Inhibition of topoisomerase I by antibodies in sera from scleroderma patients

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Purified type I topoisomerase from calf thymus as well as nuclear and cytoplasmic extracts from EGF-stimulated human and mouse fibroblasts in cell culture efficiently convert supercoiled plasmid DNA to the relaxed form. The purified IgG fraction from the sera of Japanese patients with the rheumatic disease scleroderma were shown to inhibit this relaxation activity. Thus, these patients likely produce autoantibodies to topoisomerase I. In addition, the human, bovine and murine enzymes share antigenic determinants recognized by the antisera.

Topoisomerase I *Scleroderma antiserum* *Epidermal growth factor*

1. INTRODUCTION

Topoisomerases are enzymes that alter the topological conformation of DNA; they have been implicated in replication, gene expression and chromosome segregation (review [1–3]). Type I topoisomerases generate transient single-strand nicks in duplex DNA that allow for relaxation of supercoiled molecules as well as other topological manipulations [2–5]. Recently, two groups [6,7] have identified Scl-70, a nuclear antigen recognized by the sera of patients with diffuse scleroderma, as topoisomerase I. We have shown that the autoantibodies from Japanese patients neutralize the relaxation of supercoiled plasmid DNA and that they cross react with topoisomerase I from human, bovine and murine sources.

2. MATERIALS AND METHODS

2.1. *Materials*

Purified calf thymus topoisomerase I was obtained from BRL (Maryland). Supercoiled plasmid DNA was prepared by cesium chloride/ethidium bromide density gradient centrifugation [8].

2.2. *Preparation of IgG fraction*

IgG was purified from the sera of various patients by ammonium sulfate precipitation and DEAE-cellulose column chromatography as described [9].

2.3. *Cell culture*

Human fibroblasts (HFO) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum (FBS), 0.3 mg/ml glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Swiss/3T3 mouse fibroblasts were grown in DMEM containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were grown to confluency in 10 cm dishes at 37°C in 5% CO_2 and 100% humidity. EGF (Collaborative Research) was added to a final concentration of 15 ng/ml 6 h before harvesting EGF-induced Swiss/3T3 cells and 9 or 12.5 h before collecting HFO cells.

2.4. *Cell fractionation*

Cells were harvested and lysed as described previously [10], except intact cells were frozen at −70°C for 1–30 days, and HFO was lysed in 1 ml.

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2.5. *Immunoneutralization*

Either 5 μl of cell lysate or 10 μl of diluted calf thymus topoisomerase I (0.25 U/μl) were incubated in 10 mM potassium phosphate, pH 7.5, with various amounts of purified IgG for 30 min at 4°C, with slow rotation, in a final volume of 10 or 20 μl.

2.6. *Topoisomerase I assay*

Relaxation activity was measured by a modification of previous protocols [4,11]. The reaction mixture contained in 20 or 40 μl final volume 50 mM Tris-HCl, pH 7.8, 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 30 μg/ml BSA and 20 μg/ml supercoiled plasmid DNA (BpMTVdhfrHiHi3 # 1, 7 kb, a derivative of pMTVdhfr [12]). Relaxation reactions were initiated by addition of 10 or 20 μl of concentrated reaction mixture to the immunoneutralization and incubated for 30 min at 30°C. Reactions were terminated by addition of 5 or 10 μl of 5% SDS, 50% glycerol, 0.1% bromophenol blue and 0.1% xylene cyanole, heated to 60°C for 2 min and cooled on ice.

2.7. *Electrophoresis*

Samples were loaded onto a 0.7% agarose gel and electrophoresed for 13–15 h at 30 V (2.2 V/cm) in 40 mM Tris-acetate, 1 mM EDTA at room temperature. The gels were stained in 1 μg/ml ethidium bromide for 45 min, destained in 1 mM $MgSO_4$ for 1 h, visualized on a long wave UV transilluminator (Ultra-violet Products) and photographed through Kodak Wratten filters, nos25 and 12, as well as 3 mm of Lucite, with a Polaroid MP-3 land camera using Polaroid type 57 film [8].

3. RESULTS

Type I topoisomerase activity is measured as the ability to relax supercoiled DNA (see [2]). The relaxation reaction results in the formation of a series of topoisomers, DNA molecules with the same nucleotide sequence but that differ in topological conformation. The relaxed molecules migrate slower in agarose gels due to their decreased compaction [13].

Fig.1 shows the relaxation of supercoiled (form I) plasmid DNA by 2.5 U (180 ng) of purified calf thymus topoisomerase I (lane 3). Normal human IgG has no effect on the topoisomerase activity (lane 4); however, at high concentrations, scleroderma IgG causes a tailing of nicked (form II) DNA (lanes 2 and 5). In a titration of purified IgG from FO sera with topoisomerase I (lanes 5–10), inhibition first appears at 1 μg IgG and topoisomerase activity is completely neutralized by 10 μg IgG.

