T Cell Proliferative Response Induced by DNA Topoisomerase I in Patients with Systemic Sclerosis and Healthy Donors

Masataka Kuwana,* Thomas A. Medsger, Jr.,* and Timothy M. Wright*‡
*Division of Rheumatology and Clinical Immunology, Department of Medicine; and ‡Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Abstract

The in vitro T cell proliferative response to DNA topoisomerase I (topo I) was examined in 26 systemic sclerosis (SSc) patients with anti–topo I antibody, 10 SSc patients without anti–topo I antibody, and 21 healthy donors. Using recombinant fusion proteins encompassing the entire human topo I amino acid sequence, a topo I–specific proliferative response was detected in PBMC cultures from 25 (96%) anti-topo I–positive SSc patients, 4 (40%) anti-topo I–negative SSc patients, and 13 (62%) healthy donors. Molecular typing at MHC class II loci revealed that all SSc patients and healthy donors having either DRB1*1501,2 (DR15), DRB1*1101,3,4 (DR11), or DRB1*07 (DR7) were responders. Characterization of the topo I–induced T cell proliferative response showed that (a) the responding cells were CD4+ T cells; (b) antigen-presenting cells were necessary for the response; (c) the response was restricted by HLA-DR, and to a lesser extent by HLA-DQ; and (d) the estimated frequency of the responding T cells determined by limiting dilution analysis was 1/9,277–1/24,853. PBMC cultures from anti-topo I–positive SSc patients showed a high T cell proliferative response after only 3 d of culture with topo I. Anti-topo I–negative SSc patients and healthy donors had no proliferative response after 3 d, but did respond after 7 d of culture. T cell proliferative responses to six truncated topo I fragments tested individually showed different patterns of T cell proliferation that were dependent upon the responder's HLA-DR alleles. These results indicate that T cells reactive with topo I are components of the normal T cell repertoire, and that the topo I–specific T cell proliferative response is not associated with the presence or absence of SSc or anti–topo I antibody, but is restricted by MHC class II alleles. (*J. Clin. Invest.* 1995. 96:586–596.) Key words: autoantibody • antinuclear antibody • HLA • T cell tolerance • cryptic epitope

1. *Abbreviations used in this paper:* APC, antigen-presenting cell; MBP, maltose-binding protein; SI, stimulation index; snRNP, small nuclear ribonucleoprotein; SSc, systemic sclerosis; topo I, DNA topoisomerase I.

Introduction

Systemic sclerosis (scleroderma; or SSc[1]) is an autoimmune disease characterized by microvascular damage and fibrosis of the dermis and internal organs (1). One prominent immunologic abnormality of SSc is the presence of serum antinuclear antibody, which is found in > 90% of patients (1). The autoantigens to which these antibodies bind include DNA topoisomerase I (topo I); centromere/kinetochore; RNA polymerases I, II, and III; U3 ribonucleoprotein; and Th ribonucleoprotein (1–5). It is well established that each distinctive SSc-related antinuclear antibody is closely associated with its own unique combination of clinical features (1–5). These findings suggest that the production of SSc-related antinuclear antibodies may be related to the fundamental disease process of SSc.

Antinuclear antibodies directed against topo I (6), formerly termed Scl-70 (7), are detected in the sera from ~ 20–30% of SSc patients (8). Anti–topo I antibody is specific to SSc sera and is associated with diffuse cutaneous involvement, peripheral vascular disease, and pulmonary interstitial fibrosis (4, 5). Human autoimmune sera containing anti–topo I antibodies recognize multiple B cell epitopes on the topo I molecule (9), suggesting that anti–topo I antibody production is an antigen-driven process. T cell "help" is presumed to be required for the production of this autoantibody since topo I is a protein antigen, anti–topo I antibody is usually present in SSc sera at high titer (6, 10), and the most abundant anti–topo I antibody isotypes are IgG and IgA (10). Recent MHC class II gene typing analyses of anti-topo I–positive SSc patients showed that the presence of serum anti–topo I antibody was associated with specific MHC class II alleles. In two studies examining this relationship in North American populations, anti–topo I antibody was associated with the broad specificity of DR11 and DQ3 (11), or more specifically specific DR11 and DQ3 suballeles DRB1*1104 and DQB1*0301 (12). In a study of Japanese SSc patients, anti–topo I antibody was associated with DRB1*1502 and DQB1*0601 (13). Furthermore, we found that anti–topo I antibody titer and reactivities against certain B cell epitopes on topo I were related to amino acid sequences shared among several HLA-DR molecules (13). These observations strongly suggest that an interaction of MHC class II molecules and autoreactive T cells via the T cell receptor plays an important role in the anti–topo I antibody production in SSc patients. Although T cell responses to self-proteins have been well characterized in organ-specific autoimmune diseases such as multiple sclerosis and autoimmune thyroid diseases, only a limited number of such analyses have been performed for systemic autoimmune diseases. Moreover, all of these latter studies examined autoantigen-specific T cell response in patients with SLE or mixed connective tissue disease (14–16), and an investigation of autoantigen-specific T cell responses has not performed in SSc patients. One potential reason for this may be

the difficulty in obtaining a sufficient amount of highly purified soluble autoantigen for such studies. Recently, we have prepared recombinant fusion proteins encompassing the entire topo I molecule and have used these recombinant topo I proteins to develop an anti–topo I antibody ELISA (10). In the present study, these recombinant proteins were used to examine the topo I–specific T cell proliferative response in SSc patients with or without serum anti–topo I antibody and in healthy donors.

Methods

Patients and healthy donors. 26 SSc patients with serum anti-topo I antibody (19 women and 7 men; age 28–69 yr, mean 48 yr), 10 SSc patients without serum anti-topo I antibody (8 women and 2 men; age 28–68 yr, mean 43 yr), and 21 healthy donors (10 women and 11 men; age 26–60 yr, mean 39 yr) were included in this study. All SSc patients visited the Scleroderma Clinic, Division of Rheumatology and Clinical Immunology, University of Pittsburgh School of Medicine for follow-up evaluation during January through May, 1994, and fulfilled the American Rheumatism Association preliminary classification criteria for SSc (17). Anti-topo I–positive SSc patients included 23 North American Caucasians, 2 North American Blacks, and one Japanese, whereas all 10 anti-topo I–negative SSc patients were North American Caucasians. Healthy donors consisted of 19 North American Caucasians and 2 Japanese living in the Pittsburgh area.

Clinical and laboratory findings were prospectively collected on all anti-topo I–positive SSc patients. These included disease duration at the time of the analysis, organ involvement (peripheral vasculature, skin, joint/tendon, muscle, intestine [esophagus and small bowel], lung, heart, and kidney), and previous and current medications (corticosteroids, immunosuppressive agents, and D-penicillamine). Disease duration was measured from the time of onset of the first symptom attributable to SSc. SSc disease classification (limited or diffuse cutaneous involvement variant of SSc) was determined by one of the authors (T. A. Medsger, Jr.) according to the previously published criteria (1). Criteria for organ involvement were based on our definition reported elsewhere (2).

Anti-topo I antibody assays. Anti-topo I antibody was analyzed using serum samples obtained at the time of PBMC isolation for the cell proliferation assay. Serum anti-topo I antibody was assayed by three different methods including double immunodiffusion (Ouchterlony) using rabbit thymus extract as an antigen (9), immunoprecipitation using ^{35}S-labeled HeLa cell extract (3, 18), and ELISA using recombinant topo I (10). The levels of IgG, IgA, IgM, and "total" (total of IgG, IgA, and IgM isotypes) anti-topo I antibodies were determined by quantitative ELISA (10). Sera from all anti-topo I–positive SSc patients were confirmed to be positive by all three methods, and those from all anti-topo I–negative SSc patients and healthy donors were confirmed to be negative by all three methods.

