Distinct Autoreactive T Cell Responses to Native and Fragmented DNA Topoisomerase I: Influence of APC Type and IL-2

Timothy B. Oriss, Paul Q. Hu and Timothy M. Wright

*J Immunol* 2001; 166:5456-5463; doi: 10.4049/jimmunol.166.9.5456

http://www.jimmunol.org/content/166/9/5456

References

This article **cites 47 articles**, 21 of which you can access for free at:

http://www.jimmunol.org/content/166/9/5456.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Distinct Autoreactive T Cell Responses to Native and Fragmented DNA Topoisomerase I: Influence of APC Type and IL-2

Timothy B. Oriss, Paul Q. Hu, and Timothy M. Wright

Systemic sclerosis (SSc), or scleroderma, is an autoimmune disease characterized by the presence of Abs against a variety of autoantigens including, but not limited to, DNA topoisomerase I (Topo I; also known as Sc1-70), centromere, RNA polymerase I and III, and U3 RNA/fibrillarin (1). SSc is characterized by overproduction of collagen by affected fibroblasts, resulting in fibrosis of the skin and internal organs including the heart, lungs, kidneys, and gastrointestinal tract (2). Ten-year mortality rates can approach 50% in the most severe systemic forms of the disease (3).

The role of T cells in the pathogenesis of SSc is supported by: 1) the presence of CD4+ T cells in early skin lesions and increased T cells in bronchoaveolar lavage fluid of patients with pulmonary fibrosis; 2) disease-associated autoantibodies of multiple isotypes that correlate with clinical findings and disease course; and 3) responses to T cell-targeted therapies (e.g., cyclosporin A and antithymocyte globulin), although thus far this is limited to case reports and small series (4–8). In several autoimmune diseases in which autoantigens have been identified, including multiple sclerosis and SSc, autoreactive T cells can be detected and expanded in vitro (9, 10). Interestingly, these same autoreactive T cells can also be found in the peripheral circulation of healthy individuals (9, 11). The finding of autoreactive cells in healthy individuals suggests that additional immunologic events must occur to break T cell tolerance during the initiation of disease. These events may involve cross-reactivity of bacterial or viral Ags with autoantigens (12–15), environmental influences such as chemical toxins (3, 16), altered forms of autoantigen as may occur during apoptosis (17–19), and changes or defects in Ag processing (20–26).

Our laboratory has studied the autoreactive T and B cell responses to the SSc-associated Ag DNA Topo I. We previously reported that Topo I-specific T cells can be identified in the peripheral blood of SSc patients and healthy subjects whose cells bear one or more MHC class II HLA-DR responder alleles, notably HLA DR*11, DR*15, or DR*7 (9). These prior studies were performed using a Topo I Ag preparation consisting of a set of five overlapping maltose binding protein (MBP) fusion proteins, termed F3 through F7, spanning the entire length of the 766 amino acid Topo I molecule. A series of CD4+ T cell clones derived using this Ag were specific for a portion of the Topo I molecule represented by fragments F5 and F6 (spanning aa 209–386 and aa 363–563, respectively), and all but one of 15 clones expressed the same TCR β-chain variable gene fragment (vβ20.1) (27). The biologic relevance of the Ag preparation used in these studies was confirmed when it was determined that Abs in the serum of Topo I Ab-positive SSc patients also reacted with this portion of the molecule and that the T cell clones could provide help to autoreactive B cells, leading to anti-Topo I Ab production (28).

As part of our efforts to understand the initial phases of SSc, we examined dendritic cell (DC) presentation of Topo I to T cells, because DCs are believed to prime immune responses in vivo (29). As a component of these studies, we undertook the production of a native full-length form of Topo I using a baculovirus vector in insect cells. The initial goal of these studies was to compare the presentation of Topo I to T cells by DCs and APCs present in PBMC. However, unexpected differences were found regarding...
the form of the Topo I Ag and the APC type involved in presentation. These findings are presented herein, and their potential significance is discussed.

