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Autoimmunity to the p53 Protein is a Feature of Systemic Lupus Erythematosus (SLE) Related to Anti-DNA Antibodies

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The induction of anti-DNA autoantibodies in systemic lupus erythematosus (SLE) patients is problematic because mammalian DNA is poorly immunogenic at best. Here we demonstrate a chain of connected antibodies in SLE patient sera that could account for the induction of anti-DNA antibody, and possibly for some of the pathogenic features of SLE. We now report that SLE patients, in addition to anti-DNA, produce antibodies to the carboxy-terminal domain of the tumour suppressor molecule p53; this p53 domain recognizes damaged DNA. Hence, these anti-p53 antibodies could mimic damaged DNA immunologically. Indeed, SLE sera do contain anti-idiotypic antibodies to a prototypic anti-p53 antibody. Moreover, SLE anti-DNA antibodies also recognize this type of anti-p53 antibody. Indeed, binding of affinity-purified anti-DNA both to DNA and to the anti-p53 antibody could be blocked by a p53 peptide derived from the DNA-binding domain. This mimicry of the p53 DNA-binding domain by the SLE anti-DNA antibodies is functional: activation of the p53 molecule could be inhibited by such anti-DNA antibodies. Thus, anti-DNA antibodies may arise in SLE patients by a chain of idiotypic autoimmunity centered around p53 autoimmunity. The SLE anti-DNA and anti-p53 antibodies can functionally block p53 activation, and so could affect apoptosis.

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Introduction

Antibodies to various autoantigens, including DNA, are a common feature of systemic lupus erythematosus (SLE), and the anti-DNA autoantibodies are believed to be major factors in the pathogenesis of the disease [1]. Autoimmunity to DNA, however, is problematic, since native DNA itself is only poorly immunogenic [2]. Thus, the immunogen that actually drives the autoimmune reaction to DNA is probably not DNA. A current view is that SLE autoimmunity might be related to dysregulated apoptotic cell death. In SLE patients, phagocytosis of apoptotic cells appears to be impaired [3], and this aberrant clearance of apoptotic cells might expose otherwise inaccesible immunogens to the immune system [4]. Systemic exposure of mice to apoptotic cells has been shown to induce autoantibodies [5]. Indeed, at least some of the target molecules of SLE-associated autoantibodies are proteins that are involved in apoptosis [6], including the p53 molecule [7]. The p53 molecule, when activated by damaged DNA, acts as a tumour suppressor by in-

ducing growth arrest and DNA repair, or apoptosis of aberrant cells [8]. The malfunction of apoptosis in SLE, however, remains to be clarified.

We recently reported that MRL/MpJ-Fas*lpr* mice, which spontaneously develop murine lupus, spontaneously make rising titers of antibodies to the carboxy-terminal domain of the p53 molecule [9]. This domain of p53 recognizes damaged DNA [10]. The mice also make antibodies to anti-p53 antibodies (anti-idiotypes), and some of these anti-idiotypes also recognize DNA [9]. In other words, an anti-p53 antibody that binds the carboxy-terminal DNA-binding domain of p53 [11] could mimic DNA; the p53 carboxy terminus binds both DNA and the specific anti-p53 antibody. Some anti-DNA antibodies, therefore, could mimic the carboxy-terminal DNA-binding domain of p53. The point of the mimicry we discovered in mice between p53, anti-p53 antibody and DNA is that an autoimmune response to the p53 carboxy terminus could lead to the induction of anti-DNA antibody by way of an idiotypic network (Figure 1). This idiotypic response can be pathogenic; immunization of standard BALB/c mice to an anti-p53 monoclonal autoantibody induced anti-DNA antibodies and a lupus-like clinical syndrome [9].

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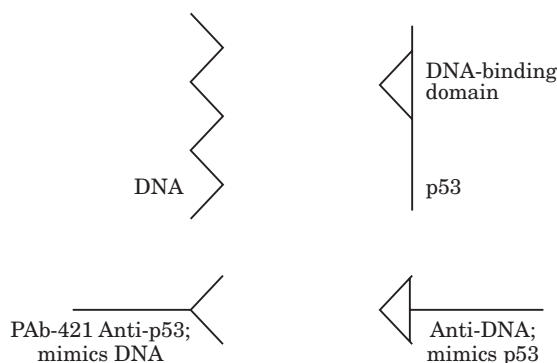
Figure 1. Anti-p53 antibody can mimic DNA and anti-DNA antibody can mimic p53. The p53 molecule (right) features a DNA-binding domain that allows it to recognize damaged DNA (left). In the course of an immune response to p53, antibodies to p53 could arise (left) that might bind the DNA-binding domain of p53. These antibodies immunologically mimic the structure of damaged DNA [9]. Thus, a DNA-mimicking anti-p53 antibody might induce anti-DNA antibody.

