secreting anti-DNA antibody was isolated after fusion between a nonsecreting myeloma line (P3 x 63-Ag 8653) and (NZB x NZW) F1 spleen cells. The selection of hybrid, cloning and subcloning of the line, purification of the antibody, characterization of its class and subclass, and its specificity study have all been described in detail (6, 7). PME77 mAb was shown to be an IgG2b, κ-chain antibody and sharply specific for DNA (7). The IgG2b, κ MOPC 195 mAb (Lilton Bioletics) was used as control in all binding experiments.

Cell-Binding Experiments. Cell binding experiments were done as described (3). Briefly, 50 µl (2 x 10^6 cells) of the suspension was aliquoted in polypropylene test tubes (12 x 75 mm). Twenty-five microliters of graded concentrations of PME77 and MOPC 195 mAb was added, and the cell suspensions were incubated for 45 min at 37°C. Cells were then washed free of unbound material and resuspended in 50 µl of Hanks’ solution/bovine serum albumin. Cell-bound IgG2b was evaluated by incubating the cells for 30 min at 37°C and then for 30 min at 4°C with 25 µl (20,000 cpm) of 125I-labeled protein A (Amersham) (specific activity, 10-50 µCi/µg; 1 Ci = 37 GBq). Cells were washed free of unbound radiolabeled protein and resuspended in Hanks’ solution/bovine serum albumin. The amount of radioactivity bound to the cell pellet was quantified in a gamma scintillation counter. All determinations were done in duplicate.

PME77 Fab Fragments. Fab fragments were prepared as described (3). Briefly, Fab fragments of PME77 mAb were prepared by digestion with mercuric papain (Sigma) (Ab/enzyme ratio, 100:1) (8). The digestion was in 10 mM EDTA/10 mM cysteine/phosphate-buffered saline (P/NaCl), pH 7.2.

The digestion was terminated after 2 hr by dialysis in 0.005 M phosphate (pH 8). The digest was passed through a DEAE cellulose column (Whatman) and the Fab fragments were isolated as the first peak by gradient elution in phosphate buffer from 5 M to 500 M. The DNA-binding capacity of the Fab fragments was checked in a cellulose ester filter radioimmunoassay (9). Fab fragments were incubated with Raji cells as described above. Cells were washed free of unbound material and incubated with a rabbit anti-mouse κ light chain (Lilton Bioletics) radiiodinated by the chloramine-T method (specific activity, 10^6 cpm/µg).

Proteinase K Digestion of Raji Cells. Proteinase K (Boehringer Mannheim) was dissolved in P/NaCl at 1 mg/ml. Forty million Raji cells were centrifuged at 200 x g at 4°C, and the pellet was incubated with proteinase K in P/NaCl (final concentration, 100 µg/ml) for 1 hr on ice. The cells were washed free of the enzyme and resuspended in P/NaCl. Viability was assessed by trypan blue exclusion.

Gel Electrophoresis and Immunoreplica Analysis. The extract of Raji cells was prepared by the procedure of Garrels

Abbreviations: SLE, systemic lupus erythematosus; mAb, monoclonal antibody; P/NaCl, phosphate-buffered saline.
RESULTS

PME77 Anti-DNA mAb Binds to Different Mammalian Cell Types. We have previously reported the binding of PME77 mAb to the surface of Raji cells (3). Similarly, cell-binding experiments, using 125I-labeled protein A as a tracer, demonstrated that PME77 mAb binds to a number of other human cell types: human erythrocytes, neuroblastoma, a T-lymphoblastoid cell line (Ramos), and normal T and B lymphocytes (Table 1). PME77 Fab fragments were shown to react with these cell types, using 125I-labeled rabbit anti-κ light chain as the tracer (Table 2). These results indicate that putative Fc receptors cannot account for the binding.

TREATMENT OF RAJI CELLS BY PROTEINASE K. We previously showed that treatment with Raji cells with DNase I did not decrease the binding of PME77 mAb to the cells (3). In contrast, when Raji cells were pretreated with proteinase K, the binding of PME77 mAb was completely suppressed (Table 3). Taken together, these results suggest that the antigenic determinants recognized by PME77 mAb at the cell surface are distinct from DNA. At the end of the treatment, at least 95% of the cells were shown to be viable, a result indicating the specificity of the cell-surface treatment.

Immunoreplica Analysis. After electrotransfer of polypeptides to the nitrocellulose sheet, the supernatant of PME77 mAb was added and incubated. As shown in Fig. 1, five bands could be detected at Mr, 14,000, 16,000, 17,000, 33,000, and 34,000; four of these bands showed a strong reaction and the fifth showed a weaker one. The same pattern was observed when unredused samples were used in a similar experiment. Two controls were used: (i) When PME77 mAb was preincubated with DNA (1 mg/ml), the staining of all bands was strongly decreased (Fig. 1). (ii) Using a DNA unrelated mAb; for this purpose, a mAb raised against a minor membrane protein of the Golgi apparatus was applied to the nitrocellulose sheets (15). No band appeared under these conditions (Fig. 1).