Materials and Methods

Human subjects

Peripheral blood was obtained from healthy adults and SSc patients by venipuncture according to all applicable Institutional Review Board guidelines. SSc subjects fulfilled the American College of Rheumatology preliminary criteria for the classification of SSc (30) and were judged to be either positive or negative for serum anti-Topo I Ab (31). Data presented herein represent results obtained with cells from healthy individuals. In certain cases, cells from SSc patients were used to confirm results that had been obtained using healthy donors’ cells, as noted in the text.

Ag preparation

An overlapping set of five MBP fusion proteins representing the entire length of the human Topo I molecule was prepared by subcloning Topo I cDNA fragments into the pMAL-C2 expression vector (New England Biolabs, Beverly, MA). These were expressed in Escherichia coli strain DH5α (Life Technologies, Rockville, MD) and were purified as previously described (30). This set of fusion proteins was used together in equimolar concentrations and was termed MBP-Topo I mix. In several experiments, one of the MBP fusion proteins, MBP-F5 (aa 209–386), was used alone. MBP was similarly generated by transforming E. coli with pMAL-C2 vector lacking Topo I cDNA and was purified for use as a control Ag. Purified native full-length Topo I was prepared using the MaxBac baculovirus expression system (Invitrogen, Carlsbad, CA) as described by P. Q. Hu T. A. Medsger, Jr., and T. M. Wright, (manuscript in preparation). Briefly, cDNA corresponding to the entire open reading frame of Topo I (30) was cloned into the multiple cloning site of the shuttle vector pBlue-Bac4.5 (Invitrogen), which was then cotransfected along with Bac-N-Blue DNA (Invitrogen) into insect Sf9 cells to produce recombinant virus. Full-length recombinant baculovirus-derived Topo I (rTopo I) was produced by infection of Sf9 cells with the recombinant virus, followed by isolation of cell nuclei, precipitation of cellular genomic DNA, and purification of the rTopo I from the resulting supernatant using three columns in succession: a heparin Sepharose CL-6B column (Amersham Pharmacia Biotech, Uppsala, Sweden), followed by phenyl Sepharose 6 and Mono S H 5/5 columns (Amersham Pharmacia Biotech). The concentration of rTopo I was measured by a colorimetric protein assay (Bio-Rad, Richmond, CA), and purity was estimated to be >95% by SDS-PAGE and Coomassie blue staining. Purified rTopo I was reconstituted in 50% glycerol with 1 mM DTT, filter-sterilized, and stored at −80°C. The MBP-F5 fusion construct was also produced using the baculovirus system as described above. Tetanus toxoid, used as a control Ag in some experiments, was purchased from Massachusetts State Biological Laboratories (Jamaica Plain, MA).

Synthetic peptides representing the entire length of the Topo I molecule were purchased from Chiron Mimotopes (Victoria, Australia). The peptides were synthesized in 0.1 M HEPES (pH 7.4), 80°C.

DC generation and Ag loading

PBMC were obtained from heparinized peripheral blood by isolation on Ficoll-Paque gradients (Amersham Pharmacia Biotech). DCs were obtained from cultures of PBMC (5–10 × 10⁶ cells/ml) in 75 cm² plastic cell-culture flasks (Falcon; BD Biosciences, Franklin Lakes, NJ) for 2 h at 37°C in AIM V culture medium (Life Technologies) with penicillin/streptomycin (100 U/ml, 100 μg/ml; Life Technologies), followed by removal of nonadherent cells and culture of adherent cells for 7 days in AIM V supplemented with IL-4 (30 ng/ml; Life Technologies) and GM-CSF (10 ng/ml; BD Pharmingen, San Diego, CA). Additional AIM V cultures were added on days 4 and 6 of culture, DCs were harvested by removing nonadherent cells and collecting adherent cells by incubation with PBS/15 mM EDTA for 15 min at 4°C, followed by scraping with a cell scraper. The phenotype of DC preparations was determined by staining and flow cytometry for CD1c, CD14, CD80, CD86, and HLA-DR. DCs expressed all of the markers except CD14, which was expressed at fresh monocytes/macrophages or cells cultured in GM-CSF alone. The cells, 90–95% of which had a DC phenotype by flow cytometry, were not further purified and demonstrated no proliferative response to Ag without the addition of T cells.