Antigens. Six truncated soluble recombinant topo I fusion proteins (Fig. 1) were prepared as described (10). These included F3 (amino acids 1–93 of 765 amino acids of topo I), F4 (amino acids 43–224), F5 (amino acids 209–386), F6 (amino acids 363–563), F7 (amino acids 541–765), and F11 (amino acids 658–765). Briefly, portions of the topo I cDNA prepared by restriction endonuclease digestion were subcloned in frame into the pMAL-c2 expression vector (New England Biolabs, Beverly, MA) and expressed in *Escherichia coli* as maltose-binding protein (MBP)–topo I fusion proteins. After affinity purification using amylose resin chromatography (19), fusion proteins were dialyzed against Tris-buffered saline (20 mM Tris/HCl, 140 mM NaCl, pH 7.4) and sterilized by passage through 0.22-μm pore-size syringe filters. To minimize proteolytic degradation, all recombinant fusion proteins were immediatley aliquoted and stored at −80°C until use. SDS-PAGE analyses showed that the purity of all recombinant proteins was > 95%, and all recombinant proteins except for F4 contained intact recombinant protein and minimal degradation fragments. F4 was prone

Figure 1. DNA topoisomerase I and truncated recombinant topo I fusion proteins (*F3, F4, F5, F6, F7,* and *F11*) that were used in this study. Numbers denote the amino acid residues of the whole topo I amino acid sequence. Maltose-binding protein was fused to the NH_2 terminus (*N*) of the topo I amino acid sequence in all recombinant proteins.

to degradation, but the preparations used in these studies contained ≥ 10% intact F4 protein. Since F3–F7 encompassed the entire topo I amino acid sequence, an equimolar mixture of these five recombinant proteins was regarded as topo I, and was used to measure topo I–specific cell proliferation. MBP was expressed in *E. coli* bearing the pMAL-c2 vector without insert DNA and was affinity purified (as above) for use as a control. In some experiments, purified tetanus toxoid (Massachusetts Public Health Biological Laboratories, Boston, MA) was used as a control antigen for the cell proliferation assay.

Cell preparations. PBMC and PMN were isolated from heparinized blood using Histopaque (Sigma Chemical Co., St. Louis, MO) double-density gradient centrifugation. PBMC were cultured in RPMI 1640 containing 10% FBS, 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37°C, 5% CO_2. PMN were stored at −20°C and used as sources for genomic DNA. Monocytes, T cells, and B cells were isolated from some PBMC samples. Briefly, PBMC were cultured on plastic dishes for 1 h, and nonadherent cells were harvested. After three washes with TBS, adherent cells were removed by scraping with a rubber policeman and served as the monocyte fraction. T cells were separated from nonadherent cells by passage through a nylon wool column. B cells were removed from the nylon wool by compression against the sides of the tubes. More than 80% of cells in the monocyte fraction were viable monocytes determined by phagocytosis of yeast. Flow cytometry revealed that the T cell fraction contained > 95% CD3+ T cells, and CD3+ T cell contamination in the B cell fraction was < 5%. PBMC samples that were depleted of CD4+ or CD8+ cells were prepared by incubating PBMC with anti-CD4 or anti-CD8 mAb-coupled magnetic beads (Dynal, Oslo, Norway), respectively, at 4°C for 1 h, followed by magnetic removal of bead-bound cells (20). After depletion using 5.6 × 10^6 beads/10^6 PBMC, the residual CD4+ or CD8+ cells in the fractions were each < 3% by flow cytometry. In some experiments, CD4+ or CD8+ cells were depleted using serial amounts of magnetic beads (7.0 × 10^4–5.6 × 10^6/10^6 PBMC).

Cell proliferation assay. PBMC (10^5 per well) were cultured in complete medium in 96-well flat-bottomed tissue culture plates (Corning Glass Works Inc., Corning, NY). Cells were cultured for 7 d (or, as indicated, 3 d) with medium alone, MBP (20 μg/ml), or topo I (the mixture of F3–F7; 20 μg/ml) and then incubated with 1 μCi/well of [^{3}H]thymidine during the final 16 h of culture. Cells were harvested and [^{3}H]thymidine incorporation was measured by liquid scintillation counting. All cultures were carried out in triplicate, and topo I–specific cell proliferation was expressed as the stimulation index (SI), which was calculated as cpm incorporated in topo I–stimulated cultures/cpm incorporated in MBP-stimulated cultures. On the basis of a previous report of antigen-specific T cell response (21), an SI > 3 was considered to be positive. F3, F4, F5, F6, F7, and F11 fusion proteins were also

used separately as antigens for the cell proliferation assay. The molar concentration of each protein was equalized on the basis of the estimated molecular weight ratio (F3, F4, F5, F6, F7, and F11 were used at concentrations of 9.8, 12.2, 11.5, 12.5, 13.4, and 10.2 μg/ml, respectively). Fractionated cell populations (monocytes, T cells, and B cells), and the mixture of these cell populations in various ratios were also tested in the cell proliferation assay.

Secondary stimulation of PBMC was performed in some experiments. After the priming stimulation of PBMC (10^5 per well) with topo I for 7 d, the viable cells were then cultured for 3 d in the presence of 50 U/ml rIL-2 (GIBCO BRL, Grand Island, NY) and irradiated (9,000 rad) autologous EBV-transformed B cell line cells (5×10^4) as antigen-presenting cells (APCs) in medium alone, or supplemented with topo I (20 μg/ml) or MBP (20 μg/ml). Proliferation was measured by incorporation of [^{3}H]thymidine as described above.

mAb blocking of topo I–specific cell proliferation. The effect on topo I–induced cell proliferation produced by blocking interactions with the MHC class II molecules was determined by the addition of mAbs specific for nonpolymorphic regions of the MHC class II molecules (0.2 μg/ml, unless indicated otherwise) to the PBMC cultures. The mAbs used were anti-HLA-DR (L243; IgG$_{2a}$), anti-HLA-DQ (1a3; IgG$_{2a}$), and anti-HLA-DP (B7/21; IgG$_3$) (Leinco, St. Louis, MO). Purified mouse IgG$_{2a}$ and IgG$_3$ (Leinco) were used as isotype controls.

Limiting dilution analysis. To estimate the frequency of topo I–responding T cells, a limiting dilution analysis was carried out according to the method of Brett et al. (22). Aliquots containing serial numbers of T cells (10^3, 2.5×10^3, 5×10^3, and 10^4) were cultured in 96-well plates in the presence of monocytes (10^3/well) and topo I (20 μg/ml). Each aliquot of T cells was dispensed in 48 wells. The wells showing an SI > 3 were considered to be positive. On the basis of the assumption that topo I–responding cells are randomly distributed among the culture wells, the frequency of responding cells was calculated according to a Poisson distribution formula.

MHC class II allele genotyping. Genomic DNA isolated from PMN (23) was amplified by PCR using primers specific for the polymorphic exon 2 domains of the DRB1, DRB3, DRB5, DQB1, and DPB1 genes (24). MHC class II alleles were determined based on the results of RFLP of PCR-amplified products as described previously (24) with some modifications. The restriction endonuclease BsrBI (New England Biolabs) was used for discrimination of DRB1*1101 and *1104.

Statistical analyses. Differences in frequencies were tested by Fisher's two-tailed exact test, and significant P values were corrected by multiplying by the number of comparisons made. Mean values were compared by Student's t test. Correlation coefficients (r) were determined using a single regression model. Unless stated otherwise, all values represent the mean of triplicate determinations and SD are < 15% of the mean.

Results

MHC class II alleles in anti-topo I–positive SSc patients versus anti-topo I–negative SSc patients and healthy donors. The MHC class II alleles of the SSc patients and healthy donors who participated in this study are listed in Table I. Since the majority of the SSc patients examined were Caucasian, the MHC class II allele frequencies were compared among the 23 anti-topo I–positive Caucasian SSc patients, the 10 Caucasian anti-topo I–negative SSc patients, and the 19 Caucasian healthy donors. The frequencies of DRB1*1501 and DRB1*1104 alleles were increased in anti-topo I–positive SSc patients compared with anti-topo I–negative SSc patients and healthy donors, but these differences did not reach statistical significance (35% vs. 20% and 26%, and 39% vs. 10% and 11%, respectively). All but one Caucasian anti-topo I–positive SSc patient (T26) had either DR15 (DRB1*1501), DR11 (DRB1*1101, *1103, and *1104), or DR7 (DRB1*07). The frequency of

having one of these three alleles was significantly greater in anti-topo I–positive SSc patients compared with anti-topo I–negative SSc patients and healthy donors (96% vs. 40% and 47%; $P = 0.001$, corrected $P = 0.05$ in both comparisons). One of 2 Black anti-topo I–positive SSc patients had DRB1*1501, and one Japanese anti-topo I–positive SSc patient had DRB1*1502, another suballele of DR15. There were no associations between DR15, DR11, or DR7 and clinical findings in Caucasian SSc patients.

