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Detection of anti-topoisomerase I antibodies using a full length human topoisomerase I recombinant protein purified from a baculovirus expression system

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SUMMARY

Topoisomerase I (topo I) is a major systemic sclerosis (SSc)-associated autoantigen. A cDNA construct encoding full length human topo I in a recombinant baculovirus transfer vector was used to infect insect cells in culture from which recombinant protein was purified. An ELISA using recombinant protein was evaluated in 340 sera including sera from 134 patients with SSc, of whom 33 had anti-topo I antibodies detected by immunodiffusion. A high yield of pure topo I of expected molecular mass and catalytic activity was obtained. The recombinant topo I ELISA was 92% sensitive and 98% specific in detecting anti-topo I antibodies which were present almost exclusively in patients with SSc. Therefore, the potential advantages of expressing human autoantigens in eukaryotic systems for diagnostic purposes were confirmed.

Keywords scleroderma recombinant autoantigen topoisomerase I

INTRODUCTION

Topoisomerase I (topo I) is a nuclear enzyme responsible for interconverting different topological forms of DNA [1]. Antibodies to topo I are found in about 20–30% of patients with systemic sclerosis (SSc), and are associated with the presence of diffuse skin involvement and pulmonary disease [2–5]. Anti-topo I antibodies are highly specific for SSc, although they have been found occasionally in a small number of patients with systemic lupus erythematosus (SLE) [6]. Also, the presence of anti-topo I antibodies in patients with Raynaud's phenomenon may be associated with an increased risk of later development of SSc [7]. Therefore, a sensitive and specific solid-phase assay for detecting anti-topo I antibodies should provide a useful diagnostic and prognostic tool.

The availability of cloned material for human topo I has provided a rich source of recombinant fusion proteins for use in diagnostic assays [8–15]. Most such studies have used partial length topo I fusion proteins expressed in prokaryotic systems, which have the potential disadvantage of excluding important antigenic epitopes, or may give rise to false positive results by including non-relevant bacterial antigens. In the current study we have used a full length human topo I protein purified from a eukaryotic baculovirus system as the source of antigen in a solid-phase ELISA for the detection of anti-topo I antibodies. We have evaluated the ELISA in sera from a large collection of patients with SSc and related conditions, including patients with anti-topo I antibodies detected by other means. Our results confirm that eukaryotic expression systems may provide a valuable source of recombinant autoantigens that can be used in diagnostic assays for screening populations at risk of developing SSc.

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PATIENTS AND METHODS

Subjects and controls

Sera were analysed from 134 patients with SSc [16], 104 patients with SLE [17], 20 patients with rheumatoid arthritis (RA) [18] and 40 healthy controls. Sera were also available for study from 15 patients with silicosis-associated SSc (SA-SSc) and 27 patients with silicosis without any clinical signs of SSc [19].

Immunodiffusion and functional assay for detecting anti-topo I antibodies

Anti-topo I antibodies and precipitating antibodies to other antigens (including U1RNP, Sm, Ro (SAA), La (SSB), Pm-Sci, Jo-1 and Ku) were measured by Ouchterlony double diffusion using a rabbit thymus extract (Bradshaw Biologicals, Market Harborough, UK), calf thymus extract (Bradshaw Biologicals) and human spleen extract as the sources of antigen.

Lines of identity were sought with known autoantibody prototypes.

All sera with anti-topo I antibodies were further assayed for their ability either to directly inhibit or to absorb out the enzymatic activity of bovine topo I as previously described [19].

Recombinant topo I

Isolation of the cDNA clone for human topo I has been previously described [20]. Construction of the topo I-expressing baculovirus and the purification and characterization of the recombinant human topo I will be described in detail elsewhere (L. Stewart, personal communication). Briefly, insect (Sf9) cells (4×600 ml) were grown to a density of 3×10^6 cells/ml and infected with a high titre virus stock. The cells were harvested at 50 h post-infection and nuclei isolated following lysis with Triton X-100. The nuclei were lysed with high salt (1 M NaCl) and the DNA precipitated with a final concentration of 6% PEG 8000 [21]. The supernatant was subjected to successive chromatography on phosphocellulose, phenyl Sepharose and Mono-S. The final pooled fractions were dialysed against 50% glycerol in 10 mM Tris/HCl pH 7·5, 2 mM dithiothreitol and 1 mM EDTA and stored at −20°C.