In the present study, we assayed human SLE sera to learn whether idiotypic immunity to p53 could be a feature of human SLE, and whether human anti-DNA antibodies, like those in mice, might mimic immunologically the DNA-binding domain of p53.

Materials and methods

Human sera

Sera were obtained from 48 consecutive SLE clinic patients attending the SLE Clinic of the Johannes Gutenberg University, Mainz, Germany, and from 16 healthy subjects. Anti-DNA antibodies from pooled patient sera with anti-DNA activity or from the serum of single patients, chosen for sufficient volume, were purified by affinity-chromatography on DNA-cellulose (Pharmacia, Freiburg, Germany), according to the instructions of the manufacturer.

Antigens

Escherichia Coli BL21 (DE3) cells were transformed with the T7 expression vector containing human p53 cDNA [12]; purification of p53 was done as described [13]. The p53 peptides, amino-terminal p16-35 and carboxy-terminal p363-382, were prepared using an automated synthesizer (Abimed AMS 422; Langenfeld, Germany) according to the manufacturer. Peptide purity was confirmed by analytical reverse phase HPLC and mass spectroscopic analysis. The sequence of the p53 amino peptide (p16–35) is: PLSQETFSGLWKLLPPEDIL; that of the p53 carboxy peptide (p363–382) is: HSSYLKTKKGQSTSRHKKTM. Peptide p363–382 was used because it is recognized by the anti-p53 monoclonal antibody PAb-421 [9]. The monoclonal antibodies PAb-421 [11] and PAb-248 [14] were purified from ascitic fluid by Protein A affinity

chromatography (Sigma, Rehovot, Israel), and $(Fab)_2$ fragments of the antibodies were prepared as described [15]. Calf thymus DNA was purchased from Sigma (Rehovot, Israel).

ELISA

ELISA assays were done in 96-well Maxisorp plates (Nunc, Roskilde, Denmark), in which each well was coated with 10 µg/ml test antigen in PBS. After washing and blocking with 1% (w/v) BSA in PBS for 1 h at 37°C, diluted test sera (1:1,000; 0.1 ml per well) were added for 1 h at 37°C. Alternatively, 10 µg of affinity-purified SLE anti-DNA antibodies, and as indicated, 20 µg per ml of p53 peptides were added. There then followed 1 h of incubation with a goat anti-human immunoglobulin specific secondary antibody conjugated to horseradish peroxidase (DAKO, Copenhagen Denmark), diluted 1:5,000. A substrate solution containing 1 mg/ml ABTS in 0.2 M citric acid, 0.2 M Na_2HPO_4, and 3‰ (v/v) H_2O_2 (all from Sigma) was added, and the plates were read at 405 nm. The test antigens used were calf thymus DNA after precoating with methylated BSA (Sigma), $(Fab)_2$ fragments of the monoclonal antibodies PAb-421 or PAb-248, recombinant p53, or the p53 peptides p16-35 or p363-382. For absorption experiments, the test sera were preincubated for 1 h with $(Fab)_2$ fragments of PAb-421 or PAb-248 prior to testing for residual antibody of DNA.

Band shift assay

The p53-responsive element consensus sequence oligonucleotide, TCGAGAGGCATGTCTAGGCATGT CTC [16], was synthesized, prepared in a double stranded form and end-labeled [13]. The DNA probe (120 pg) was incubated with 1 µg of recombinant p53 for 15 min on ice, and for 15 min at room temperature in a buffer containing 2 µg of non-specific DNA (poly dI-dC), 12.5 mM Tris-HCl, 25 mM KCl, 0.25 mM EDTA, 0.5 mM DTT and 5% (w/v) glycerol. As indicated, 1 µl of PAb-421 antibody ascitic fluid, purified anti-DNA antibodies, or non-specific human IgG (Sigma, Rehovot, Israel) were added to the reaction. The reaction products were separated by electrophoresis on a 4% polyacrylamide gel for 3 h in a 0.4% TBE running buffer. The bands were analysed densitometrically with the NIH Image 1.61 software package.