PBMC proliferative response to topo I. The proliferative responses of PBMC cultured for 7 d with recombinant topo I expressed as [^{3}H]thymidine incorporation and SI are shown in Table I. A topo I–specific response (SI > 3) was detected in 25 (96%) of 26 anti-topo I–positive SSc patients. Four (40%) anti-topo I–negative SSc patients and 13 (62%) healthy donors also responded to topo I. The frequency of PBMC proliferation to topo I was significantly higher in anti-topo I–positive SSc patients than those in anti-topo I–negative SSc patients and healthy donors ($P = 0.001$ and 0.007, respectively), but the mean SI levels of responders were not different among these three groups (7.6 ± 3.3, 6.0 ± 2.6, and 10.6 ± 7.2, respectively). When the MHC class II alleles of topo I responders and nonresponders were examined, it was found that all anti-topo I–positive SSc patients, anti-topo I–negative SSc patients, and healthy donors possessing either DR15 (DRB1*1501 and *1502), DR11 (DRB1*1101, *1103, and *1104), or DR7 (DRB1*07) were responders. The remaining responders included one Black anti-topo I–positive SSc patient with DRB1*0405/*1001 and three Caucasian healthy donors with DRB1*1302/*0801, DRB1*0404/*0801, and DRB1*0402/ *1301. In contrast, DR1, DR3, and most alleles of DR4 and DR6 seemed to be associated with nonresponsiveness to topo I.

To confirm the presence of topo I–specific T cells in both anti-topo I–positive SSc patients and healthy donors, a secondary stimulation of PBMC with topo I was performed. In these experiments, topo I–dependent proliferation of primary and secondary cultures of PBMC from two anti-topo I–positive SSc patients (T1 and T4) and one healthy donor (D2) was determined. As shown in Table II, when [^{3}H]thymidine incorporation was measured after 3 d of the secondary culture, topo I, but not MBP, induced proliferation of PBMC from the anti-topo I–positive SSc patients and a healthy donor.

We examined the potential relationship between SI levels and clinical findings in 26 anti-topo I–positive SSc patients. Although there was no significant association of SI levels with SSc disease subset or organ involvement, a weak negative correlation between SI levels and disease duration was found ($P = 0.04$, $r = 0.404$; Fig. 2). At the time the blood samples were obtained for the cell proliferation assay, four, seven, and two anti-topo I–positive patients were on low dose corticosteroids (< 20 mg/d prednisolone), D-penicillamine, and azathioprine, respectively (2 took both corticosteroids and D-penicillamine and one took both D-penicillamine and azathioprine). There were no differences in the SI levels of PBMC from patients taking these potentially immunosuppressive agents and the remaining patients. We also found no correlation between IgG, IgA, IgM, or total anti-topo I antibody levels and the PBMC proliferative response (SI) to topo I (data not shown).

Dose dependency of topo I–induced proliferation. PBMC from three SSc patients with anti-topo I antibody were stimulated with various concentrations of topo I and MBP (0.01–

No.	Race	Disease subset	DR	DQ	DRB1[‡]	DRB3	DQB1	DPB1	[³H]Thymidine incorporation (cpm)*			SI	Response
									Medium	MBP	Topo I		
						Anti-topo I–positive SSc patients							
T1	Caucasian	Diffuse	2, 5	1, 3	1601/1104	0202	0502/0301	0402/1001	91	51	810	15.9	+
T2	Caucasian	Limited	5	3	1101/1104	0202	0301	0401/0402	327	184	2631	14.3	+
T3	Black	Diffuse	4, 10	1, 3	0405/1001	—	0501/0302	0101/0301	196	165	2227	13.5	+
T4	Caucasian	Limited	2	1	1501	—	0602	0401	112	147	1582	10.8	+
T5	Caucasian	Limited	7	2, 3	07[§]	—	0201/0303	0901/1301	192	136	1401	10.3	+
T6	Caucasian	Diffuse	2, 6	1, 3	1501/1301	0202	0602/0301	1001/1301	393	191	1816	9.5	+
T7	Japanese	Diffuse	2, 8	1, 4	1502/0802	—	0601/0402	0301/0901	82	109	1032	9.5	+
T8	Caucasian	Limited	2, 7	1, 2	1501/07	—	0602/0201	0501/0601	321	179	1511	8.4	+
T9	Caucasian	Diffuse	1, 7	1, 3	0101/07	—	0504/0303	0401/1301	183	134	1038	7.7	+
T10	Caucasian	Diffuse	1, 5	1, 3	0101/1104	0202	0501/0301	0401/1701	209	112	857	7.7	+
T11	Caucasian	Diffuse	5, 6	1, 3	1103/1302	0202/0301	0604/0301	0401	156	185	1409	7.6	+
T12	Caucasian	Limited	2, 3	1, 2	1501/0301	0101	0602/0201	0401	171	211	1417	6.7	+
T13	Caucasian	Limited	2, 5	1, 3	1601/1104	0202	0502/0301	0402/1701	130	62	408	6.6	+
T14	Caucasian	Diffuse	2, 7	1, 3	1501/07	—	0602/0303	0201/0402	185	151	967	6.4	+
T15	Caucasian	Limited	2, 5	1	1501/1101	0202	0602/0603	0402/0501	115	98	624	6.4	+
T16	Caucasian	Diffuse	5, 6	1, 3	1104/1302	0202/0301	0604/0301	0301/0402	762	1883	11442	6.1	+
T17	Caucasian	Limited	1, 5	1, 3	0102/1101	0202	0501/0301	0301/0401	191	173	969	5.6	+
T18	Caucasian	Limited	4, 5	3	0401/1103	0202	0301/0302	0201/0401	186	176	967	5.5	+
T19	Caucasian	Limited	2, 5	1, 3	1501/1104	0202	0602/0301	0401	306	297	1595	5.4	+
T20	Caucasian	Diffuse	5, 6	1, 3	1101/1302	0202/0301	0501/0301	0101/1301	151	201	1005	5.0	+
T21	Caucasian	Limited	5, 6	1, 3	1104/1302	0202/0301	0604/0301	0401/1301	918	925	4440	4.8	+
T22	Caucasian	Diffuse	5, 7	2, 3	1104/07	0202	0201/0301	0401/0402	95	76	341	4.5	+
T23	Caucasian	Diffuse	5, 7	3	1101/07	0202	0301/0303	0401	225	158	676	4.3	+
T24	Caucasian	Diffuse	1, 2	1	0101/1501	—	0501/0602	0401/1301	171	158	648	4.1	+
T25	Black	Diffuse	2	1	1501	—	0602	0401	79	86	344	4.0	+
T26	Caucasian	Diffuse	4, 6	1, 3	0401/1302	0301	0604/0302	0901/1301	100	144	275	1.9	−
						Anti-topo I–negative SSc patients							
S1	Caucasian	Diffuse	5, 7	3	1101/07	0202	0301/0303	0401	119	173	1513	8.7	+
S2	Caucasian	Diffuse	1, 2	1	0103/1501	—	0501/0602	0401	195	109	836	7.7	+
S3	Caucasian	Diffuse	1, 2	1	0101/1501	—	0501/0602	0401	128	265	1087	4.1	+
S4	Caucasian	Diffuse	5, 6	1, 3	1104/1302	0101/0202	0301/0603	0401/0402	173	166	587	3.5	+
S5	Caucasian	Limited	3, 4	2, 3	0301/0401	0101	0201/0302	0301	179	234	497	2.1	−
S6	Caucasian	Diffuse	1, 4	1, 3	0101/0401	—	0501/0301	0301/0402	169	892	1686	1.9	−
S7	Caucasian	Diffuse	4	3	0401/0404	—	0301/0302	0301/1001	109	173	230	1.3	−
S8	Caucasian	Limited	3, 6	1, 2	0301/1301	0101	0603/0201	0401	98	101	115	1.1	−
S9	Caucasian	Limited	2, 3	1, 2	1602/0301	0101	0502/0201	0201/0301	159	344	348	1.0	−
S10	Caucasian	Limited	4, 6	1, 3	0404/1401	0202	0503/0301	0401/0402	133	141	138	1.0	−
						Healthy donors							
D1	Caucasian		6, 8	1, 4	1302/0801	0202	0603/0402	0301/0401	81	50	1391	27.8	+
D2	Caucasian		3, 5	2, 3	0301/1104	0101/0202	0201/0301	0201/0301	266	434	5803	21.8	+
D3	Caucasian		4, 8	3, 4	0404/0801	—	0302/0402	0401/1501	174	138	1819	13.2	+
D4	Caucasian		1, 5	1, 3	0101/1101	0202	0501/0301	0401/1701	562	627	7652	12.2	+
D5	Caucasian		4, 6	1, 3	0402/1301	0101	0603/0303	0201/0401	94	72	881	12.2	+
D6	Caucasian		5, 7	3	1103/07	0202	0301/0303	0401/0402	164	176	1929	11.0	+
D7	Caucasian		1, 2	1	0103/1501	—	0501/0602	0401	307	525	4309	8.2	+.
D8	Caucasian		2, 5	1, 3	1501/1101	0202	0602/0301	0201/0401	331	239	1817	7.6	+
D9	Caucasian		6, 7	1, 2	1301/07	0101	0603/0201	0201/0402	643	814	5011	6.2	+
D10	Caucasian		2, 5	1, 3	1501/1101	0202	0602/0301	0101/0201	204	300	1793	6.0	+
D11	Caucasian		2, 5	1, 3	1501/1104	0202	0602/0301	0401/0402	160	162	797	4.9	+
D12	Japanese		2, 6	1	1502/1405	0202	0503/0601	0501/0901	135	180	648	3.6	+
D13	Caucasian		1, 2	1	0101/1501	—	0501/0602	0401/1401	224	185	625	3.4	+
D14	Caucasian		4, 6	1, 3	0407/1301	0202	0603/0302	0402/1901	150	163	342	2.1	−
D15	Caucasian		3, 10	1, 3	0301/1001	0101	0501/0302	0401	64	58	62	1.1	−
D16	Caucasian		4	3	0401	—	0302	0201/0301	92	94	95	1.0	−
D17	Caucasian		3	2, 3	0301	0101	0201/0302	0101/0401	141	180	164	0.9	−
D18	Caucasian		1, 6	1	0101/1302	0301	0501/0604	0301/0401	140	172	157	0.9	−
D19	Caucasian		3, 4	2, 3	0301/0404	0101	0201/0302	0101/0301	163	205	151	0.7	−
D20	Japanese		8, 9	1, 3	0803/0901	—	0601/0303	0501	86	163	121	0.7	−
D21	Caucasian		6	1	1302	0301	0501/0604	0401/1401	94	92	55	0.6	−