DCs were exposed to Ag directly in the 96-well plates that were used for the assays. For some experiments, variable numbers of cells (30–30,000 cells/well) were used, but for most experiments 10⁵ DCs/well were used. Cells were plated in 100 µl of AIM V medium with 100 U/ml of TNF-α (0.9 U/ng; BD Pharmingen) and the indicated Ag and were incubated overnight at 37°C. The following day the medium was gently removed from the wells, leaving behind the plastic-adherent DCs.

Primary T cell stimulation

DCs were exposed to Ags as described above. Plastic-nonadherent PBMCs were used as a source of T cells and were added to wells (10⁵ cells/well) containing Ag-exposed DC in a total volume of 200 µl of RPMI 1640 medium (Life Technologies) supplemented with 10% heat-inactivated (60°C, 1 h) human AB serum (Cellgro, Herndon, VA) and penicillin/streptomycin (RPMI/huAB). The T cell-enriched plastic-nonadherent cell population typically was 72 ± 11.4% T cells, 6.6 ± 3.9% monocytes, 2.8 ± 0.4% B-cells, and 23.2 ± 3.5% NK cells (n = 10) as analyzed by flow cytometry. Using a wide range of Ag concentrations (0.1–10 μg/ml), no detectable T cell proliferation was observed in the plastic nonadherent fraction unless additional APCs were added (data not shown).

Primary stimulation of T cells with MBP APC was conducted by directly plating whole, unseparated PBMC (2 × 10⁶ cells/well) with Ag in RPMI/huAB. Twice as many unspurred PBMC were used as for DC stimulation because roughly one half of the PBMC were typically plastic nonadherent. In some experiments, IL-2 was also added to the cultures (20 U/ml, 10⁶ U/µg; Life Technologies) as indicated. Primary stimulation cultures with either APC type were incubated for 7 days at 37°C with the addition of [methyl-3H]thymidine ([3H]thymidine) (1 µc/well; NEN, Boston, MA) during the final 18 h of incubation. Incorporation of [3H]thymidine was determined by harvesting cells onto filters followed by liquid scintillation counting. Allogeneic immune responses were measured in the absence of exogenous Ag using an MLR with T cells and DCs from two individuals with mismatched MHC class II alleles. Cells that were undergo secondary stimulation were treated in a manner identical to that described above, except that the [3H]thymidine addition was eliminated.

Secondary T cell stimulation

T cells were stimulated with Ag and APC for 7 days, were then washed three times with PBS, and were used in secondary stimulation assays. DCs were exposed to Ag as described for primary T cell stimulation. PBMC APCs in these assays consisted of unseparated PBMCs (10⁵ cells/well) that were γ-irradiated at 2000 rad and were mixed with Ag at the time of adding T cells. Irradiated, unseparated PBMC did not proliferate in response to any Ag without the addition of T cells. Primary-stimulated T cells (3 × 10⁵ cells/well) were added in RPMI/huAB, and culture volumes were maintained at 200 µl. Secondary stimulation assays were incubated for 48 h, and the [3H]thymidine addition, harvesting, and scintillation counting was performed as described above.

T cell line generation

Short-term T cell lines were generated by repeated stimulation with Ag. Plastic-nonadherent PBMC were incubated with Ag-loaded DCs under conditions identical with those described for primary T cell stimulation. At intervals of 7–10 days, the T cells were restimulated with Ag, APC, and IL-2 (10 U/ml) as described for secondary T cell stimulation above. After three to four rounds of stimulation, the cells were routinely found to be >95% CD4+ T cells by flow cytometry (data not shown). These cells were tested in proliferation assays (3 × 10⁵ cells/well) with DCs (1 × 10⁴ cells/well) as the APC for their ability to be stimulated by synthetic peptides. In these assays, the peptides, DCs, and T cells were added simultaneously because Ag uptake and processing by the APCs was not required. These assays were incubated for 48 h, and the [3H]thymidine addition, harvesting, and scintillation counting was performed as described above.