Statistics

The differences between experimental groups were tested for significance using the *t*-test, when applicable, or the Mann-Whitney test (Figure 2). The comparison of densitometrically assessed band areas above threshold was performed with Fisher's exact probability test. Correlation coefficients were calculated using the Spearman rank correlation analysis.
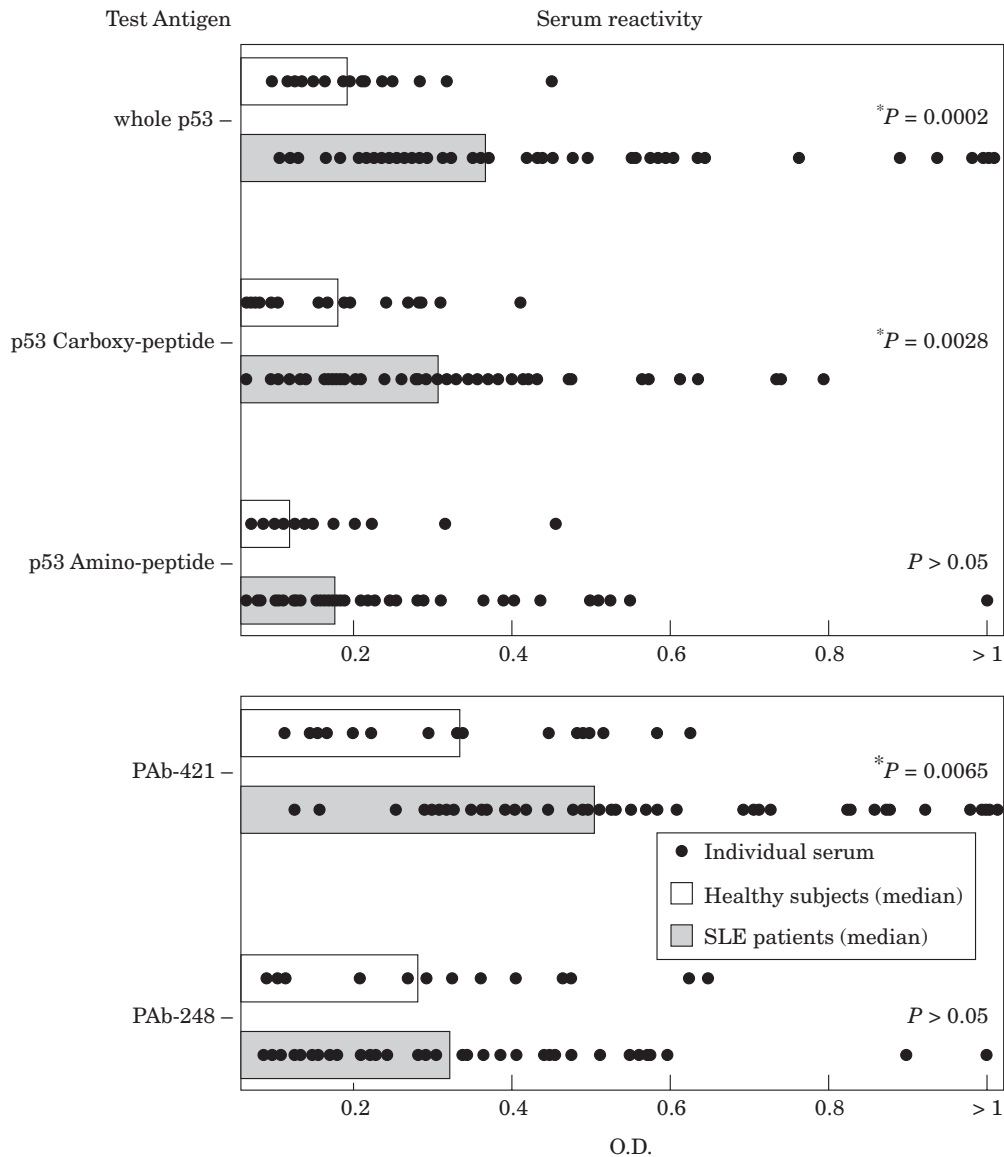
Test Antigen   Serum reactivity