* Results are expressed as the mean of triplicates (SD < 20% of the mean). ‡ All subjects with DRB1*1501 or *1502 had DRB5*0101 or *0102, respectively; and all subjects with DRB1*1601 or *1602 had either DRB5*0201 or *0202. § DRB1*0701 and *0702 were not discriminated in this study.

Table II. Primary and Secondary Stimulation of PBMC with Topo I and MBP

	Primary culture			Secondary culture		
	Medium	MBP	Topo I	Medium	MBP	Topo I
		cpm			*cpm*	
Anti-topo I–positive SSc patients						
T1	184±30	192±18	2748±312 (14.5)	1816±212	2060±201	37080±629 (18.0)
T4	136±21	189±24	1304±96 (6.9)	782±41	986±103	10996±1329 (11.1)
Healthy donor						
D2	369±24	518±104	8316±1201 (16.0)	1855±128	1909±62	28468±1024 (14.9)

Results in medium alone and MBP-induced responses are shown as mean±SD. Results in topo I–induced responses are shown as mean±SD (SI). SI was calculated as cpm incorporated in topo I–stimulated cultures/cpm incorporated in MBP-stimulated cultures.

100 μg/ml). As shown in Fig. 3, the dose–response of topo I–induced proliferation was different among these three patients. PBMC from two anti-topo I–positive SSc patients, T9 and T18, responded to 1 μg/ml topo I, whereas PBMC from another anti-topo I–positive SSc patient, T15, required 12.5 μg/ml of topo I to induce proliferation. To examine differences in the dose–response relationship of topo I–induced proliferation, PBMC from 26 responders, including 14 anti-topo I–positive SSc patients, 4 anti-topo I–negative SSc patients, and 8 healthy donors, were stimulated with two different concentrations of topo I (1 and 20 μg/ml). The results indicated that the concentration-dependence of the topo I proliferative response was independent of the presence and duration of disease (SSc), the presence of anti-topo I antibody, and MHC class II alleles (data not shown).

Determination of cell population responding to topo I. T cells, monocytes, and B cells were separated from the PBMC of five anti-topo I–positive SSc patients and three healthy donors, and the topo I–induced proliferative response was measured in different combinations of these cell populations. Similar results were obtained from all individuals, and representative results from three anti-topo I–positive SSc patients and one healthy donor are shown in Fig. 4. We found that T cells or monocytes cultured alone did not respond to topo I. The topo I–induced proliferative response was detected in the samples in which T cells and monocytes were cultured together and

proliferation was enhanced in a dose-dependent manner as the number of monocytes increased. Similar results were obtained when B cells were co-cultured with T cells in the absence of monocytes and B cells alone did not respond to topo I (data not shown).

To identify the T cell subset responding to topo I, CD4+ T cell–depleted PBMC and CD8+ T cell–depleted PBMC obtained from eight anti-topo I–positive SSc patients and four healthy donors were cultured with topo I. Representative results for four anti-topo I–positive SSc patients and two healthy donors are shown in Fig. 5. The topo I–induced proliferative response was lost when CD4+ cells were depleted, but not when CD8+ cells were depleted. The topo I–induced proliferative response was enhanced after depletion of CD8+ cells in all anti-topo I–positive SSc patients, whereas only one of four healthy donors showed response enhancement after depletion of CD8+ cells. Reduction of the proliferative response by CD4+ cell depletion using serial amounts of magnetic beads was dose dependent (data not shown). Taken together, these observations indicate that the responding cells are CD4+ T cells and that APCs (monocytes or B cells) are necessary for the in vitro T cell proliferation to topo I.

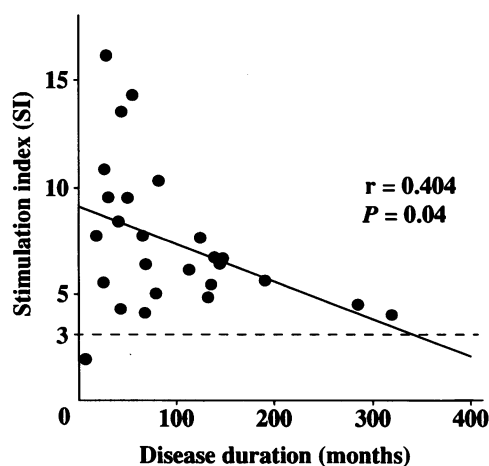*Effect of anti-MHC class II mAbs on topo I–induced T cell*

Figure 2. Negative correlation between SI induced by DNA topoisomerase I and disease duration in 26 SSc patients with anti-topo I antibody.
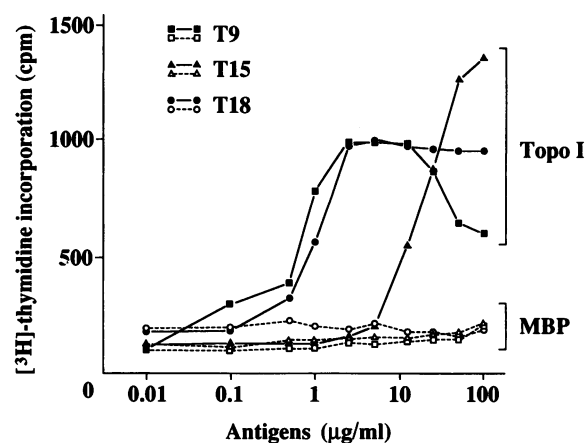