Purity and catalytic activity of recombinant topo I

The purity of the recombinant topo I was determined by SDS–PAGE with coomassie staining. Protein was determined by the method of Lowry *et al.* [22] and was calculated to be 5·76 mg from approx. 7×10^9 Sf9 cells. Catalytic activity of the purified protein was measured by its ability to relax a supercoiled form of plasmid DNA (ϕX174 RF DNA; GIBCO, Paisley, UK) as previously described [19].

Topo I ELISA

All sera were tested for the presence of IgG and IgM anti-topo I antibodies by ELISA using recombinant topo I. Those sera with IgG or IgM anti-topo I antibodies by ELISA were also tested for IgA anti-topo I antibodies. Recombinant topo I was used to coat 96-well Nunc Maxi-sorp plates (0·25 μg/ml) in carbonate buffer pH 9·6, and left overnight at 4°C. Plates were washed three times in PBS containing 0·05% Tween-20 and blocked for 1 h in the same buffer. Primary anti-serum was added to each well (1/1000 in PBS/Tween-20) and left for 1 h at room temperature. Plates were washed three times in PBS/Tween-20 followed by the addition of secondary anti-serum (goat anti-human IgG, IgM or IgA alkaline phosphatase conjugate (Sigma, St Louis, MO) 1/1000) for 1 h at room temperature. Plates were washed as before and substrate (*p*-nitrophenyl phosphate, 0·5 mg/ml in carbonate buffer, pH 9·6) was added. The resulting colour was read at 410 nm using a Dynatech plate reader and results were expressed as a percentage of a standard high titre serum. Samples were considered positive if readings were > 3 s.d. above the mean of 40 healthy controls.

SDS–PAGE and immunoblotting

Twenty-three sera positive for anti-topo I antibodies, a range of prototype sera with other well defined autoantibodies that were negative for anti-topo I antibodies, and 40 normal control sera were used in immunoblotting experiments for detecting recombinant topo I. Following separation on 10% SDS–PAGE, recombinant topo I was transferred to nitrocellulose [23] at 250 mA constant current for 2 h and stained with Ponceau-S. Sheets of nitrocellulose were blocked in PBS containing 5% milk powder and cut into strips. Strips were incubated in primary anti-sera (1/100 in PBS/milk powder) for 2 h with shaking. Following three washes in PBS, the strips were incubated in secondary anti-sera (goat anti-human IgG, IgM, IgA (1/1000) or polyvalent (1/350) alkaline phosphatase conjugate) for 2 h, washed as before and the colour developed in 5-bromo-4-chloro-3-indoyl-phosphate/nitro-tetrazolium blue in 50 mM Tris/HCl pH 9·6 containing 100 mM NaCl and 5 mM $MgCl_2$.

RESULTS

Purification and biological activity of recombinant topo I

Following elution from the Mono-S column, a single band representing recombinant topo I was visualized on SDS–PAGE running at an expected apparent molecular weight of 100 kD (Fig. 1). On the coomassie-stained gel, faint bands of lower relative molecular mass were apparent. These were likely to be contaminating proteins rather than breakdown products of recombinant topo I, as sera with anti-topo I antibodies recognized only the 100-kD band on nitrocellulose. After transfer to nitrocellulose the 100-kD protein was recognized by all 23 sera monospecific for anti-topo I antibodies and not by other control sera (Fig. 2). Also the purified protein was able to relax supercoiled DNA consistent with the known enzymatic properties of topo I (Fig. 3).