Immunoreplica Patterns of Raji Cells Untreated or Treated with Proteinase K. The comparison of immunoreplica patterns of Raji cells untreated or treated with proteinase K was studied to determine which of the polypeptide(s) recognized by PME77 mAb is accessible at the cell surface. A mild proteinase K treatment that does not alter cell viability is an assay that probes for a portion of membrane protein(s) accessible at the cell surface. When Raji cells were pretreated with proteinase K at 100 μg/ml (>95% living cells), two of the five bands (Mr, 33,000 and 34,000) were almost completely removed; the band at Mr, 16,000 was decreased. In contrast, one band remained unchanged (Mr, 17,000). This suggests that the Mr, 17,000 polypeptide is either resistant to proteinase K or it lies inside the cell.

DISCUSSION

As long as the predominant autoantibodies in murine and human SLE were considered to be directed exclusively against

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Lymphoblastoid T cell (HSB2)</th>
<th>Lymphoblastoid B cell (Ramos)</th>
<th>E+ cells</th>
<th>E- cells</th>
<th>Neuroblastoma</th>
<th>Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME77 mAb</td>
<td>100 μg/ml</td>
<td>4825 ± 54</td>
<td>5085 ± 423</td>
<td>3421 ± 392</td>
<td>3116 ± 572</td>
<td>2819 ± 311</td>
</tr>
<tr>
<td></td>
<td>30 μg/ml</td>
<td>2237 ± 326</td>
<td>2118 ± 228</td>
<td>2718 ± 217</td>
<td>2019 ± 163</td>
<td>1912 ± 104</td>
</tr>
<tr>
<td></td>
<td>10 μg/ml</td>
<td>1532 ± 213</td>
<td>1553 ± 112</td>
<td>1628 ± 173</td>
<td>1187 ± 104</td>
<td>1182 ± 125</td>
</tr>
<tr>
<td></td>
<td>3 μg/ml</td>
<td>631 ± 32</td>
<td>770 ± 88</td>
<td>850 ± 78</td>
<td>872 ± 126</td>
<td>481 ± 74</td>
</tr>
<tr>
<td>MOPC 195</td>
<td>100 μg/ml</td>
<td>452 ± 77</td>
<td>638 ± 92</td>
<td>767 ± 79</td>
<td>746 ± 81</td>
<td>446 ± 73</td>
</tr>
<tr>
<td></td>
<td>10 μg/ml</td>
<td>429 ± 17</td>
<td>624 ± 29</td>
<td>603 ± 114</td>
<td>624 ± 29</td>
<td>481 ± 37</td>
</tr>
</tbody>
</table>

Results are expressed as cpm bound per plate. MOPC 195 is a control myeloma mAb.
Immunology: Jacob et al.

FIG. 1. Electrophoretic protein patterns and specific immuno-replica analysis of Raji cell extract. In each case, the gels were loaded with 40 μg of Raji cell extract. Lanes: a, untreated Raji cell extract; total protein pattern; b, untreated Raji cell extract transferred to a nitrocellulose sheet and incubated with PME77 mAb; c, proteinase K-treated Raji cell extracts; total protein pattern; d, proteinase K-treated Raji cell extract transferred to a nitrocellulose sheet and incubated with PME77 mAb; e, control with PME77 mAb preincubated with DNA; f, control with anti-Golgi mAb. Molecular weight markers are in far left lane.

DNA, it appeared difficult to understand how the production of these antibodies could be triggered. The recognition by anti-DNA mAb of phospholipids provided the first evidence of a possible triggering mechanism (16). Here, we show that a mAb with strict specificity for double-stranded DNA (4) binds to various mammalian cell types and recognizes a few (five) polypeptides. Three of these polypeptides appear to be sensitive to proteinase K action and, therefore, are expressed at the cell surface.

The nature of the antigenic determinant common to DNA and the polypeptides and whether the epitope(s) detected at the Raji cell surface by PME77 mAb are identical to those present at the surface of the other human cell types shown to bind PME77 remain to be determined. The affinity of the anti-DNA mAb for protein(s) that we have shown might ac-count for some of the autoantibody cross-reactions observed in SLE, such as that found between anti-neuronal and anti-erythrocyte or anti-lymphocyte antibodies (17), and that described between anti-lymphocyte and anti-nuclear antibodies (18).

Finally, these results raise the possibility that the triggering antigen in SLE could be a protein rather than DNA. The role of autoantigens in the onset of autoimmune response is indicated by the prevention of spontaneous anti-thyroglobulin autoantibody production in the obese chicken by neonatal thyroidec-tomy (19). One must thus speculate that the protein(s) cross-reacting with DNA is involved in the onset of SLE. This hypothesis would account for the low immunogenicity of nucleic acids as outlined above.

We wish to thank Marie-Annick Lety and Béatrice Legrand for their excellent technical assistance. This work was supported by Institut National de la Santé et de la Recherche Médicale Grant 811050 (to L.J.).