Figure 3. Dose–response of DNA topoisomerase I (*Topo I*)–induced PBMC proliferation in 3 SSc patients with anti-topo I antibody (*T9, T15,* and *T18*). PBMC (10^5) were stimulated with serial concentrations (0.01–100 μg/ml) of topo I (*closed symbols*) or MBP (*open symbols*) for 7 d.
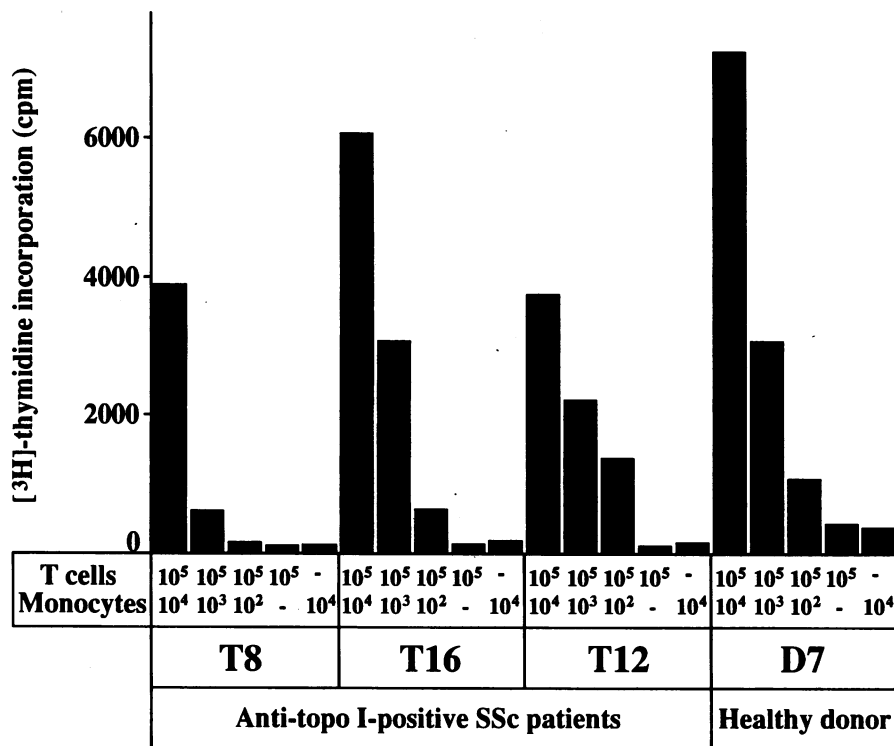
Figure 4. Topo I–induced proliferative response of T cells alone, monocytes alone, and the mixture of T cells (10^5) and various numbers of monocytes (10^2–10^4) from three SSc patients with anti-topo I antibody (T8, T16, and T12) and one healthy donor (D7). Cells were stimulated with 20 μg/ml of topo I and cultured for 7 d.

T cells	10^5 10^5 10^5 10^5 -	10^5 10^5 10^5 10^5 -	10^5 10^5 10^5 10^5 -	10^5 10^5 10^5 10^5 -
Monocytes	10^4 10^3 10^2 - 10^4	10^4 10^3 10^2 - 10^4	10^4 10^3 10^2 - 10^4	10^4 10^3 10^2 - 10^4
	T8	T16	T12	D7
	Anti-topo I-positive SSc patients			Healthy donor

proliferation. Specific HLA-DR (12, 13) and HLA-DQ (11–13) alleles were reported to be associated with anti-topo I antibody. Our analysis of the T cell proliferative response to topo I revealed an association with HLA-DR15, DR11, and DR7. These findings suggested that HLA-DR (and/or HLA-DQ) alleles may be important in the presentation of topo I peptides to the responding T cells. To investigate this further, we examined the effect of MHC class II–specific mAbs on the topo I–in-
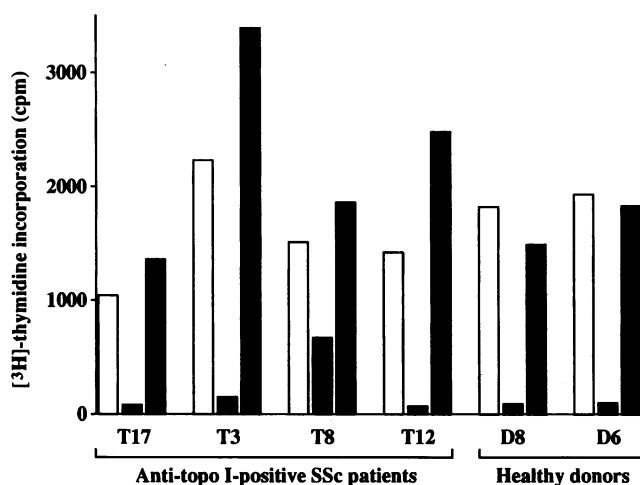
Figure 5. Topo I–induced proliferative response of PBMC (open bars), CD4+ cell–depleted PBMC (hatched bars), and CD8+ cell–depleted PBMC (solid bars) in four SSc patients with anti-topo I antibody (T17, T3, T8, and T12) and two healthy donors (D8 and D6). CD4+ cells and CD8+ cells were removed from PBMC using anti-CD4 and anti-CD8 mAb-coupled magnetic beads, respectively. Cells were stimulated with 20 μg/ml of topo I for 7 d.

duced T cell proliferative response in two anti-topo I–positive SSc patients and two healthy donors. As shown in Fig. 6, the anti-HLA-DR mAb was highly effective in inhibiting topo I–induced proliferation compared with anti-HLA-DQ and anti-HLA-DP mAbs and isotype controls. Similar results were obtained in experiments using an additional five anti-topo I–positive SSc patients, two anti-topo I–negative SSc patients, and four healthy controls. Anti-HLA-DQ mAb inhibited topo I–induced T cell proliferation between 20 and 42% in six (86%) anti-topo I–positive SSc patients and three (50%) healthy donors. The effects of various concentrations of anti-HLA-DR and anti-HLA-DQ mAbs and a control mouse IgG_{2a} on the topo-I proliferative response of PBMC from one anti-topo I–positive SSc patient (T17), are shown in Fig. 7. The topo I–induced proliferation was inhibited by anti-HLA-DR mAb in a dose-dependent fashion with maximum inhibition (75%) at 0.2 μg/ml. In contrast, the maximum inhibition by anti-HLA-DQ mAb was only 24% at 1 μg/ml.

Frequency of T cells responding to topo I. The frequency of T cells responding specifically to topo I was estimated by limiting dilution analysis. The frequency of topo I–reacting T cells ranged between 1/9,277 and 1/24,853 (mean 1/15,187) in four anti-topo I–positive SSc patients (T1, T7, T12, and T13). The frequency of topo I–reacting T cells was 1/23,755 in one anti-topo I–negative SSc patient (S1), and 1/9,770–1/21,645 (mean 1/15,077) in five healthy donors (D3, D4, D7, D9, and D12). Thus, we found no differences in the frequencies of topo I–specific T cells among anti-topo I–positive SSc patients, anti-topo I–negative SSc patients, and healthy donors.