Detection of IgG anti-topo I antibodies using the recombinant topo I ELISA

SSc. By immunodiffusion there were 24 patients with SSc who had IgG anti-topo I antibodies (Table 1). All were able to directly inhibit or absorb out bovine topo I enzymatic activity (data not shown). There were 23 SSc sera that were positive for IgG anti-topo I antibodies by recombinant ELISA (Table 1, Fig. 4). Two sera from patients with SSc that were positive for anti-topo I antibodies by immunodiffusion were negative by ELISA. When these sera were used to probe an immunoblot containing recombinant topo I (Fig. 2, lane 3) a faint band was seen at 100 kD. Therefore, it was likely that anti-topo I antibodies were present in these sera, but at levels not detectable by ELISA. One other serum was positive by the recombinant topo I ELISA but negative by immunodiffusion and the functional topo I assay, and did not recognize recombinant topo I on an immunoblot (data not shown).

SLE and RA. Two of 104 sera from patients with SLE and one of 20 sera from patients with RA were positive for anti-topo I IgG by the recombinant ELISA. None of these sera was positive for anti-topo I antibodies by immunodiffusion or by the functional topo I assay, and they did not recognize a 100-kD polypeptide on a blot containing recombinant topo I (data not shown).

Detection of anti-topo I antibodies in patients with silicosis-associated SSc and silicosis

Eight of the 15 sera from individuals with SA-SSc had antibodies to topo I when screened by the IgG ATA ELISA (Table 1, Fig. 4). These sera were all positive for anti-topo I antibodies by immunodiffusion [19]. One additional serum was positive by immunodiffusion but negative by the ELISA. When

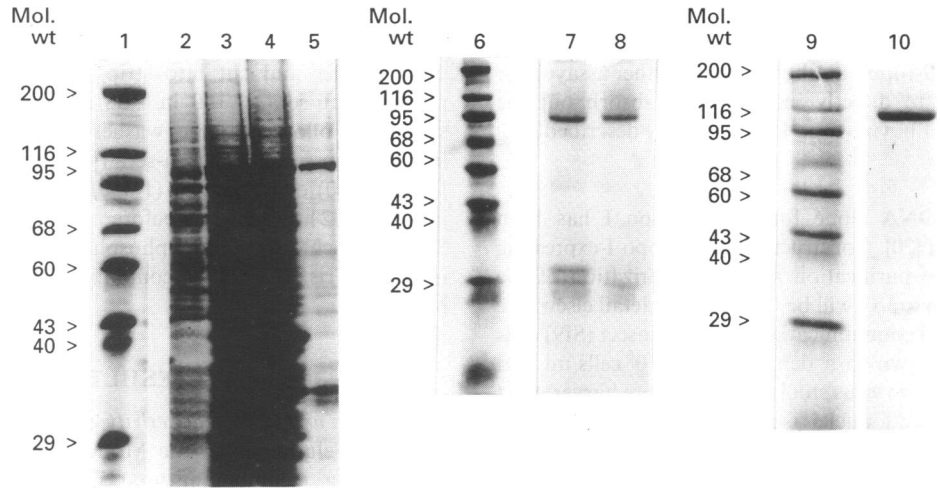

Fig. 1 Stages in the purification of recombinant topo I. Proteins are separated on 10% polyacrylamide gels and visualized by coomassie staining. Topo I migrates as a 100-kD band. Lanes 1, 6 and 9, molecular weight markers; lane 2, insect (Sf9) cell extract; lane 3, Si9 cell extract infected with the baculovirus/topo I construct; lane 4, polyethylene glycol pellet; lane 5, supernatant containing topo I; lane 7, pooled peak from phosphocellulose column; lane 8, eluant following passage through a phenyl Sepharose column; lane 10, topo I following salt elution from a mono-S column.

used to probe an immunoblot containing recombinant topo I, a faint band was observed at 100 kD (data not shown). None of the individuals with silicosis alone had anti-topo I antibodies detected by ELISA or by immunodiffusion.

Detection of IgM and IgA antibodies using the recombinant topo I ELISA

There were three SSc sera with both IgG anti-topo I antibodies and anti-topo I antibodies of another isotype (three with IgM anti-topo I antibodies, of which one had IgA anti-topo I antibodies). However, only IgG anti-topo I antibodies were detected on an immunoblot of the recombinant protein. In contrast, others have reported high levels of IgA and IgM antibodies to calf thymus topo I [24] and to a recombinant topo I fragment [11]. Differences in the source of autoantigen or in experimental procedure may account for the disparity. For instance, it is possible that IgA antibodies recognize epitopes on topo I not accessible on the baculovirus recombinant protein.