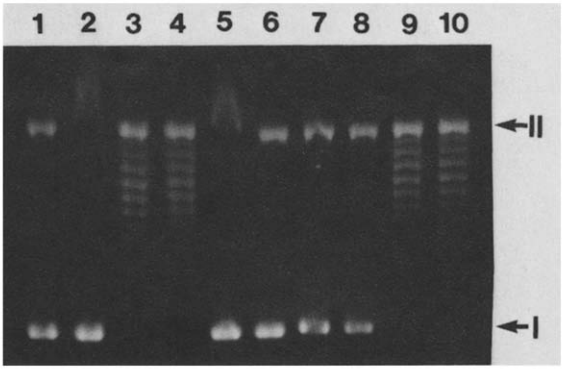

Fig.1. Inhibition of purified calf thymus topoisomerase I. Reactions contained in 40 μl either no topoisomerase (lanes 1 and 2) or 2.5 U of topoisomerase (lanes 3–10). Lanes: 1 and 3, no IgG;. 4, 20 μg of control IgG from normal human sera; 2 and 5, 20 μg of FO IgG; 6, 10 μg FO IgG; 7, 2 μg FO IgG; 8, 1 μg FO IgG; 9, 0.2 μg FO IgG; 10, 0.1 μg FO IgG. Nicked and relaxed molecules comigrate.

IgG from MI sera have a similar response (not shown). A third serum, KK, inhibits topoisomerase I at only 0.2 μg IgG (not shown). KK sera may have a higher titer of autoantibodies or the antibodies may have a higher affinity for the enzyme.

We next examined the ability of these antibodies to inhibit topoisomerase I activity in crude extracts from EGF-stimulated human and mouse cells. We previously have shown that EGF induces topoisomerase activity in contact-inhibited fibroblasts [10]. Type II topoisomerase activity was eliminated by omission of ATP from the reaction mixture as well as by freezing the cell pellets prior to lysis. The type II enzyme is, in contrast to topoisomerase I, ATP dependent as well as labile in frozen whole cells [11,14].

Topoisomerase I activity in the cytoplasm of HFO cells treated for 8 h with EGF (fig.2, lane 3) or in the nucleus of cells treated for 12.5 h (lane 6)

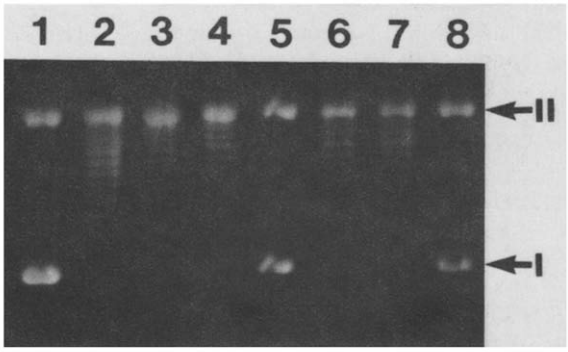

Fig.2. Inhibition of topoisomerase I activity in HFO lysates. Reactions contained in 20 μl no lysate (lane 1), 5 U of calf thymus topoisomerase I (lane 2), 5 μl of 12.5 h EGF-induced HFO post-nuclear fraction (lanes 3–5), or 5 μl of 8 h EGF-induced HFO nuclear extract (lanes 6–8). Lanes: 1, 2, 3 and 6, no IgG; 4 and 7, 10 μg control IgG; 5 and 8, 10 μg of FO IgG.

is effectively inhibited by IgG from FO sera (lanes 5 and 8).

FO antibodies also recognize and inhibit the relaxation activity from the cytoplasm of Swiss/3T3 mouse cells induced by EGF for 6 h and from the nucleus of uninduced cells (fig.3). Immunoblotting of cytoplasmic and nuclear extracts from Swiss/3T3 cells with FO IgG revealed bands of 100, 110 and 66 kDa, similar to the known molecular masses of intact mammalian topoisomerase I, its phosphorylated form and its proteolytic cleavage product, respectively (not shown) [2,5,15].

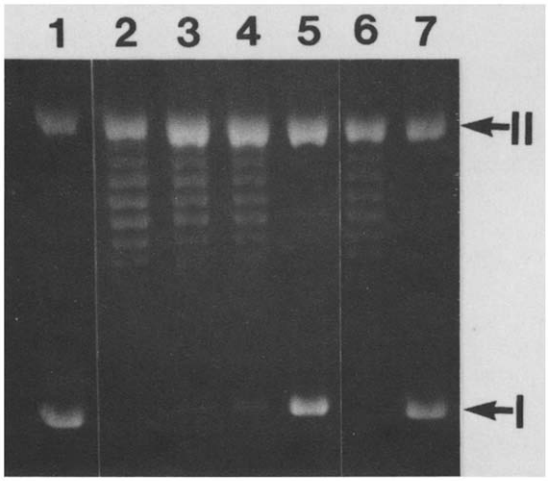

Fig.3. Inhibition of topoisomerase I activity in Swiss/3T3 lysates. Reactions contained in 20 μl no lysate (lane 1), 2.5 U of calf thymus topoisomerase I (lane 2), 5 μl of 6 h EGF-induced Swiss/3T3 post-nuclear fraction (lanes 3–5), or 5 μl of Swiss/3T3 nuclear extract (lanes 6 and 7). Lanes: 1, 2, 3 and 6, no IgG; 4, 10 μg control IgG; 5 and 7, 10 μg of FO IgG.