Factor Xa digestion

The MBP fusion partner was removed from the MBP-Topo I fusion protein MBP-F5 by enzymatic cleavage with Factor Xa (New England Biolabs). Cleavage was performed according to the manufacturer’s instructions. Briefly, 1 mg of MBP-F5 was suspended in a total volume of 1 ml reaction buffer (20 mM Tris-HCl, 100 mM NaCl, and 2 mM CaCl₂ (pH 8.0)) to which 20 μg of Factor Xa was added. The reaction was allowed to proceed for 1 h at 23°C. Reaction products, as well as uncut control, were visualized by electrophoresis on 10% SDS-polyacrylamide gels followed by staining with Coomassie blue.
**Results**

**DC phenotype and function**

The mature myeloid DCs used in this study were generated from peripheral blood monocytes by the commonly used method of culture with GM-CSF and IL-4 (29). DCs generated by this method had the expected gross morphology and cell surface phenotype, including expression of MHC class II, costimulatory molecules CD80 and CD86, CD11c, and the absence of CD14 expression (data not shown). DCs initiated vigorous allogeneic immune responses (Fig. 1A) and efficiently presented various Ags, including recombinant Topo I fusion proteins and tetanus toxoid to T cells (Fig. 1, B–D). Also, as is typical of DCs, these cells presented Ags to T cells much more efficiently than PBMC APCs (Fig. 1, C and D). Thus, the cells used in our experiments have phenotypic and functional properties typical of DCs described by others (29). This is important because our findings using a full-length rTopo I clearly differ from these typical observations (see the following section and Fig. 2D).

**T cell response to rTopo I requires exogenous IL-2 and is preferentially initiated by PBMC APCs**

Previous work in our laboratory used Topo I Ag in the form of a mix of five MBP fusion proteins, which together constitute the entire length of the Topo I molecule (MBP-Topo I mix) in an overlapping fashion (31). The MBP-Topo I mix Ag preparation stimulated T cell proliferation from both SSc patients as well as healthy subjects with certain MHC class II alleles and reacted with Ab in the sera of SSc patients but not healthy controls (9, 28, 31). As part of our efforts to better understand the events that initiate disease pathogenesis in SSc, we wanted to examine T cell responses to the native form of Topo I. Therefore, we produced full-length human Topo I protein in insect cells (rTopo I). We found that rTopo I was recognized by anti-Topo I Ab-positive SSc patient sera in ELISA, had the expected Mr of 100 kDa on SDS-PAGE, and was enzymatically active in a plasmid relaxation assay (P.Q. Hu, T. A. Medsger, Jr., and T. M. Wright, manuscript in preparation). Therefore, the rTopo I represented native, functional DNA Topo I, which was recognized by disease-specific sera.

We next examined the T cell proliferative response to native rTopo I and compared it to the response to the MBP-Topo I mix. In contrast to proliferative responses to Topo I fragments, T cell responses were not observed to rTopo I presented by DCs (Fig. 2A). This experiment was repeated with cells from seven healthy subjects and two SSc patients, and the results were similar (data not shown). T cells from all individuals tested failed to respond to rTopo I at Ag concentrations as high as 100 μg/ml and at DC concentrations as high as 3 × 10⁵ cells/well (data not shown). We hypothesized that the autoreactive T cells specific for peptides generated from rTopo I may have been deleted or rendered anergic. To address the possibility that these T cells were anergized in vivo, we examined T cell proliferation in response to rTopo I with the addition of IL-2, which is reported to reverse T cell anergy (32–35).
As shown in Fig. 2B, cultures of T cells stimulated with Ag presented by DCs and supplemented with IL-2 (20 U/ml) responded to rTopo I in a concentration-dependent manner.

Similar observations were made regarding the requirement for IL-2 when PBMCs were used as the source of APCs (Fig. 2C). However, in contrast to the T cell responses to Topo I fragments (Fig. 1D), PBMC APCs stimulated greater T cell response to rTopo I in the presence of IL-2 compared with DCs (Fig. 2D). This may reflect the ability of PBMC APCs to process and present rTopo I peptide fragments to T cells much more efficiently than DCs. Similar observations, including the requirement for IL-2 and the preference for PBMC APCs, have been made using cells from a limited number of SSc patients examined to date (data not shown).

For both APC types, magnetic bead depletion studies determined that the proliferative response to rTopo I plus IL-2 was mediated by CD4+ T cells (data not shown), as had been previously observed for the MBP-Topo I mix Ag (5). Addition of IL-2 to the MBP-Topo I mix resulted in an increased rate of [3H]thymidine incorporation but did not alter the shape of the Ag dose-response curve (data not shown). Even in the presence of exogenous IL-2, T cell responses from any given individual were generally much lower in response to rTopo I plus IL-2 than to the MBP-Topo I mix Ag regardless of the APC used (data not shown).