DISCUSSION

The requirements of an autoantigen for optimal use in diagnostic assays are purity and retention of sites for recognition of autoantibody (epitopes). The eukaryotic baculovirus expression system is ideally suited for generation of high yields of full length protein lacking the extraneous sequences commonly present in fusion proteins. The absence of foreign sequences in the protein eliminates the need to screen sera with the portion of the polypeptide not encoded by the autoantigen gene in order to eliminate false-positive results. A further advantage of eukaryotic systems is that contaminating host cell proteins, which may co-purify with the recombinant antigen, are unlikely to be a target for antibodies in patients with autoimmune disease. For instance, *Escherichia coli* is a common organism found in the gastrointestinal tract to which the human immune system may be primed, whereas background autoreactivity to insect cell proteins may be less likely to occur. Post-translational modifications of the recombinant protein such as phosphorylation and glycosylation are not typically seen in prokaryotic systems, and may be important for autoantibody recognition.

Using a baculovirus expression system we have purified full

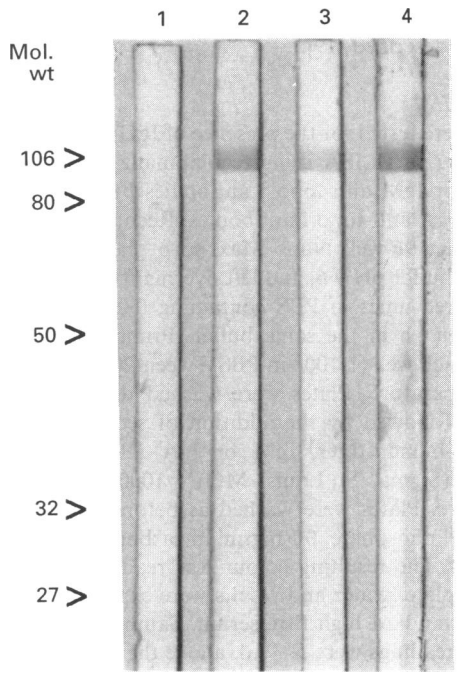

Fig. 2. Immunoblot of recombinant topo I separated on 10% polyacrylamide gels, transferred to nitrocellulose and probed with various anti-sera. Lane 1, serum from a healthy control; lane 2, systemic sclerosis (SSc) serum positive for anti-topo I antibodies by recombinant ELISA and immunodiffusion; lane 3, serum negative for anti-topo I antibodies by recombinant ELISA but positive by immunodiffusion weakly recognizes recombinant topo I on immunoblot; lane 4, silicosis-associated (SA)-SSc serum with anti-topo I antibodies by ELISA and immunodiffusion.

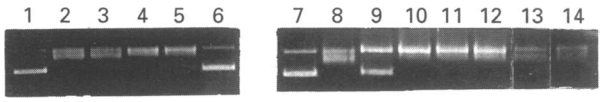

Fig. 3. Relaxation of supercoiled DNA by recombinant topo I and inhibition of relaxation by anti-topo I antibodies. Lanes 1 and 7, supercoiled plasmid DNA alone; lanes 2 and 8, plasmid DNA plus topo I (240 ng); lanes 3–6, plasmid DNA plus decreasing amounts of topo I (50 ng, 10 ng, 2 ng, 0·4 ng); lane 9, plasmid DNA, topo I and prototype anti-topo I serum; lanes 10–13, plasmid DNA, topo I and sera positive for anti-topo I antibodies by recombinant ELISA but negative by immunodiffusion (lanes 10 and 11; SLE sera, lane 12; rheumatoid arthritis serum, lane 13; systemic sclerosis (SSc) serum); lane 14; plasmid DNA, topo I and normal control serum.

length human topo I, a major antoantigen formerly known as Scl-70 [25,26] and recognized by patients with SSc. The protein had an apparent molecular weight (100 kD) consistent with its predicted size. A high level of purity was obtained following extraction and chromatography. It has been shown that the catalytic site of autoantigens including topo I are favoured sites for autoantibody recognition [19,27–29]. In the current study all the anti-topo I antibodies detected by immunodiffusion were able to inhibit or absorb out anti-topo I enzymatic activity. Therefore it may be of importance that a major part of the biological activity of the purified protein was retained.

