CONCISE REPORT

The binding of lupus-derived autoantibodies to the C-terminal peptide (83–119) of the major SmD1 autoantigen can be mediated by double-stranded DNA and nucleosomes

J W Dieker, C C Van Bavel, G Riemekasten, J H Berden, J van der Vlag

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Objectives: To evaluate the binding of lupus-derived autoantibodies, double-stranded DNA and nucleosomes to the positively charged C-terminal SmD1(residues 83–119) peptide and the full-length SmD protein.

Methods: The binding of lupus-derived monoclonal antibodies, sera from patients with systemic lupus erythematosus, rheumatoid arthritis and systemic sclerosis, dsDNA and nucleosomes to the SmD1(83–119) peptide or the full-length SmD protein was determined using different ELISA methods.

Results: Monoclonal anti-dsDNA antibodies and the serum of patients with systemic lupus erythematosus that are positive for anti-dsDNA antibodies react with the SmD1(83–119) peptide in ELISA. However, DNaseI treatment of the blocking reagents leads to a decreased reactivity. Purified dsDNA and nucleosomes bind to the SmD1 peptide but not to the full-length SmD protein.

Conclusions: The SmD1(83–119) peptide is able to bind dsDNA and nucleosomes, and dsDNA or nucleosomes in applied reagents lead to an apparent reactivity of anti-dsDNA, anti-histone or nucleosome-specific antibodies with the SmD1(83–119) peptide in ELISA.

In systemic lupus erythematosus (SLE), a variety of autoantibodies against nuclear components such as nucleosomes (histones and double-stranded DNA (dsDNA)) and ribonucleoproteins (eg, Sjögren syndrome A/Ro, Sjögren syndrome B/La, small nuclear ribonucleoproteins, Sm and heterogenous nuclear ribonucleoproteins) exist. The autoantibody production seems to be antigen driven and T cell dependent. Recently, the glycine–arginine-rich C-terminal part (residues 83–119) of the spliceosomal SmD1 protein has been identified as a major autoepitope targeted by sera from 36–70% of patients with SLE.^{1–3} The reactivity with the SmD1(83–119) peptide correlates with anti-dsDNA reactivity and disease activity.^{1–3} Furthermore, SmD1(83–119)-reactive T cells can stimulate the production of pathogenic anti-dsDNA antibodies and administration of the SmD1(83–119) peptide to (NZB/NZW) F1 lupus mice accelerates the progression of the disease.^{4–5}

Anti-Sm reactivity has been included as one of the classification criteria for SLE by the American College of Rheumatology and at present the anti-SmD1(83–119) reactivity is routinely measured by ELISA. The binding of dsDNA to the positively charged C-terminal tail of the SmD1 protein may have a role in the development of the anti-chromatin immune response in SLE,^{5–6} and may also interfere with ELISA measurements.⁷ Therefore, we evaluated the binding of lupus-derived monoclonal anti-dsDNA, anti-histone and anti-nucleosome antibodies, sera from patients with SLE,

rheumatoid arthritis and systemic sclerosis (SSc), dsDNA and nucleosomes to the SmD1(83–119) peptide and full-length SmD protein, using different ELISA methods.

METHODS

Monoclonal antibodies and patients' sera

Monoclonal anti-dsDNA (#36, #42 and #56), anti-histone (KM-2 and #34) or anti-nucleosome (PL2-3, #32 and LG10-1) antibodies were derived from (NZB×NZW) F1 lupus mice, except PL2-3 and LG10-1 that were derived from MRL/+ and MRL/lpr lupus mice, respectively.⁷ The monoclonal antibodies were DNaseI-treated and thoroughly purified as described previously.⁸ WT-32 (anti-CD3) was used as a control. A rabbit anti-SmD1(83–119) peptide antiserum was described.² Serum samples from patients with SLE (n = 45), rheumatoid arthritis (n = 15) and SSc (n = 15) were collected at the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, and the Charité University Hospital, Berlin, Germany, which was approved by the local ethics committees. Anti-dsDNA and anti-SmD titres in patients' sera were routinely determined by the Farr assay, and counterimmunoelectrophoresis, immunoblot or RNA precipitation, respectively.

Anti-SmD1(83–119) peptide, anti-SmD protein and anti-dsDNA ELISA

Anti-SmD1(83–119) and anti-SmD reactivity was determined in ELISA as described previously.² Coated SmD1(83–119) peptide (5 µg/ml) or SmD protein (5 µg/ml) was blocked with PBS/0.05% (vol/vol) Tween-20 with 1% non-fat dry milk (Bio-Rad Laboratories, Veenendaal, The Netherlands), casein (Sigma, Zwijndrecht, The Netherlands) or bovine serum albumin (BSA; Sigma) for 2 h at room temperature. Wells were incubated with serially diluted monoclonal antibody (starting concentration of 5 µg/ml), 1:100 diluted patients' serum or rabbit serum for 2 h at room temperature. Horseradish peroxidase-conjugated goat anti-mouse IgG, goat anti-human IgG or goat anti-rabbit IgG (Southern Biotechnology Associates, Birmingham, USA) was used for detection. Anti-dsDNA ELISA and DNaseI treatment of reagents were carried out as described.⁷