Together, these data raise important questions about the form of the Topo I Ag and the APC that are responsible for the initiation of the autoimmune response in SSc.

The protein expression system is not important in the differential T cell response to different forms of DNA Topo I

An important consideration regarding the two Topo I Ag preparations was whether the different methods by which the proteins were expressed affected their relative immunogenicity. Full-length Topo I could not be produced as an MBP fusion protein in bacteria due to bacterial toxicity and enzymatic degradation (31), prompting the use of a baculovirus expression system that should promote natural glycosylation and folding of the protein (36). When rTopo I failed to elicit T cell proliferation, several possible explanations for the greater efficiency of MBP-Topo I mix presentation to T cells relative to rTopo I were considered. First, because the MBP-Topo I mix Ag was produced in a bacterial expression system and the rTopo I in a baculovirus expression system, there could have been differences in protein folding and glycosylation between the two systems. Second, the bacterially expressed Topo I fragments may contain LPS, which could affect APC function and possibly enhance T cell responses to this form of Ag. Third, the MBP fusion partner itself could have directed proteins into a processing pathway that allowed for more efficient Ag presentation to T cells and/or the presentation of different epitopes. Finally, MBP might have a direct effect on the APC and enhance Ag processing and/or presentation.

To address these issues, we performed a series of experiments using one of the Topo I fragments contained in the MBP-Topo I mix Ag preparation, MBP-F5, which was used because we have observed strong T cell proliferative responses to this Ag, and most of the Topo I-specific T cell clones generated in our laboratory previously have reacted with this portion of the Topo I molecule (27). The construct that was used to generate bacterial MBP-F5 was cloned into a baculovirus vector and was expressed in the S91 insect cell line. No difference in T cell response was observed regardless of the expression system used to generate MBP-F5 (Fig. 3A). We also found that the addition of purified LPS did not stimulate or enhance T cell responses to any of our Ag preparations (data not shown). Furthermore, enzymatic cleavage of MBP from F5 also did not reduce T cell response to this fragment (Fig. 3C).

Finally, exogenous MBP produced in the bacterial expression system and used at the same molar concentration as that in the MBP-Topo I mix preparation failed to augment T cell responses to rTopo I or the control Ag tetanus toxoid (Fig. 3B). Taken together, these results indicate that the observed differences in T cell responses to rTopo I and the MBP-Topo I mix do not arise from differences in methods by which the proteins were expressed, and that MBP itself does not act as an “adjuvant” or a chaperone of proteins into a particular APC Ag-processing pathway.

IL-2, which is required for rTopo I response, appears to act directly on T cells

The absolute requirement for exogenous IL-2 to elicit T cell responses to rTopo I may be due to effects on the T cells or on the APCs. To examine this further, DCs were incubated overnight with Ag in the presence or absence of exogenous IL-2. Unbound IL-2 was washed away before the addition of T cells. Untreated and IL-2-treated DCs both failed to induce T cell responses to rTopo I (Fig. 4A). As shown in Fig. 4B, we observed variable levels of background (no Ag) incorporation of [3H]thymidine in T cell cultures supplemented with IL-2. In this figure, only the samples in which IL-2 was added at time zero resulted in T cell responses with a stimulation index (cpm of Ag-stimulated cultures/cpm of cultures with no Ag added) >2. However, as shown in Fig. 4B, exogenous IL-2 added as late as 48 h following T cell/DC coincubation resulted in significant T cell proliferation (Fig. 4B).
These results indicate that the exogenous IL-2 did not affect Ag uptake, processing, or MHC class II loading because these events are largely complete in DC after overnight incubation with TNF-α before the addition of T cells. The results suggest that IL-2 acted directly on T cells to promote proliferation following Ag-specific TCR engagement.