The full length topo I protein was used to optimize a highly sensitive assay for the detection of anti-topo I antibodies. All but three sera positive by immunodiffusion were positive by recombinant ELISA, giving a sensitivity of 92%. Furthermore, the recombinant protein was recognized weakly on immunoblot by the three sera that were negative by ELISA. Therefore the recombinant topo I did contain epitope(s) for all anti-topo I antibodies used in the present study. Several previous works have suggested that there are multiple epitopes on topo I for anti-topo I antibodies, some of which appear to be immunodominant [8,9,11–15]. However, only a few studies have been able to locate an epitope that was recognized by all anti-topo I antibodies studied [12]. Also, most groups have used methods which favour the recognition of linear or denatured epitopes, yet conformational epitopes are thought to be more representative of what human autoantibodies recognize *in vivo*. Therefore, the full length and functional topo I protein described here represents an excellent substrate for the detection of anti-topo I antibodies to native autoantigen.

The recombinant topo I ELISA was highly specific (98%) with only four sera positive on ELISA and negative by other methods. All four sera were just above the cut-off point for positivity, and none recognized recombinant topo I in an immunoblot. The specificity of the ELISA may be attributed to the high degree of purity obtained at the end of the isolation procedure, and the lack of antigenic contaminants that accompanies recombinant proteins expressed in bacterial systems. For instance, antibodies to bacterial proteins were thought to account for false-positive autoantibody levels to another scleroderma autoantigen, CENP-B, expressed in *E. coli*. [30]. The relative lack of false-positive results in our ELISA underlines the advantage of generating recombinant autoantigens in eukaryotic systems, although contamination with other eukaryotic autoantigens may potentially still occur.

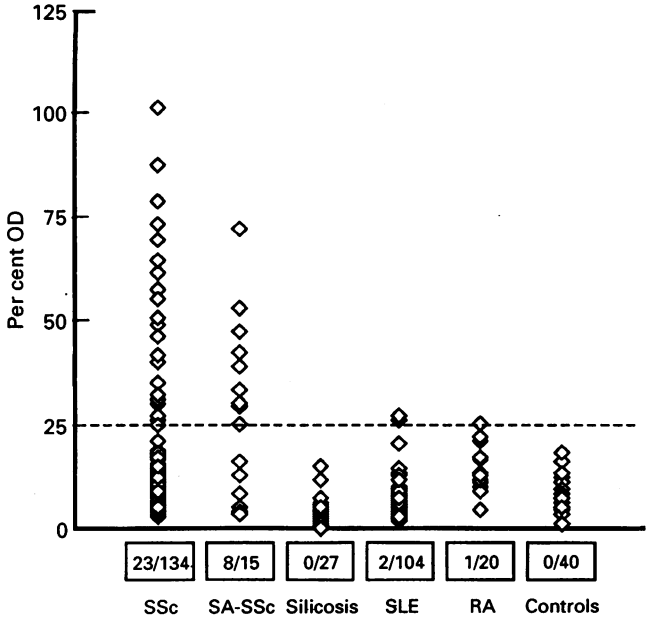

Fig. 4. Levels of IgG anti-topo I antibodies measured by recombinant topo I ELISA. Results are expressed as a percentage of the absorbance of a high titre serum. Samples were considered positive if they were > 3 s.d. (dashed line) above the mean of 40 healthy controls. The numbers in boxes represent the numbers of positives from the total number in the group. SSc, Systemic sclerosis; SA-SSc, silicosis-associated SSc; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.