Figure 2. Idiotypic immunity to p53 in SLE patients. Sera from SLE patients (grey bars) and sera from healthy subjects (open bars) were assayed in an ELISA test for antibodies to human p53, to the p363–382 peptide of the carboxy-terminal DNA-binding domain of p53, and to a peptide (p16–32) of the amino terminus of p53 (upper panel). SLE patients showed significantly higher reactivities to p53 ($P=0.0002$) and to the carboxy-terminal p363–382 peptide ($P=0.0028$). No significant difference in the reactivity to the amino-terminal p16–32 peptide was detected. Sera were also assayed for the presence of p53-mimicking antibodies (lower panel), by testing reactivity to the monoclonal antibody PAb-421 that binds the carboxy-terminal p53 domain, and to the monoclonal antibody PAb-248 that binds the amino terminus of p53. SLE patients showed significantly higher reactivity to PAb-421 ($P=0.0065$). No significant difference in binding to PAb-248 was found.

Results

The sera of 48 SLE patients and the sera of 16 healthy subjects were tested for the presence of p53-associated antibodies. The SLE group sera manifested significantly higher titers of IgG antibodies to p53 than did controls ($P=0.0002$, Figure 2, upper panel). The SLE antibodies to p53 recognized a peptide (p363-382) from the p53 carboxy-terminal domain that binds damaged DNA ($P=0.0028$). In contrast, there was no significant recognition of a control peptide from the amino terminus of p53 (p16-32; $P=0.0685$). The presence of antibodies that mimic the carboxy-terminal domain of p53 was detected through their idiotypic reactivity to the PAb-421 monoclonal antibody; this antibody binds to the domain of p53 that recognizes damaged DNA (Figure 2, lower panel; $P=0.0065$ compared to control sera). In contrast, there was no significant difference between the SLE patients and the healthy subjects in their reactivity to an antibody that binds the p53 amino terminus (PAb-248; $P=0.2813$), or to monoclonal antibodies that do not bind p53 (not shown). There appeared to be a positive correlation between the amounts of anti-PAb-421 and anti-p53 antibodies made by the patients: eighty percent of the sera with high binding activity to PAb-421
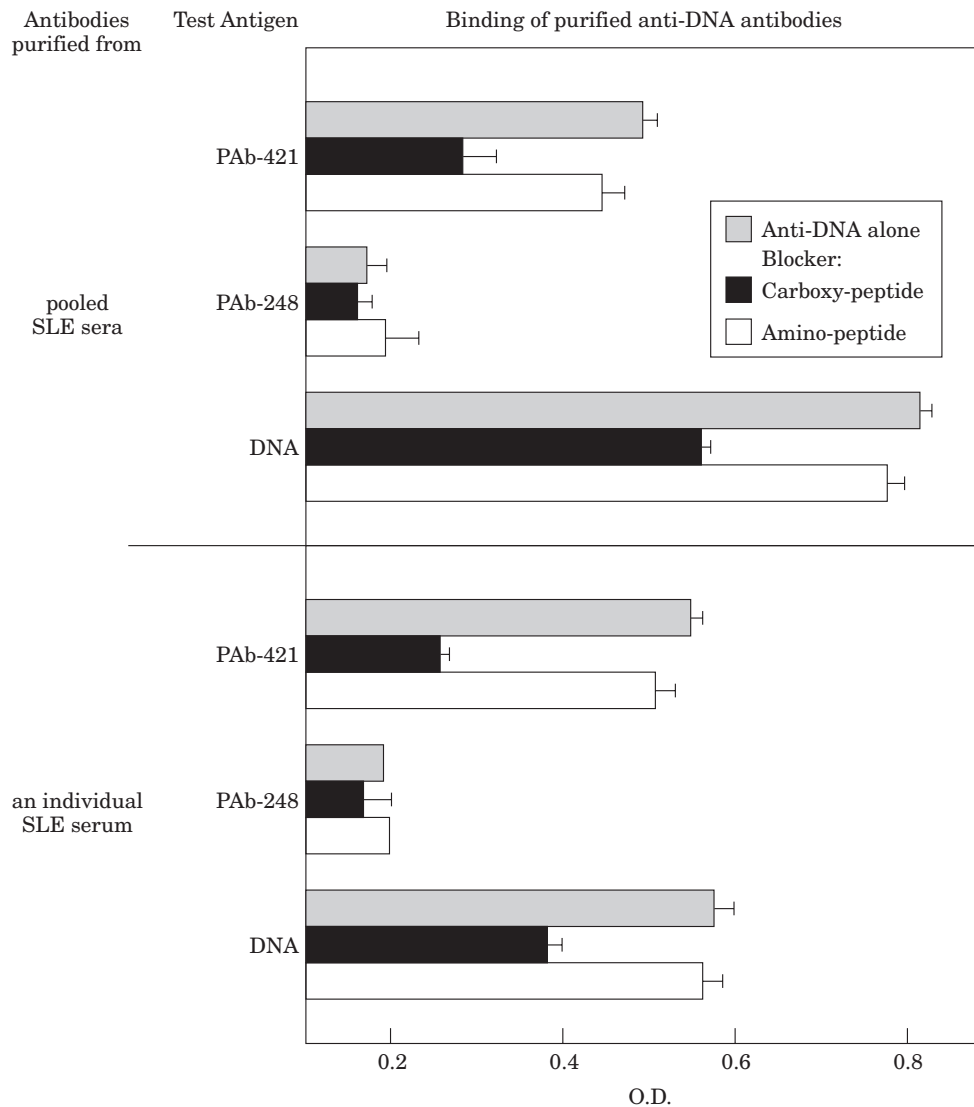
Figure 3. Anti-DNA antibodies from SLE patients mimic the carboxy-terminal DNA-binding domain of p53. Affinity purified anti-DNA antibodies from 30 pooled SLE sera (upper) or from a single patient (lower) were tested for reactivity to DNA, to the PAb-421 monoclonal antibody that binds the p53 carboxy-terminal domain, or to the monoclonal antibody PAb-248 that binds the p53 amino terminus. The purified antibodies (grey bars) showed reactivity not only to DNA, but also idiotypic reactivity to PAb-421, and not to PAb-248. Addition to 20 µg/ml of the p363–382 peptide (black bars), which is derived from the p53 carboxy terminus, could significantly inhibit the binding of the purified antibodies both to DNA ($P<0.0002$) and to the PAb-421 antibody ($P<0.0011$). No inhibition was found when 20 µg/ml of the p16-32 peptide were added ($P>0.06$; open bars). Samples were measured in triplicate. Values are shown as mean±SD.