Binding of dsDNA, biotin-labelled dsDNA or nucleosomes to the SmD1(83–119) peptide or full-length SmD protein

dsDNA was labelled with biotin according to the manufacturer's instructions (Roche, Almere, The Netherlands). Coated SmD1(83–119) peptide or SmD protein was blocked with DNaseI-treated milk or casein, or BSA (1%), and incubated with serially diluted calf thymus dsDNA (Roche),

Abbreviations: BSA, bovine serum albumin; dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus; SSc, systemic sclerosis

Figure 1 Binding of lupus-derived monoclonal anti-double-stranded DNA (dsDNA), anti-histone and anti-nucleosome (anti-nuc) antibodies to the SmD1(83-119) peptide. Average reactivity of three anti-dsDNA (#36, #42, and #56), two anti-histone (KM-2 and #34) and three anti-nucleosome (PL2-3, #32 and LG10-1) antibodies with the SmD1(83-119) peptide in ELISA with milk or casein (1%), either treated with DNaseI or not, or with bovine serum albumin (BSA) (1%) as blocking reagent. WT-32 served as a control antibody. Values are given as means SD of six experiments (A). Average reactivity of anti-dsDNA, anti-histone and anti-nucleosome antibodies with the SmD1(83-119) peptide, using BSA (1%) as blocking reagent, after the addition of dsDNA, starting at 100 ng/ml (B), or nucleosomes, starting at 74 ng/ml (C). Binding of biotin-labelled DNA to the SmD1(83-119) peptide or the full-length SmD protein determined in ELISA with DNaseI-treated milk (1%) as blocking reagent (D). The values represent means of six experiments (B-D).

biotin-labelled dsDNA or nucleosomes for 30 min at room temperature. The ELISA was carried out as described.⁷

Statistical methods

Significance was determined by Kruskal-Wallis and Mann-Whitney U test using Graphpad Prism V.4.0 (GraphPad Software, Inc., San Diego, California, USA).

RESULTS

Apparent reactivity of anti-dsDNA antibodies and binding of dsDNA and nucleosomes to the SmD1(83-119) peptide

The reactivity of lupus-derived monoclonal anti-dsDNA, anti-histone and anti-nucleosome antibodies with the SmD1(83-119) peptide and full-length SmD protein was determined by ELISA. With milk or casein as blocking reagent, all anti-dsDNA antibodies showed a high reactivity with the peptide, whereas the anti-histone and anti-nucleosome antibodies hardly reacted (fig 1A). With BSA as blocking reagent, a low reactivity of anti-dsDNA and anti-histone antibodies was observed (fig 1A). Until recently, it seemed that the application of different blocking reagents led to different reactivities of serum samples from patients with SLE with the SmD1(83-119) peptide,⁹ whereas we showed that the presence of nucleosomal material in commonly used blocking reagents interfered with the reactivity of anti-chromatin autoantibodies in ELISA.⁷ Indeed, DNaseI treatment of the applied blocking reagent—that is, milk and casein—abolished the reactivity of all monoclonal anti-dsDNA antibodies with the SmD1(83-119) peptide (fig 1A). The addition of purified dsDNA (fig 1B) or nucleosomes (fig 1C) to the coated SmD1(83-119) peptide not only restored the reactivity of anti-dsDNA antibodies but also showed the reactivity of

anti-nucleosome and anti-histone antibodies. The addition of purified dsDNA or nucleosomes to the full-length SmD protein did not result in any reactivity of monoclonal anti-dsDNA antibodies, irrespective of the applied blocking (not shown). We also showed the direct binding of biotin-labelled dsDNA to the SmD1(83-119) peptide, but not to the full-length SmD protein (fig 1D).

DNaseI treatment of blocking reagent abolishes anti-SmD1(83-119) reactivity in anti-dsDNA-positive sera from patients with SLE

We questioned whether the dsDNA and nucleosomes present in blocking reagents could also interfere with the reactivity of sera from patients with SLE with the SmD1(83-119) peptide. On the basis of the anti-dsDNA and anti-SmD reactivities, as determined by standard non-ELISA methods, sera from 45 patients with SLE were divided into 4 groups—that is, anti-SmD-/anti-dsDNA-, anti-SmD-/anti-dsDNA+, anti-SmD+/anti-dsDNA- and anti-SmD+/anti-dsDNA+. DNaseI treatment of the blocking reagent—that is, 1% milk—largely decreased the anti-SmD1(83-119) reactivity, particularly in sera positive for anti-dsDNA in patients with SLE, whereas the reactivity of sera negative for anti-dsDNA in patients with SLE was hardly affected (fig 2A). The high reactivity of the rabbit anti-SmD1(83-119) antiserum was not affected by DNaseI treatment (fig 2A), and sera from patients with rheumatoid arthritis or SSc hardly reacted (not shown).