Anti-topo 1 antibodies were highly specific for SSc. Two patients with Raynaud's phenomenon were positive for anti-topo I antibodies (data not shown). According to previous work these patients may be at risk of later development of SSc [7]. Anti-topo I antibodies have occasionally been reported in patients with SLE [6]. There were two patients with SLE who had low levels of anti-topo I antibodies by ELISA unconfirmed by other methods. The close association of anti-topo I antibodies with the disease phenotype of SSc is further underlined by the complete absence of these antibodies in patients with silicosis who have not developed SSc. In the future, the highly sensitive and specific ELISA will be a valuable tool for screening large populations either geneti-

Table 1. Anti-topo I antibodies in disease groups and healthy controls detected by immunodiffusion and by a recombinant topo I ELISA

Condition	*n*	Anti-topo I antibodies by ELISA	Anti-topo I antibodies by immunodiffusion
SSc	134	23	24
SLE	104	2	0
RA	20	1	0
SA-SSc	15	8	9
Silicosis	27	0	0
Healthy controls	40	0	0

SSc, Systemic schlerosis; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SA-SSc, silicosis-associated SSc.

cally or environmentally at risk of developing SSc, who may have Raynaud's phenomenon or other early manifestations of a connective tissue disease.

Recombinant topo I was recognized by antibodies predominantly of the IgG isotype, in keeping with the notion that autoantibody generation is driven by antigen [31,32]. The mechanisms accounting for generation of an autoimmune response to a nuclear antigen are not known, let alone the relationship of antibody specificity to disease. The availability of relatively pure and intact recombinant antigens such as topo I in large amounts provides a valuable source of immunogens to explore autoimmune mechanisms.