4. DISCUSSION

Topoisomerases are probably involved in many aspects of cell proliferation [10,16–19]. However, the role of the type I eukaryotic enzyme has not been elucidated. The clinical significance of the anti-topoisomerase I antibodies in the sera of scleroderma patients is also not understood [6]. *Escherichia coli* [20], *Saccharomyces cerevisiae* [21,22] and *Schizosaccharomyces pombe* [23] topoisomerase I mutants are viable; but DNA synthesis is blocked during the early phase of elongation in a temperature sensitive topoisomerase I mutant of BALB/3T3 cells [24]. A topoisomerase-like activity has been found to be associated with the EGF receptor [25,26]. We are currently using the autoantibodies as a tool to study the intracellular localization and regulation of topoisomerase I in EGF-induced cells in order to provide greater insight into mitogenic signal transduction as well as topoisomerase I function.

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REFERENCES

[1] North, G. (1985) Nature 316, 394–395.
[2] Vosberg, H.-P. (1985) Curr. Top. Microbiol. Immunol. 114, 19–102.
[3] Wang, J.C. (1985) Annu. Rev. Biochem. 54, 664–697.
[4] Liu, L.F. (1983) Methods Enzymol. 100, 133–136.
[5] Liu, L.F. and Miller, K.G. (1981) Proc. Natl. Acad. Sci. USA 78, 3487–3491.
[6] Shero, J.H., Bordwell, B., Rothfield, N.F. and Earnshaw, W.C. (1986) Science 231, 737–740.

[7] Maul, G.G., French, B.T., Van Venrooij, W.J. and Jimenez, S.A. (1986) Proc. Natl. Acad. Sci. USA 83, 5145–5149.
[8] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
[9] Fahey, J.L. and Terry, E.W. (1978) in: Handbook of Experimental Immunology, vol.1 (Weir, D.M. ed.) chapter 8, Blackwell, London.
[10] Miskimins, R., Miskimins, W.K., Bernstein, H. and Shimizu, N. (1983) Exp. Cell Res. 146, 53–62.
[11] Goto, T., Laipis, P. and Wang, J.C. (1984) J. Biol. Chem. 259, 10422–10429.
[12] Lee, F., Mulligan, R., Berg, P. and Ringold, G. (1981) Nature 294, 228–232.
[13] Keller, W. (1975) Proc. Natl. Acad. Sci. USA 72, 2550–2554.
[14] Samuels, D.S. and Shimizu, N., unpublished results.
[15] Durban, E., Mills, J.S., Roll, D. and Busch, H. (1983) Biochem. Biophys. Res. Commun. 111, 897–905.
[16] Nelson, W.G., Liu, L.F. and Coffey, D.S. (1986) Nature 322, 187–189.
[17] Nagata, K., Guggenheimer, R.A. and Hurwitz, J. (1983) Proc. Natl. Acad. Sci. USA 80, 4266–4270.
[18] Duguet, M., Lavenot, C., Harper, F., Mirambeau, G. and De Recondo, A.-M. (1983) Nucleic Acids Res. 11, 1059–1075.
[19] Taudou, G., Mirambeau, G., Lavenot, C., Der Garabedian, A., Vermeersch, J. and Duguet, M. (1984) FEBS Lett. 176, 431–435.
[20] Sternglanz, R., DiNardo, S., Voelkel, K.A., Nishimura, Y., Hirota, Y., Becherer, K., Zumstein, L. and Wang, J.C. (1981) Proc. Natl. Acad. Sci. USA 78, 2747–2751.
[21] Thrash, C., Voelkel, K., DiNardo, S. and Sternglanz, R. (1984) J. Biol. Chem. 259, 1375–1377.
[22] Goto, T. and Wang, J.C. (1985) Proc. Natl. Acad. Sci. USA 82, 7178–7182.
[23] Uemura, T. and Yanagida, M. (1984) EMBO J. 3, 1737–1744.
[24] Zeng, G.-C., Zannis-Hadjopoulos, M., Ozer, H.L. and Hand, R. (1985) Som. Cell Mol. Genet. 11, 557–569.
[25] Mroczkowski, B., Mosig, G. and Cohen, S. (1984) Nature 309, 270–273.
[26] Basu, M., Frick, K., Sen-Majumdar, A., Scher, C.D. and Das, M. (1985) Nature 316, 640–641.