Molecular recognition patterns of anti-topoisomerase I-antibodies in patients with systemic sclerosis before and after autologous stem cell transplantation

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ABSTRACT

Objective. *To evaluate the effect of autologous stem cell transplantation (aSCT) on antibody (ab) reactivity towards linear epitopes of topoisomerase-I (topo-I/Scl70) in patients with systemic sclerosis (SSc) and to correlate antibody reactivities with clinical outcome after aSCT.*

Methods. *Fourteen anti-topo-I/Scl70-positive SSc-patients were analysed before and after non-myeloablative aSCT. Five patients showed ongoing good response (group 1), 9 had primarily responded but later relapsed or did not respond (group 2). Patients' sera were tested by ELISA against full length (fl) topo-I and 45 overlapping 25-mer peptides. Furthermore, for comparison sera from patients with anti-topo-I-negative SSc (n=12), other collagen disorders (n=6), and from 21 healthy controls (HC) were analysed.*

Results. *Anti-topo-I-positive SSc-sera showed significantly higher IgG-reactivity as compared to HC towards 34 of the 45 peptides. Especially peptide 39 (aa647-671) emerged as a immunodominant epitope being recognised predominantly by anti-topo-I-positive SSc-sera. Reactivity towards 17 of the 45 peptides decreased after aSCT in group 1- and 2-patients. Before aSCT, group 1-patients had lower antibody reactivity towards peptide 39 than group 2-patients. There was no change in peptide-specificity after aSCT.*

Conclusion. *Reactivity towards topo-I-epitopes is heterogeneous in SSc, but peptide 39 (aa647-671) may be another immunodominant epitope besides the published epitope aa489-573. Antibody reactivity to this peptide 39 was higher in group 2- than in group 1-patients. Peptide recognition pattern did not change after aSCT.*

Introduction

Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterised by prominent inflammatory and fibrotic changes involving skin, the vascular system and internal organs. It is characterised by different autoantibodies (1), notably to DNA topoisomerase I (topo-I), formerly termed anti-Scl70 (2, 3). These are present in 20-30% of SSc patients and are preferentially associated with diffuse cutaneous involvement and pulmonary interstitial fibrosis indicating an unfavourable course (4-6). DNA topoisomerase I is synthesised as a precursor with a molecular weight of 100 kDa which is then proteolytically processed to a 70-kDa protein, from which the Scl-70 antigen has derived its name. It is a 765-amino-acid (aa) nuclear enzyme consisting of five distinct regions: the N-terminal domain (aa 1-215), core subdomains I-II (aa 216-435), core subdomain III (aa 436-636), the linker domain (aa 637-713), and the C-terminal domain (aa 714-765) (7, 8). Topo-I contains several epitopes recognised by anti-topo-I-antibodies, and especially the epitope aa489-573 has been shown to be an immunodominant site (9-17).

There are hardly any therapeutic options for general improvement or termination of fibrosis (18). During the last years, autologous stem cell transplantation (aSCT) emerged as a treatment option for refractory patients (19-23). In a recent study we analysed the presence of antibodies to topo-I and the immunodominant epitope aa489-573 in patients with SSc before and after aSCT, and it emerged that anti-topo-I-antibodies were hardly altered in their reactivity by aSCT while the anti-topo489-573 significantly decreased

(24). Moreover, the presence of the latter antibodies before aSCT indicated a less favourable course after aSCT (24). In this study, we examined the influence of aSCT on autoantibody reactivity towards peptides spanning the whole sequence of topo-I in SSc patients in order to see, whether there might be an antigen shift to distinct peptides after aSCT and whether distinct peptide patterns may correlate with the clinical outcome after aSCT.

Table I. Demographic and clinical data in 14 anti-topo-I positive SSc patients at the time of decision for autologous stem cell transplantation.

Characteristics	
Median age in years (range)	34 (19-57)
No. women/men	9 / 5
Baseline modified Rodnan skin score (mRSS): median (range)	16 (2-26)
Baseline CRP, mg/dl: median (range)	2.2 (0.1-5.5)
Baseline ESR, mm/H: median (range)	21 (6-28)
Baseline DLCO, % expected: median (range)	58 (38-83)
Raynaud's syndrome (number patients)	14
Heart manifestation (number patients)	3
Oesophageal manifestation (number patients)	6

Patients

Eighteen SSc patients in whom aSCT had been performed because of severe SSc had been recently analysed for anti-topo-I-antibodies. Detailed clinical data including exclusion criteria, clinical and biochemical data, treatment and outcome measures as well as the transplant regimen have been described (19, 24, 25).