**T cells stimulated by the MBP-Topo I mix and rTopo I are specific for Topo I peptide amino acid sequences**

In order make certain that the observed T cell responses were specific for the Topo I molecule, we generated short-term T cell lines by three to four rounds of stimulation with either rTopo I or the MBP-Topo I mix. Overlapping synthetic peptides representing the entire length of the rTopo I molecule were then presented to the T cell lines to determine whether they could induce proliferation. The first step in the epitope mapping process involved screening pools of 10 peptides each. Fig. 5A demonstrates that for a single rTopo I-responsive line, a single peptide pool stimulated T cell proliferation. In the second step of mapping, the individual peptides within the pool that was stimulatory in Fig. 5A were each tested for their ability to induce T cell line proliferation. Three overlapping peptides (Fig. 5C) were found to stimulate the T cell line (Fig. 5B). Each of these peptides is within the F6 fragment of the MBP-Topo I mix (9). Similar results were obtained with lines generated using the MBP-Topo I mix (data not shown). Additionally, we have previously shown that T cell lines stimulated by the MBP-Topo I mix are very likely to be peptide-specific because they can provide help to SSc patient B cells for the production of anti-Topo I Ab (28).

**Unique antigenic responses are generated from the MBP-Topo I mix and rTopo I**

T cell responses are generally amplified in secondary stimulations relative to primary stimulations, due to higher numbers of Ag-specific T cells that were expanded during the initial stimulation. We next examined whether there were differences between rTopo I and the MBP-Topo I mix in secondary T cell stimulations and whether the responding T cells were specific for the form of Topo I Ag. Secondary stimulations were performed using each of the Topo I Ag preparations and resulted in higher levels of T cell proliferation.
proliferation than primary stimulations (data not shown). In the case of rTopo I, the absolute requirement for exogenous IL-2 in primary stimulations was abrogated in secondary stimulations, suggesting that a nonresponsive state to this Ag was reversed or that the small number of IL-2-producing rTopo I-specific T cells were expanded during the primary stimulation (Fig. 6B). Interestingly, cells that received a primary stimulation with one Ag preparation were unable to respond to the other Ag preparation in the secondary assay (Fig. 6). The results in Fig. 6 were obtained using DCs as the APCs, and similar results were obtained when PBMC APCs were used (data not shown). In addition, several short-term T cell lines were generated using DC or PBMC APCs, and these lines only responded to the Ag with which they were initiated (data not shown). These results suggest that unique sets of antigenic peptides are derived from the two forms of Topo I Ag.

**DC and PBMC APCs generate unique epitopes from the same form of Topo I Ag**

As described above, the T cell responses to the two Topo I Ag preparations were distinct and not cross-reactive. We next examined the role of the APC in determining the response to the two different forms of Topo I autoantigen. For typical Ags, such as tetanus toxoid (Fig. 7), primary stimulation with DCs results in a population of T cells that is able to respond the Ag presented by either DC or PBMC APCs in a secondary stimulation. However, we found that for both the MBP-Topo I mix and rTopo I, secondary proliferative responses were dependent upon using the same APC as that used in the primary stimulation (Fig. 8). This was true whether DCs (Fig. 8, A and B) or PBMCs (Fig. 8, C and D) were used as the APC in the primary stimulation. This was unexpected in the case of DCs, because these cells are widely believed to initiate immune responses in vivo. These results indicate that different sets of antigenic peptides are generated from each of the two forms of the autoantigen Topo I by DC and PBMC APCs, resulting in unique responder T cell populations.

**Discussion**

To date, there is limited information regarding the processing and presentation of disease-related autoantigens and the mechanisms regulating autoreactive T cell responses. In this regard, there are several significant findings in the present work. First, two forms of the autoantigen Topo I were very different in their ability to induce T cell proliferation. We found that a fragmented form (the MBP-Topo I mix) efficiently elicited a response, but a full-length form requires the addition of exogenous IL-2 (Fig. 2, A–C). Second, even in the presence of IL-2, T cell responses to rTopo I were much lower when presented by DC vs PBMC APCs (Fig. 2D). Finally, the two forms of Ag (native full-length vs fragmented) appear to be processed differently, with each resulting in a unique set of antigenic peptides being presented to T cells in the context of MHC class II. T cells primed with one Ag preparation were completely unable to subsequently respond to the second preparation (Fig. 6). DCs and PBMC APCs (monocytes and B cells) also appear to process the Topo I Ags differently, because T cells stimulated by one Ag type could not subsequently respond to the same Ag presented by a different APC (Fig. 8).