Accelerated proliferative response of topo I–reactive T cells in anti-topo I–positive SSc patients. In order to evaluate the kinetics of the in vitro T cell response to topo I, T cell proliferation was measured on days 3 and 7 of culture with topo I fusion proteins or tetanus toxoid as a control (Table III).
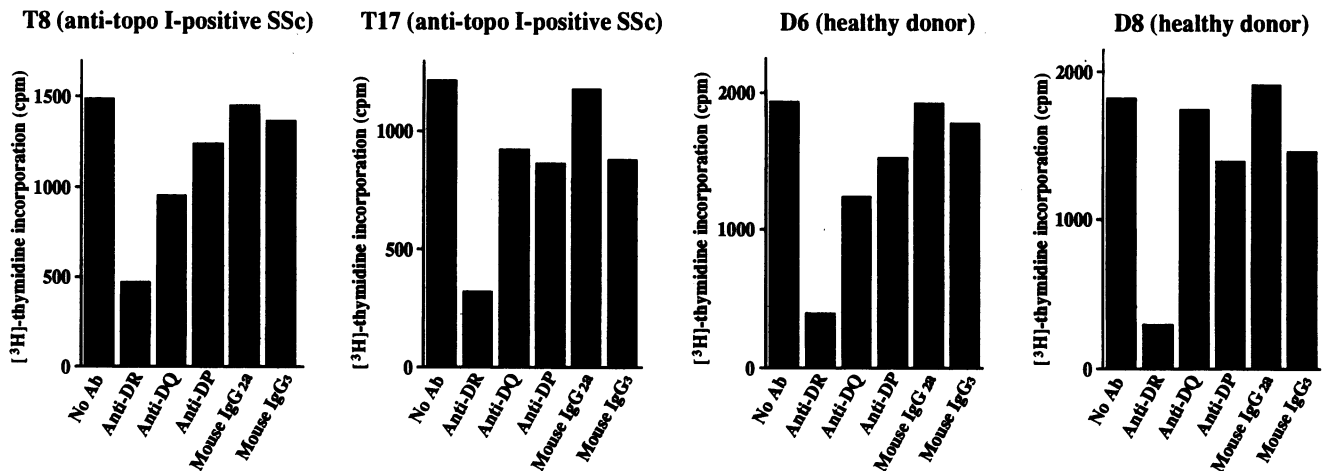
Figure 6. Effect of MHC class II–specific mAbs on topo I–induced T cell proliferation. PBMC (10^5 per well) obtained from two SSc patients with anti–topo I antibody (*T8* and *T17*) and two healthy donors (*D6* and *D8*) were stimulated with 20 μg/ml of topo I for 7 d in the presence of anti–HLA-DR mAb (L243), anti–HLA-DQ mAb (1a3), anti–HLA-DP mAb (B7/21), mouse IgG$_{2a}$, or mouse IgG$_3$ (0.2 μg/ml). The isotype of L243 and 1a3 is IgG$_{2a}$, and isotype of B7/21 is IgG$_3$.

PBMC from three anti-topo I–positive SSc patients, one anti-topo I–negative SSc patient, and two healthy donors were evaluated. The anti-topo I–positive SSc patients showed a marked proliferative response at day 3, which was higher than that observed at day 7. In contrast, T cells from the anti-topo I–negative SSc patient and the healthy donors did not respond to topo I at day 3 (SI < 3), but did show proliferation at day 7. This is similar to kinetics of the PBMC proliferative response to tetanus toxoid, a typical foreign recall antigen. The decline in the proliferative response seen in PBMC cultures from the anti-topo I–positive SSc patients from day 3 to day 7 may have been due to activation-dependent T cell apoptosis or early cell proliferation with exhaustion of the medium, which was not
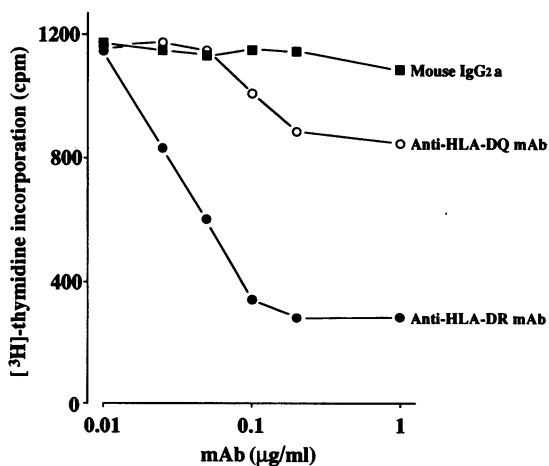

Figure 7. Dose-dependence of anti–MHC class II mAb effects on the topo I–induced PBMC proliferation in anti-topo I–positive SSc patient T17. PBMC (10^5 per well) were stimulated with 20 μg/ml of topo I for 7 d in the presence of serial concentrations (0.01–1 μg/ml) of anti–HLA-DR mAb (L243; ●), anti–HLA-DQ mAb (1a3; ○), and mouse IgG$_2$a (■). The values of [^{3}H]thymidine incorporated by cells cultured with medium alone, MBP (20 μg/ml), and topo I (20 μg/ml) were 202, 182, and 1,240 cpm, respectively.

changed during this interval. The observation that PBMC from patients with anti-topo I antibody proliferated after only 3 days of culture is consistent with recent antigen exposure (in vivo "activation") in these patients, but not in anti-topo I–negative SSc patients and healthy donors.

T cell proliferative responses to individual topo I recombinant proteins. The in vitro T cell proliferative responses to six topo I fusion proteins (F3, F4, F5, F6, F7, and F11) were examined in four anti-topo I–positive SSc patients, one anti-topo I–negative SSc patient, and one healthy donor (Fig. 8). A similar pattern of topo I fragment–induced T cell proliferation was seen in PBMC from the anti-topo I–positive SSc patient T2 (DRB1*0101/*1104), the healthy donor D4 (DRB1*0101/1104), the anti-topo I–positive SSc patient T12 (DRB1*1501/*0301), and the anti-topo I–negative SSc patient S2 (DRB1*0103/*1501). The F5-induced proliferative response exceeded the responses to the other fragments in DR11-positive individuals, whereas, the F6-induced response exceeded the responses against the other fragments in DR15-positive individuals. Moreover, the anti-topo I–positive SSc patient T5 homozygous for DRB1*07 (DR7) responded to F5 and F7 without a significant response to F6, and anti-topo I–positive SSc patient T14 (DRB1*0405/*1001) responded to F5 and F6 without responses to the other fragments. These findings strongly suggest that the topo I fragment–induced T cell response is associated with HLA-DR alleles, rather than the presence or absence of SSc or anti-topo I antibody. To extend these observations, the topo I fragment–induced T cell responses were examined in an additional 20 responders including 10 anti-topo I–positive SSc patients, 2 anti-topo I–negative SSc patients, and 8 healthy donors. Assuming that the HLA-DR alleles other than DR15, DR11, and DR7 were not major determinants of the topo I–specific T cell response, we compared the topo I fragment–induced responses of individuals with DR15 (DRB1*1501), DR11 (DRB1*1101 and *1104), and DR7 (DRB1*07) who were either homozygotes or heterozygotes with non-DR15, DR11, or DR7 alleles (Fig. 9). PBMC proliferation data from seven individuals who were heterozygotes of two of DR15, DR11, and DR7 (3 with DR15/DR11 and 4 with

Table III. PBMC Proliferation Induced by Topo I and Tetanus Toxoid at Days 3 and 7*

	Topo I		Tetanus toxoid	
	Day 3	Day 7	Day 3	Day 7
	cpm	cpm	cpm	cpm
Anti-topo I–positive SSc patients				
T1	1580±248 (14.6)	846±98 (11.4)	NT[‡]	NT
T12	1946±89 (9.7)	1317±164 (6.4)	306±30 (1.4)	7846±562 (24.9)
T15	1112±76 (11.6)	608±52 (6.1)	108±12 (1.1)	1826±190 (16.9)
Anti-topo I–negative SSc patient				
S2	309±21 (2.6)	894±94 (8.2)	NT	NT
Healty donors				
D6	398±18 (2.6)	1918±162 (10.9)	NT	NT
D10	590±42 (2.9)	1764±204 (5.9)	291±18 (1.0)	6452±742 (18.4)