With the use of full-length SmD protein as a coating, no significant reduction in the reactivity of the anti-dsDNA positive sera was observed after DNaseI treatment of the blocking reagent (fig 2B). As observed for the peptide, the high reactivity of the rabbit anti-SmD1(83-119) peptide antiserum with the SmD protein was not affected by DNaseI

Figure 2 Effect of DNaseI treatment on the reactivity of sera from patients with systemic lupus erythematosus (SLE) with the SmD1(83-119) peptide and the SmD protein. The reactivity of sera with the SmD1(83-119) peptide in ELISA with untreated milk (1%) and DNaseI-treated milk (1%) as blocking reagent. A rabbit anti-SmD1(83-119) antiserum served as a control (A). The reactivity of sera with the full-length SmD protein in ELISA with untreated milk (1%) and DNaseI-treated milk (1%) as blocking reagent. A rabbit anti-SmD1(83-119) antiserum served as a control (B). The mean (SD) of two experiments is shown. * $p < 0.05$ compared with untreated blocking reagents. +, with DNaseI treatment; -, without DNaseI treatment.

treatment (fig 2B), whereas sera from patients with rheumatoid arthritis or SSc hardly reacted (not shown). In contrast with the anti-SmD1(83-119) reactivity, DNaseI treatment of reagents did not change the reactivity of sera with dsDNA (not shown).

DISCUSSION

We showed that lupus-derived monoclonal anti-dsDNA antibodies could bind to the spliceosomal SmD1(83-119) peptide when milk or casein was used as blocking reagent, whereas anti-histone and anti-nucleosome antibodies hardly reacted. Apparently, dsDNA or nucleosomes that were present in the applied blocking reagents mediated the SmD1(83-119) reactivity of anti-dsDNA antibodies, as DNaseI treatment abolished this reactivity. We recently showed that nucleosomal material present in blocking reagents interfered with the binding of lupus-derived anti-chromatin antibodies in ELISA.⁷ The SmD1(83-119) peptide bound purified dsDNA and nucleosomes, which resulted in reactivity of anti-dsDNA, anti-histone and anti-nucleosome antibodies. The presence of 16 (of 37) positively charged

residues in the SmD1(83-119) peptide and the negative charges abundantly present in dsDNA and nucleosomes explains the binding of dsDNA and nucleosomes. It was shown previously that single-stranded or dsDNA can bind to Sm proteins.¹⁰ Also, the reactivity of anti-dsDNA-positive sera from patients with SLE with the SmD1(83-119) peptide was much lower after DNaseI treatment of the blocking reagent, although in particular anti-dsDNA-negative anti-SmD-positive sera from patients with SLE retained their reactivity. These data may explain that the use of different ELISA methods may lead to great differences (36-70%) in the prevalence of anti-SmD1(83-119) reactivity in patients with SLE.^{1,2} The differences in dsDNA binding to the SmD1 peptide and to the full-length SmD protein are striking. Our findings indicate that in the full-length SmD protein the positive charges in the C-terminal domain are less accessible for dsDNA binding, which may indicate that there are differences in the conformational structure of these two antigenic structures in ELISA. The binding of anti-dsDNA antibodies to the intact SmD protein and binding of anti-SmD1 antibodies to single-stranded or dsDNA has been shown by other methods.^{3,11-14} The in vivo formation of dsDNA-SmD1 complexes and other dsDNA or protein complexes may play an important part in the development of the anti-dsDNA response in SLE^{5,6} and anti-SmD antibodies mainly target the positively charged structures in spliceosomal proteins.¹⁵ Most importantly, it has been shown that SmD1(83-119)-specific T cells provide help for the production of anti-dsDNA antibodies.⁵

In conclusion, the measurement of reactivity of sera with the SmD1(83-119) peptide in ELISA can lead to overestimation of the anti-SmD1 reactivity, as anti-dsDNA antibodies can also react. Therefore, to distinguish anti-dsDNA reactivity from genuine anti-SmD1(83-119) reactivity in ELISA, we recommend DNaseI treatment of all reagents or the use of BSA as blocking reagent. Alternatively, the full-length SmD protein may be used as a coating. Nevertheless, recognition of the dsDNA-SmD1(83-119) complex by anti-dsDNA antibodies, and of the nucleosome-SmD1(83-119) complex by anti-dsDNA, anti-histone and anti-nucleosome antibodies, is a novel finding, which may play a part in the development of the anti-chromatin immune response in SLE.

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Authors' affiliations

J W Dieker*, C C Van Bavel*, J H Berden, J van der Vlag, Nephrology Research Laboratory, Nijmegen Centre for Molecular Life Sciences, Division of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

G Riemekasten, Department of Rheumatology and Clinical Immunology, Charité University Hospital, Berlin, Germany

*These two authors contributed equally to the study.

†Present address: CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France.

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Correspondence to: J van der Vlag, Nephrology Research Laboratory (279), Nijmegen Centre for Molecular Life Sciences, Division of Nephrology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands; J.vanderVlag@NCMLS.RU.NL

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