These findings raise questions about which form of Ag is important in vivo to initiate and perpetuate autoreactive T and B cell responses in SSc (31). Our results of increased proliferative responses to Topo I fragments and the requirement for IL-2 to induce response to full-length native Topo I suggest that cryptic epitopes generated by processing fragmented Topo I may be involved in autoimmune responses in vivo. This is supported by our previous observation that T cells from SSc patients stimulated in vitro with the MBP-Topo I mix had rapid proliferation kinetics (peak proliferation at 3–5 days after stimulation) compared with healthy controls (9).

Self proteins do not normally elicit immune responses, a state that is known as immunologic tolerance. For example, in the case of T cell-mediated diseases such as multiple sclerosis or SSc, breaking of immunologic tolerance appears to involve the activation of autoreactive T cells that are present in the peripheral circulation of otherwise healthy adults (9, 10). In animal models of autoimmune disease, such as experimental allergic encephalomyelitis and collagen-induced arthritis, tolerance is broken by activating T cells present in the repertoire by immunization with appropriate Ags under inflammatory conditions (37–41). In the case of human autoimmune diseases, many factors have been proposed to mediate the breakage of T cell tolerance including cross-reactive microbial proteins or superantigens, environmental irritants, non-specific tissue injury with exposure of normally cryptic Ags, and modification of protein Ags, which renders them immunogenic (3,
were harvested and assayed (3). These secondary stimulation proliferation assays were incubated for 48 h, and [3H]thymidine was added during the final 18 h of incubation.

It was possible that MBP mediated the uptake of the MBP fusion partner to the immunogenicity of the MBP-Topo I mix fragments. It was shown), but, again, cleavage at specific sites may be required for the MBP-Topo I mix Ag into APC, and that this effect was absent in the case of the rTopo I Ag. However, we found that MBP did not alter immune responses to the Ags used in these studies regardless of whether it was present in soluble form or covalently linked to the Ag (Fig. 3, A and B). Our findings are consistent with the concept that there are multiple pathways by which protein Ags are taken up by DCs, resulting in potentially different immune responses (29, 46—49).

T cell responses to rTopo I were detected only when exogenous IL-2 was added to cultures, regardless of whether DCs (Fig. 2B) or PBMCs (Fig. 2C) were used as APC; however, the addition of IL-2 was no longer required in secondary stimulations (Figs. 6 and 8). There are at least two possible mechanisms by which T cell responses could have been elicited to rTopo I by IL-2. First, T cells specific for rTopo I may be tolerized in vivo. Most autoreactive T cells are thought to undergo clonal deletion in the thymus. This process is not completely efficient as evidenced by the presence of autoreactive T cells in the peripheral blood of healthy individuals (9, 10). However, the fact that the incidence of autoimmune diseases is quite low in general strongly suggests that some mechanism(s) of peripheral tolerance prevents these autoreactive cells from having deleterious effects. Such “tolerized” T cells may be activated from their anergized state by treatment with IL-2, a concept supported by animal models (32—35).

The second possible mechanism explaining the role IL-2 involves the expansion of a small number of nontolerized Ag-specific T cells, which could not be detected in the absence of the cytokine. Against this hypothesis was the magnitude of the Ag-specific T cell proliferative response in cultures containing rTopo I and IL-2, which was similar to the response to tetanus toxoid. It seems unlikely that a small number of responder T cells that could not produce adequate amounts of IL-2 to provide an accessory signal for proliferation would expand in the presence of IL-2 over a 2-day period to yield [3H]thymidine incorporation comparable to the MBP-Topo I mix Ag into APC, and that this effect was absent in the case of the rTopo I Ag. However, we found that MBP did not alter immune responses to the Ags used in these studies regardless of whether it was present in soluble form or covalently linked to the Ag (Fig. 3, A and B). Our findings are consistent with the concept that there are multiple pathways by which protein Ags are taken up by DCs, resulting in potentially different immune responses (29, 46—49).