* PBMC (10^5) were stimulated with 20 μg/ml of topo I or 10 μg/ml of tetanus toxoid for 3 or 7 d. Results are shown as mean±SD with SI in parentheses. SI was calculated as cpm incorporated in topo I–stimulated cultures/cpm incorporated in MBP-stimulated cultures for topo I–induced proliferation, or cpm incorporated in tetanus toxoid–stimulated cultures/cpm incorporated in cultures without antigen for tetanus toxoid-induced proliferation. [‡] NT, not tested.
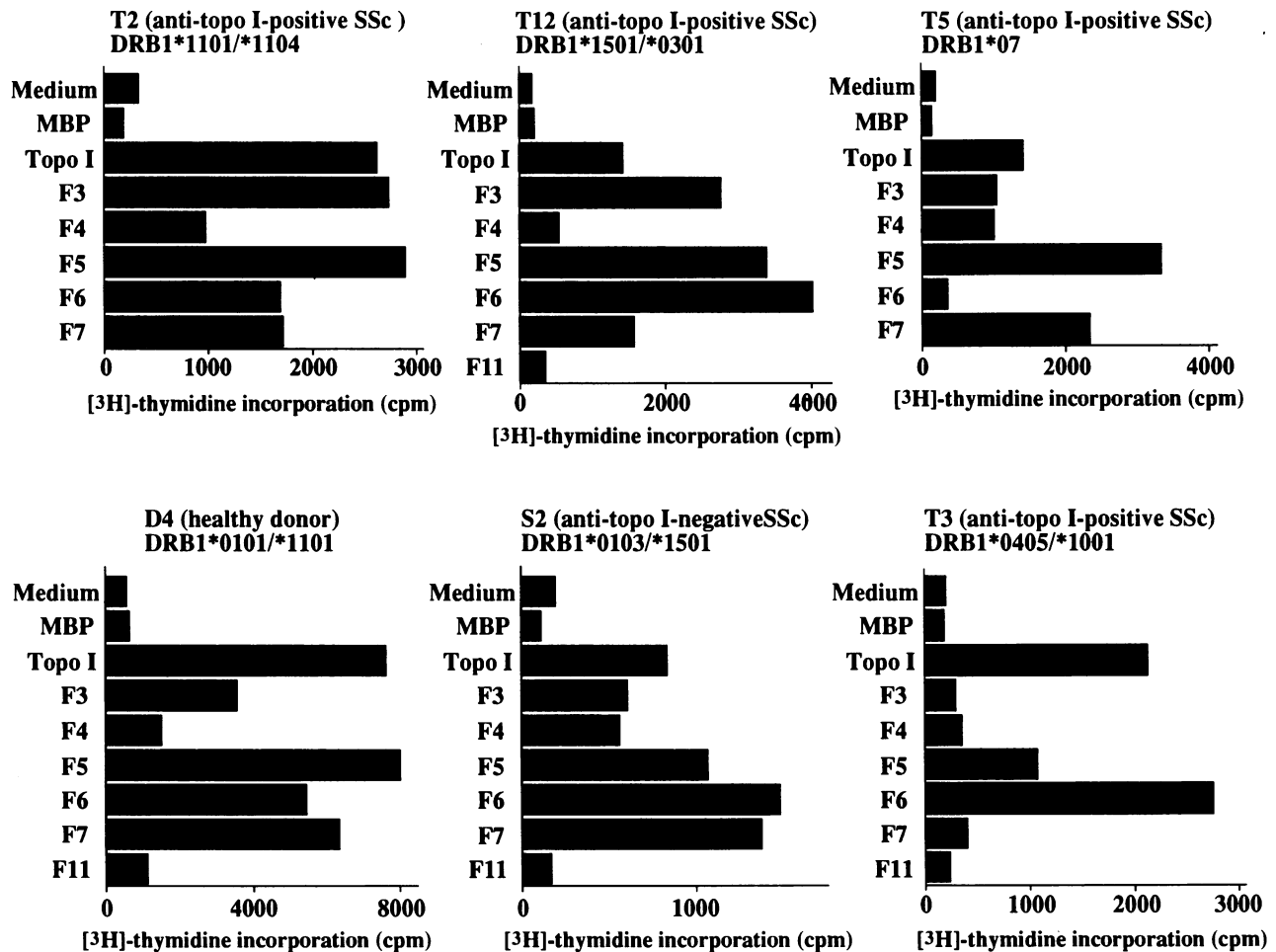

Figure 8. PBMC proliferative response induced by individual topo I fusion proteins (*F3, F4, F5, F6, F7,* and *F11*). PBMC (10^5 per well) were stimulated with MBP (20 μg/ml), topo I (20 μg/ml), F3 (9.8 μg/ml), F4 (12.2 μg/ml), F5 (11.5 μg/ml), F6 (12.5 μg/ml), F7 (13.4 μg/ml), or F11 (10.2 μg/ml) for 7 d. The molar concentration of each fragment was equivalent, based on the estimated molecular weight of the fusion protein.
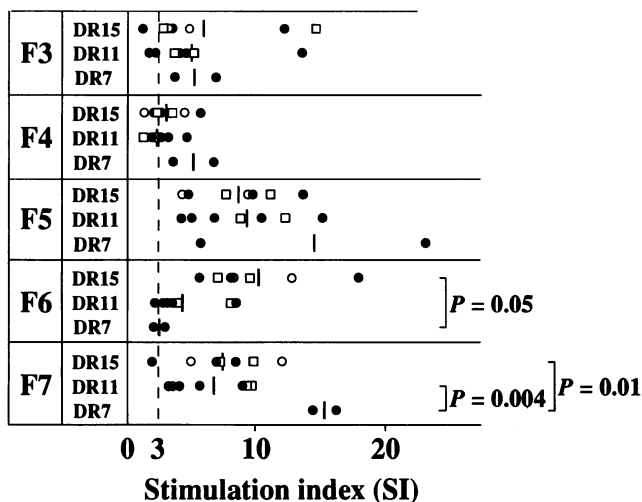
	DR15	
F3	DR11	
	DR7	
	DR15	
F4	DR11	
	DR7	
	DR15	
F5	DR11	
	DR7	
	DR15	
F6	DR11	} $P = 0.05$
	DR7	
	DR15	
F7	DR11	} $P = 0.004$ } $P = 0.01$
	DR7	

0 3   10   20

Stimulation index (SI)

Figure 9. Analysis of topo I fragment–induced PBMC proliferation according to the HLA-DR alleles DR15, DR11, and DR7. PBMC (10^5 per well) from 16 responders including 10 SSc patients with anti-topo I antibody (●), 2 SSc patients without anti-topo I antibody (○), and 4 healthy donors (□) were stimulated with MBP (20 μg/ml), F3 (9.8 μg/ml), F4 (12.2 μg/ml), F5 (11.5 μg/ml), F6 (12.5 μg/ml), and F7 (13.4 μg/ml) for 7 d. Responders were divided into three groups; individuals having DR15 ($n = 7$), those having DR11 ($n = 7$), and those having DR7 ($n = 2$), who were either homozygotes or heterozygotes with non-DR15, DR11, or DR7 allele. The mean SI level for each group is indicated shown by a solid bar. Differences between mean levels were determined using Student's *t* test.

DR11/DR7) and three without any of these three HLA-DR types were excluded from this analysis. Although there were no significant differences in the F3-, F4-, and F5-induced proliferative responses among DR15-, DR11-, and DR7-positive responders, SI levels of F6- and F7-induced responses were different among these three groups. The F6-induced responses in DR15-positive individuals were significantly higher than in DR7-positive individuals ($P = 0.05$), and F7-induced responses in DR15- and DR11-positive individuals were lower than in DR7-positive individuals ($P = 0.01$ and 0.004, respectively). F5-induced proliferative responses were detected in all 19 responders examined. In contrast, only 2 of 13 responders (15%) showed a borderline proliferative response against F11, which contains a 6-amino acid sequence homologous to the retroviral p30^{gag} protein (25).

Discussion

In this study, we analyzed the in vitro T cell proliferative response to topo I in SSc patients and healthy donors. A striking finding was that topo I–induced proliferation was independent of disease and autoantibody status. Characterization of the topo I–induced response revealed that (*a*) responding T cells had a CD4+ phenotype; (*b*) proliferation was dependent on the presence of APCs; (*c*) the T cell response was mainly restricted by HLA-DR, and to a lesser degree by HLA-DQ; (*d*) the frequency of responding T cells was 1/9,277–1/24,853; (*e*) autoreactive T cells recognized multiple T cell epitopes on topo I; and (*f*) the pattern of T cell epitope recognition was determined, at least in part, by the HLA-DR alleles. These findings were common among anti-topo I–positive SSc patients, anti-topo I–nega-

tive SSc patients, and healthy donors. These features of the topo I–specific T cell response are consistent with previously characterized human T cell responses against foreign antigens. For example, the frequency of topo I–responding T cells was similar to previous estimates of T cells reactive with *Myco-bacterium tuberculosis*–purified protein derivative (1/1,970–1/13,982) (22). A unique feature of the topo I–induced T cell response was a rapid (3 d) proliferation seen only in SSc patients positive for anti-topo I antibody. The topo I–specific T cells identified in our studies probably are involved in anti-topo I antibody production, since these T cells provide help to autologous B cells resulting in anti-topo I antibody production in vitro (Kuwana, M., T. A. Medsger, Jr., and T. M. Wright, unpublished results).