also exhibited high binding activity to p53 ($r=0.59$; $P<0.0001$) and to the p53 carboxy peptide ($r=0.70$; $P<0.0001$). Thus, some SLE patients, like MRL/MpJ-Fas^{lpr} mice, seem to produce antibodies to the carboxy-terminal domain of p53 and make related anti-idiotypic antibodies. Such anti-idiotypic antibodies might, like p53, recognize DNA.

To learn whether human SLE anti-DNA antibodies might also bind an idiotypic anti-p53 antibody, we purified anti-DNA antibodies both from pooled patient sera and from the serum of a single patient by affinity chromatography to DNA-cellulose. We then tested whether these anti-DNA antibodies, like the p53 carboxy terminus itself, might bind the anti-p53 PAb-421 antibody. As can be seen in Figure 3 (grey

bars), the purified anti-DNA antibodies bound to DNA, but also bound to the PAb-421 antibody. Thus, at least some anti-DNA antibodies could be defined as anti-idiotypic antibodies. The PAb-248 antibody, which binds to the amino-terminus of p53, in contrast, was not recognized by the purified anti-DNA antibodies. Absorption of anti-DNA on PAb-421 (not shown) confirmed that about 30% of the anti-DNA antibodies in the tested sera were anti-idiotypic to the PAb-421 anti-p53 antibody. To confirm that both DNA binding and idiotypic binding were the properties of the same antibody molecules, we tested whether both these reactivities could be blocked by the same p53 peptides. Indeed, we found that the binding of the purified antibodies both to DNA and to the PAb-421