T cell responses to rTopo I were detected only when exogenous IL-2 was added to cultures, regardless of whether DCs (Fig. 2B) or PBMCs (Fig. 2C) were used as APC; however, the addition of IL-2 was no longer required in secondary stimulations (Figs. 6 and 8). There are at least two possible mechanisms by which T cell responses could have been elicited to rTopo I by IL-2. First, T cells specific for rTopo I may be tolerized in vivo. Most autoreactive T cells are thought to undergo clonal deletion in the thymus. This process is not completely efficient as evidenced by the presence of autoreactive T cells in the peripheral blood of healthy individuals (9, 10). However, the fact that the incidence of autoimmune diseases is quite low in general strongly suggests that some mechanism(s) of peripheral tolerance prevents these autoreactive cells from having deleterious effects. Such “tolerized” T cells may be activated from their anergized state by treatment with IL-2, a concept supported by animal models (32—35).

The second possible mechanism explaining the role IL-2 involves the expansion of a small number of nontolerized Ag-specific T cells, which could not be detected in the absence of the cytokine. Against this hypothesis was the magnitude of the Ag-specific T cell proliferative response in cultures containing rTopo I and IL-2, which was similar to the response to tetanus toxoid. It seems unlikely that a small number of responder T cells that could not produce adequate amounts of IL-2 to provide an accessory signal for proliferation would expand in the presence of IL-2 over a 2-day period to yield [3H]thymidine incorporation comparable to the MBP-Topo I mix Ag into APC, and that this effect was absent in the case of the rTopo I Ag. However, we found that MBP did not alter immune responses to the Ags used in these studies regardless of whether it was present in soluble form or covalently linked to the Ag (Fig. 3, A and B). Our findings are consistent with the concept that there are multiple pathways by which protein Ags are taken up by DCs, resulting in potentially different immune responses (29, 46—49).

T cell responses to rTopo I were detected only when exogenous IL-2 was added to cultures, regardless of whether DCs (Fig. 2B) or PBMCs (Fig. 2C) were used as APC; however, the addition of IL-2 was no longer required in secondary stimulations (Figs. 6 and 8). There are at least two possible mechanisms by which T cell responses could have been elicited to rTopo I by IL-2. First, T cells specific for rTopo I may be tolerized in vivo. Most autoreactive T cells are thought to undergo clonal deletion in the thymus. This process is not completely efficient as evidenced by the presence of autoreactive T cells in the peripheral blood of healthy individuals (9, 10). However, the fact that the incidence of autoimmune diseases is quite low in general strongly suggests that some mechanism(s) of peripheral tolerance prevents these autoreactive cells from having deleterious effects. Such “tolerized” T cells may be activated from their anergized state by treatment with IL-2, a concept supported by animal models (32—35).

The second possible mechanism explaining the role IL-2 involves the expansion of a small number of nontolerized Ag-specific T cells, which could not be detected in the absence of the cytokine. Against this hypothesis was the magnitude of the Ag-specific T cell proliferative response in cultures containing rTopo I and IL-2, which was similar to the response to tetanus toxoid. It seems unlikely that a small number of responder T cells that could not produce adequate amounts of IL-2 to provide an accessory signal for proliferation would expand in the presence of IL-2 over a 2-day period to yield [3H]thymidine incorporation comparable to
a recall Ag. It may be possible to clarify these issues in future studies by examining the responses of naive vs memory T cells to the full-length Ag. Tolerized T cells would be expected to have a memory phenotype, and expansion of Topo I-specific cells from this population would indicate that they had previously encountered the Ag but were now tolerized.

In summary, we have shown that T cell responses to Topo I vary depending upon the nature of the Ag and the APC type that presents it. The mechanism by which Topo I-specific T cells do not normally become activated in vivo but do so in the setting of SSc is not known. However, our present data suggest that a specific form of Ag and APC type may be preferentially involved in the autoimmune pathogenesis of SSc.

Acknowledgments

We thank Drs. Olivera Finn and Elizabeth Hilboldt for assistance with DC culture, Dr. Joseph Ahearn and Jeanine Navrtil for assistance with flow cytometry, Dr. Thomas A. Medger, Jr., and Carol Blair, R.N., for assistance with obtaining blood samples, and Drs. Dana Ascherman and Carol Feghali for thoughtful reading of the manuscript and helpful discussions.

References