On the basis of the associations between the shared epitopes on the HLA class II alleles and the presence of serum anti-topo I antibody, the HLA-DQ molecule is postulated to play a primary role in anti-topo I antibody production (11, 12). In contrast, we have proposed that both HLA-DR and DQ molecules control the autoantibody response against topo I, since the presence of anti-topo I antibody is associated with HLA-DQ alleles and both B cell epitope reactivity and antibody titer are associated with HLA-DR alleles (12). However, it was difficult to determine which of the HLA-DR and DQ associations was primary in these studies, since HLA-DR and DQ alleles are in strong linkage disequilibrium. In our current study, the topo I–specific T cell proliferative response was associated with specific HLA-DR alleles. Furthermore, experiments using anti-HLA-DR and anti-HLA-DQ mAbs revealed that the restriction of the topo I–specific T cell response was primarily through HLA-DR, with a minor contribution by HLA-DQ. This pattern of MHC class II restriction is consistent with that of autoreactive T cells against ribosomal P2 protein in SLE patients (15).

Since SSc patients and healthy controls with DR15 or DR11 showed a similar pattern of T cell proliferation to topo I fragments, it is possible that APCs bearing DR15 or DR11 bind the common topo I peptides and present these to T cells. PBMC from two healthy donors with DRB1*0801, but without DR15, DR11, or DR7, also showed a proliferative response to topo I and had a pattern of topo I fragment recognition similar to responders with DR15 or DR11 (data not shown). Two DR15 alleles, DRB1*1501 and *1502, are in linkage disequilibrium with DRB5*0101 and *0102, respectively, and all DR11-positive SSc patients and healthy donors had either DRB1*1101, *1103, or *1104. DRB5*0101 and *0102 as well as DRB1*1101, *1103, *1104, and *0801 have amino acid residues phenylalanine and aspartic acid at positions 67 and 70 in the DRB β1 gene. These positions of the HLA-DR genes are highly polymorphic and are thought to influence peptide-binding because of their location on the surface of the peptide-binding groove (26). Interestingly, these 2-amino acid residues are included in the shared amino acid sequence that has been reported to be associated with a high anti-topo I antibody titer in Japanese SSc patients (13). APCs bearing DR7 may bind different topo I peptides since topo I responders with DR7 showed a different pattern of topo I fragment–induced T cell proliferation compared with individuals with DR15 or DR11, although only a limited number of DR7-positive individuals were available for analysis. It is likely, therefore, that the polymorphism of the HLA-DR molecules determines the binding to the processed topo I peptides and thus the topo I–specific T cell response.

The present study indicates that topo I–reactive T cells are components of the T cell repertoire in many healthy persons and, therefore, that they are not deleted during ontogeny nor are they irreversibly anergic. T cells reactive with self-proteins such as myelin basic protein and insulin have also been found in healthy individuals (21, 27). In addition, Hoffman et al. (14) have recently reported the generation of T cell clones specific for human U1 small nuclear ribonucleoprotein (snRNP) from healthy individuals having the HLA-DR alleles associated with anti-U1 snRNP antibody. There are at least two possible explanations for the detection of self-reactive T cells in the peripheral blood of healthy individuals. First, the antigen may have limited tissue distribution and sufficient antigen-derived peptides may not be presented in the thymus or the periphery resulting in a lack of T cell clonal deletion and/or anergy (immunologic ignorance). An argument against this model is that the topo I protein appears to be ubiquitously distributed and thymic extracts have traditionally been used as antigens for detection of anti-topo I antibodies (7). Under normal circumstances, however, peptides derived from topo I may not be processed and presented efficiently via the MHC class II pathway since it is a nuclear protein that is extremely sensitive to proteolytic degradation (28, and Kuwana, M., and T. M. Wright, unpublished observation). Second, it has been recently proposed that T cells responsive to minor or cryptic determinants, i.e., those not usually generated during in vivo processing, can evade tolerance because of the relatively low amounts of peptides suitable for binding to HLA molecule after in vivo processing of the antigen (29). These autoreactive T cells may become pathogenic if the cryptic determinant is revealed (30), and the immune response may subsequently diversify to recognize other areas on the same autoantigen to which there was previous T cell tolerance (31, 32). It was also reported that autoreactive T cells can be activated by artificially processed peptides, which might express cryptic determinants (33, 34). It is possible that the recombinant topo I fusion proteins used as antigens in our in vitro T cell proliferation assays were processed differently than native topo I, resulting in the generation of topo I peptides containing cryptic epitopes that are not formed normally in vivo. Our findings, therefore, are consistent with both models of autoantigen recognition since T cells from anti-topo I–positive SSc patients as well as anti-topo I–negative SSc patients and healthy donors with the appropriate HLA class II molecules were able to respond to topo I.

Our results suggest that topo I–specific T cells are activated in anti-topo I–positive SSc patients, but not in anti-topo I–negative SSc patients and healthy donors. The mechanism of antigen exposure which resulted in the activation of these T cells in vivo is unknown. One possibility is that cryptic determinants on topo I were revealed in anti-topo I–positive SSc patients due to factors that may affect normal processing of topo I such as the formation of complexes with other proteins or posttranslational modifications of topo I itself. In this regard, it has been shown recently that autoantibody to the p53 tumor suppressor protein was induced in mice immunized with complexes of murine p53 and SV40 large T antigen, but not in mice immunized with either protein separately (35). Topo I activity in host cells is known to be necessary for the replication and transcription of several viral genomes, such as adenovirus (36), SV40 (37, 38), herpes simplex virus (39), EBV (40), and HIV (41). Since topo I binds tightly to viral proteins and/or DNA during viral replication and transcription (38, 41), T cell auto-

immunity may be initiated by the complex of topo I with certain viral proteins. Another possibility is that, similar to the recent observations regarding the localization of lupus autoantigens in UVB-treated keratinocytes (42), topo I protein in a native or altered form may be clustered in blebs at the surface of apoptotic cells. It is known that a number of free radical– and proteinase-induced modifications of proteins (43, 44) occur during apoptosis. Perhaps these site-specific modifications result in the generation of unique peptide fragments and reveal potentially cryptic determinants. A third possibility is that anti-topo I–positive patients have been exposed to a foreign protein that cross-reacts with topo I. It was reported that autoreactive T cells and autoantibodies to self-snRNP can be generated in normal mice by coimmunization with a mixture of self- and foreign snRNP (45). The mechanism is thought to be the generation of cross-reactive B cells, initially primed by foreign snRNP serving as a molecular mimic, that then bind, process, and present self-snRNP with the activation of autoreactive T cells. This hypothesis is supported by our recent identification of a major T cell epitope on topo I (Kuwana, M., and T. M. Wright, unpublished observation) that is located in a region that is highly conserved among all topo I proteins for which sequence is available, including human (46), mouse (47), yeast (48), Shope fibroma virus (49), and vaccinia virus (50). Furthermore, the nucleocapsid proteins of some retroviruses have been shown to have topo I enzymatic activity and cross-react with anti-topo I autoantibody in SSc sera (51).

Based on our results, it is likely that the production of anti-topo I antibody in a subset of SSc patients is the result of a combination of factors that include (a) certain HLA-DR alleles; (b) increased presentation of topo I–derived peptides by one of a number of mechanisms described above; and (c) the activation of B cells. In conclusion, our studies provide support for the antigen-driven hypothesis for the production of autoreactive T cells and autoantibodies and further define the relationship between specific MHC class II alleles and anti-topo I antibody in SSc.

Acknowledgments

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