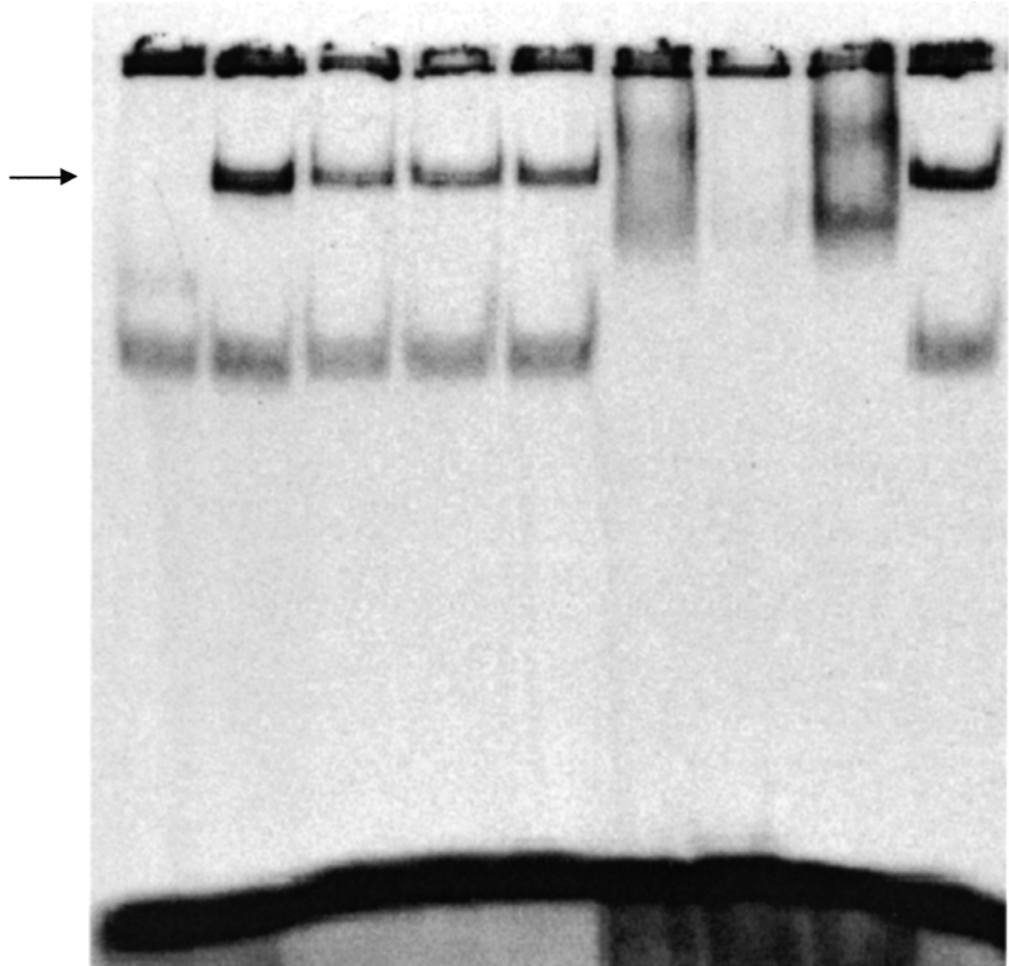| p53	+	+	+	+	+	-	-	-	+
PAb-421	-	+	+	+	+	-	-	-	+
anti-DNA	-	-	pool	pat.1	pat.2	pool	pat.1	pat.2	-
Non-specific IgG	-	-	-	-	-	-	-	-	+

Figure 4. Anti-DNA antibodies from SLE patients block activation of the p53 molecule. Binding of the p53 molecule to the specific p53-responsive nucleotide sequence (arrow) was determined in a band-shift assay as a measure of p53 activation. There was no spontaneous activation of p53 (left lane); addition of the PAb-421 monoclonal antibody induced p53 binding to the nucleotide probe (second lane from the left). Affinity-purified anti-DNA antibodies both from pooled human SLE sera or from individual patients (patient 1 and patient 2) inhibited the activation of the p53 molecule (lanes 3 to 5 from the left). Densitometric quantification revealed 49.4% inhibition by the anti-DNA from the SLE pool ($P<0.0001$), and 48.9% and 42.4% inhibition by the anti-DNA from the individual sera ($P<0.0001$). Non-specific human immunoglobulins, in contrast, did not inhibit p53 activation ($P=0.1501$; lane 9 from the left). The purified anti-DNA alone did not show specific binding.

antibody could be blocked by the addition of the p53 carboxy peptide (p363–382; black bars), but not by the addition of the amino peptide (p16–32; hatched bars). Thus, a peptide derived from the carboxy-terminal DNA-binding domain of p53 could compete with the anti-DNA for the binding not only to the anti-p53 antibody, but also to DNA; the anti-DNA antibody was an anti-idiotype to an anti-p53 antibody.