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REFERENCES

1 Wang JC. DNA topoisomerases. Annu Rev Biochem 1985; **54:**665–97.
2 Catoggio LJ, Bernstein RM, Black CM, Hughes GRV, Maddison PJ. Serological markers in progressive systemic sclerosis: clinical correlations. Ann Rheum Dis 1983; **42:**23–27.
3 McNeilage LJ, Whittingham S, McHugh N, Barnett AJ. A highly conserved 72,000 dalton centromeric antigen reactive with autoantibodies from patients with progressive systemic sclerosis. J Immunol 1986; **137:**2541–7.
4 Steen VD, Powell VD, Medsger TA Jr. Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. Arthritis Rheum 1988; **31:** 196–203.
5 Weiner ES, Earnshaw WC, Senécal J-L, Bordwell B, Johnson P, Rothfield NF. Clinical associations of anticentromere antibodies and antibodies to topoisomerase 1. Arthritis Rheum 1988; **31:**378–85.
6 Mukai M, Sagawa A, Atsumi T *et al.* 3 cases of anti-Scl-up (Topoisomerase-I) antibody associated with central nervous system lupus without renal disorder. J Rheumatol 1993; **20:**1594–7.
7 Weiner E, Hilldebrandt S, Senécal J-L *et al.* Prognostic significance of anticentromere antibodies and anti-topoisomerase 1 antibodies in Raynaud's disease. A prospective study. Arthritis Rheum 1991; **34:** 68–77.
8 D'Arpa P, White-Cooper H, Cleveland DW, Rothfield NF, Earnshaw WC. Use of molecular cloning methods to map the distribution of epitopes on topoisomerase-1 (Sci70) recognised by sera of scleroderma patients. Arthritis Rheum 1990; **10:**1501–11.
9 Verheijen R, Van den Hoogen F, Beijer R, Richter A, Penner E, Habets WJ, Van Venrooij WJ. A recombinant topoisomerase I used for autoantibody detection in sera from patients with systemic sclerosis. Clin Exp Immunol 1990; **80:**38–43.
10 Meesters TM, Hoet M, Van den Hoogen FJH, Verheijen R, Habets WJ, Van Venrooij WJ. Analysis of an immunodominant epitope of topoisomerase I in patients with systemic sclerosis. Mol Biol Rep 1992; **16:**117–23.
11 Verheijen R, De Jong BAW, Van Venrooij WJ. A recombinant topoisomerase I ELISA: screening for IgG, IgM and IgA anti-topo I autoantibodies in human sera. Clin Exp Immunol 1992; **89:**456–60.
12 Kato T, Yamamoto K, Takeuchi H *et al.* Identification of a universal B cell epitope on DNA topoisomerase I, an autoantigen associated with scleroderma. Arthritis Rheum 1993; **36:**1580–7.
13 Seelig HP, Schröter H, Ehrfeld H, Renz M. Autoantibodies against topoisomerase I detected with the natural enzyme and overlapping recombinant pepitides. J Immunol Methods 1993; **165:**241–52.
14 Kuwana M, Kaburaki J, Mimori T, Tojo T, Homma M. Autoantigenic epitopes on DNA topoisomerase I: clinical and immunogenetic associations in systemic sclerosis. Arthritis Rheum 1993; **36:**1406–13.
15 Cram DS, Fisicaro N, McNeilage LJ, Coppel RL, Harrison LC. Antibody specificities of Thai and Australian scleroderma sera with topoisomerase I recombinant fusion proteins. J Immunol 1993; **151:** 6872–81.
16 Masi AT, Rodnan GP, Medsger TA Jr *et al.* Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1992;**23:**581–90.
17 Tan EM, Cohen AS, Fries JF *e al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982; **25:**1271–7.
18 Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; **31:**315–24.
19 McHugh NJ, Whyte J, Harvey G, Haustein U-F. Anti-topoisomerase-1 antibodies in silica-associated systemic sclerosis. A model for autoimmunity. Arthritis Rheum 1994; **37:**1198–205.
20 D'Arpa P, Machlin PS, Ratrie H, Rothfield N, Cleveland DW, Earnshaw WC. cDNA cloning of human DNA topoisomerase I: catalytic activity of a 67·7-kDa carboxyl-terminal fragment. Proc Natl Acad Sci USA 1988; **85:**2543–7.
21 Champoux JJ, McConaughty BL. Purification and characterisation of the DNA untwisting enzyme from rat liver. Biochemistry 1976; **15:**4638–42.
22 Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin reagent. J Biol Chem 1951; **193:**265–75.
23 Towbin HS, Staehelin S, Gordon J. Elecrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. Proc Natl Acad Sci USA 1979; **76:**4350–5.
24 Hildebrandt S, Weiner E, Senecal J-L, Noell S, Daniels L, Earnshaw WC, Rothfield NF. The IgG, IgM, and IgA isotypes of anti-topoisomerase-1 and anticentromere autoantibodies. Arthritis Rheum 1990; **33:**724–7.
25 Shero JH, Bordwell B, Rothfield NF, Earnshaw WC. High titres of autoantibodies to topoisomerase I (Scl-70) in sera from scleroderma patients. Science 1986; **231:** 737–40.
26 Guldner H-H, Szostecki C, Vosberg H-P, Lakomek H-J, Penner E, Bautz FA. Scl 70 autoantibodies from scleroderma patients recognize a 95 kDa protein identified as DNA topoisomerase I. Chromosoma 1986; **94:** 132–8.
27 Huff JP, Roos G, Peebles CL, Houghten R, Sullivan KF, Tan EM. Insights into native epitopes of proliferating cell nuclear antigen using recombinant DNA protein products. J Exp Med 1990; **172:** 419–29.
28 Miller FW, Waite KA, Biswas T, Plotz PH. The role of an autoantigen, histidyl-tRNA synthesase, in the induction and maintenance of autoimmunity. Proc Natl Acad Sci USA 1990; **87:**9933–7.
29 Van de Water J, Gershwin ME, Leung P, Ansari A, Coppel RL. The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipamide acetyltransferase. J Exp Med 1988; **167:**1791–9.
30 Vázquez-Abad D, Wallace S, Senécal J-L, Joyal F, Roussin A, Earnshaw WC, Rothfield N. Anticentromere autoantibodies: evaluation of an ELISA using recombinant fusion protein CENP-B as antigen. Arthritis Rheum 1994; **37:**248–52.
31 Hardin JA. The lupus autoantigens and the pathogenesis of systemic lupus erythematosus. Arthritis Rheum 1986; **29:**457–60.
32 Tan EM. Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1992; **44:**93–151.