The carboxy-terminal DNA-binding domain is known to regulate the conformational activation of the p53 molecule; binding of the PAb-421 monoclonal antibody to this domain induces specific binding of p53 to p53-responsive promoter sequences [17], which can result in apoptotic cell death [18]. Therefore, we tested whether SLE anti-DNA antibodies that mimic p53 might interfere functionally with p53 activation induced by PAb-421. The ability of the anti-DNA to block p53 activation was measured by inhibition of the binding of p53 to the specific p53-responsive nucleotide sequence in a band-shift assay. Figure 4 shows that the addition of affinity purified anti-DNA from pooled patient sera or from the sera of two individual SLE patients could inhibit p53 activation. Addition of non-specific human immunoglobulin, in

contrast, did not inhibit p53 activation. Thus anti-DNA antibody from SLE patients can interfere with p53 function, at least *in vitro*.

Discussion

We have shown that antibodies that bind DNA (anti-DNA) also bind a specific antibody to p53; these anti-DNA can function as an anti-idiotype. This finding indicates that specific anti-p53 antibodies can mimic DNA; PAb-421 and DNA must look alike structurally, at least to some anti-DNA antibodies (see Figure 1). One might therefore consider the possibility that some of the anti-DNA in SLE might be driven, not by poorly immunogenic DNA, but by the DNA-mimicking antibodies to p53 that we demonstrate here. This, of course, could occur through an idiotypic network triggered by p53 autoimmunity [19, 20]. The induction of the anti-p53 antibodies can be accounted for by the accumulation of the p53 molecule, not only in tumour cells [21], but also in activated inflammatory cells [22]. The accumulation of p53 apparently can trigger an autoimmune response to the molecule [23, 24]. Exposure to sunlight could be a factor linking p53 and SLE: p53 accumulates in skin after sunburn [25], and sunburn exacerbates SLE [1].

It should be noted that the levels of the anti-p53 antibodies we found in the SLE patients seem to be lower than those reported in tumour patients [26]. More importantly, however, there seems to be a difference in specificity of the anti-p53 antibodies in these populations: the anti-p53 antibodies found in tumour patients predominantly recognize the amino terminus of p53 [27], and the anti-p53 found in SLE seem to recognize predominantly the carboxy-terminal DNA-binding domain (see Figure 2). One might speculate that the differences in p53 epitope selection between SLE and tumour patients might result from the immunogenetic predisposition of SLE patients and from the different contexts of exposure to p53. The presence and type of anti-p53 antibodies manifest in other autoimmune diseases remains to be studied.

Other DNA-binding molecules that are recognized by antibodies in SLE might also contribute to the generation of anti-DNA by a mechanism similar to the one described here; not all of the anti-DNA in SLE could be accounted for by p53 autoimmunity. Indeed, the DNA-binding site of the Ku protein has been found to be a major epitope for SLE antibodies [28], and several other DNA- and RNA-binding molecules are targets of SLE antibodies [1]. Nevertheless, a link between immunity to DNA and to DNA-binding domains of DNA-binding proteins other than p53 still needs to be established.

It is conceivable that some of the pathogenicity of anti-DNA antibodies in SLE might be due to mimicry of p53 by such antibodies. We found here that anti-DNA antibody can block p53 activation *in vitro* (see Figure 4). Of course, it would be important to learn whether anti-DNA antibody might interfere with p53-dependent apoptosis *in vivo*. It has been demonstrated that some anti-DNA antibodies can enter living cells and cause cellular damage [29]. Thus, it is possible that anti-DNA might aggravate cell damage by inhibiting p53-dependent apoptosis. Interfering with apoptosis may result in p53 accumulation [21], and hence reinforce p53 autoimmunity. Indeed, the pathogenic consequences of idiotypic immunity to the p53 DNA-binding domain are demonstrable in mice [9]. Therefore, abrogation of the immune response to the p53 DNA-binding domain might be explored as a novel approach to arrest the continuous production of pathogenic anti-DNA in SLE.

Acknowledgements

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