Fifteen of these 18 patients were positive for anti-topo-I antibodies, and from 14 of them enough serum amounts were left for the analysis of anti-peptide antibodies. Demographic and clinical data of these patients at time of decision for aSCT are given in Table I. In the present study sera from 14 anti-topo-I positive SSC patients were analysed before and at different time points after aSCT (tp0: before aSCT {n=14}, tp1: 1-9 months after aSCT {n=9}, tp2: 10-18 months {n=13}, tp3: ≥19 months {n=8}). In total, 44 sera were analysed.

The 14 patients were classified according to the outcome after aSCT as patients with a good response and no relapse (n=5), *versus* those with primary response but relapse or without response (n=9) as previously described (Supplementary material, Table S1) (24).

Furthermore, we analysed sera from 12 patients with clinically typical SSc being anti-topo I negative but showing antibodies to centromeres (n=7) or nucleoli (fibrillarin, n=5). Moreover, sera from 6 patients with different connective tissue diseases without any evidence for SSc were included into the study (Sjögren's disease positive for anti-SSA/SSB n=2, mixed connective tissue disease positive for anti-RNP n=2, systemic lupus erythematosus positive for anti-dsDNA antibodies n=2).

As controls, sera from 21 healthy individuals (kindly provided by Dr. D. Wernet, Institute for Transfusion, Tuebingen) were tested.

Ethics

The study had been approved by the local ethical committee; it was performed according to the Helsinki guidelines, and patients had given written informed consent before the study.

Materials and methods

Antigens used in the enzyme linked immunosorbent assay (ELISA)

Full-length topoisomerase (topo*fl*) was obtained from Diarect, Freiburg, Germany.

Forty-five synthetic peptides each consisting of 25 aa with 8 overlapping aa covering the entire length of topoisomerase (supplementary material Table S2) were purchased from Biotrend (Cologne, Germany).

All peptides were high-performance liquid chromatography (HPLC)-purified (more than 90% purity). An irrelevant peptide was used as background control. The peptides were reconstituted at 5 mg/ml DMSO and stored at -20°C.

Performance of ELISA

Antibodies were detected by ELISA by the published in-house assay (24). Briefly, 96-well microtiter plates (Nunc™ Maxisorp; Thermo Fisher Scientific, Waltham MA, USA) were coated with topo*fl* at a concentration of 1 µg/ml and with peptides at concentrations of 25 µg/ml.

Patients' sera were diluted 1:1,000 for topo*fl*, and 1:500 for the peptides. Bound antibodies were detected with peroxidase-conjugated anti-human IgG- and -IgM antibodies in parallel (DIANOVA, Hamburg, Germany; dilution 1:2,000). As controls, three anti-topo-I positive standard sera showing different reactivities (strong, medium, weak) and one negative serum were added in each test. All tests were performed in duplicates.

For all antigens optimal antigen- and serum dilutions had been evaluated by serial dilutions prior to the study.

Statistics

For statistical analysis SPSS v. 15.0 was used. Fisher's exact test was applied for comparing prevalence; for comparing paired data, Kruskal-Wallis analysis and for unpaired data Mann-Whitney U-tests were performed. *P*-values <0.05 were considered statistically significant.

Correlation was evaluated by determination of the Pearson correlation coefficient.

Normal values against the different antigens in the ELISA were determined with sera from 21 healthy controls; the means of their absorbance (x1,000) + threefold standard deviations were taken as the upper limits of the normal range, and their reliability was confirmed by ROC-curves.

Results

Reactivity of patients' sera with the 45 peptides of topoisomerase I

Sera from 14 anti-topo-I-positive patients were tested by ELISA against the 45 peptides spanning the whole sequence of topo-I. Strongest IgG-*reactivity* in anti-topo-I-positive SSc sera was observed with peptides 11, 14, 15, 27, 28, 31, 37, 38, 43, and 44 for IgG- (Fig. 1A) and with peptides 5, 11, 20,

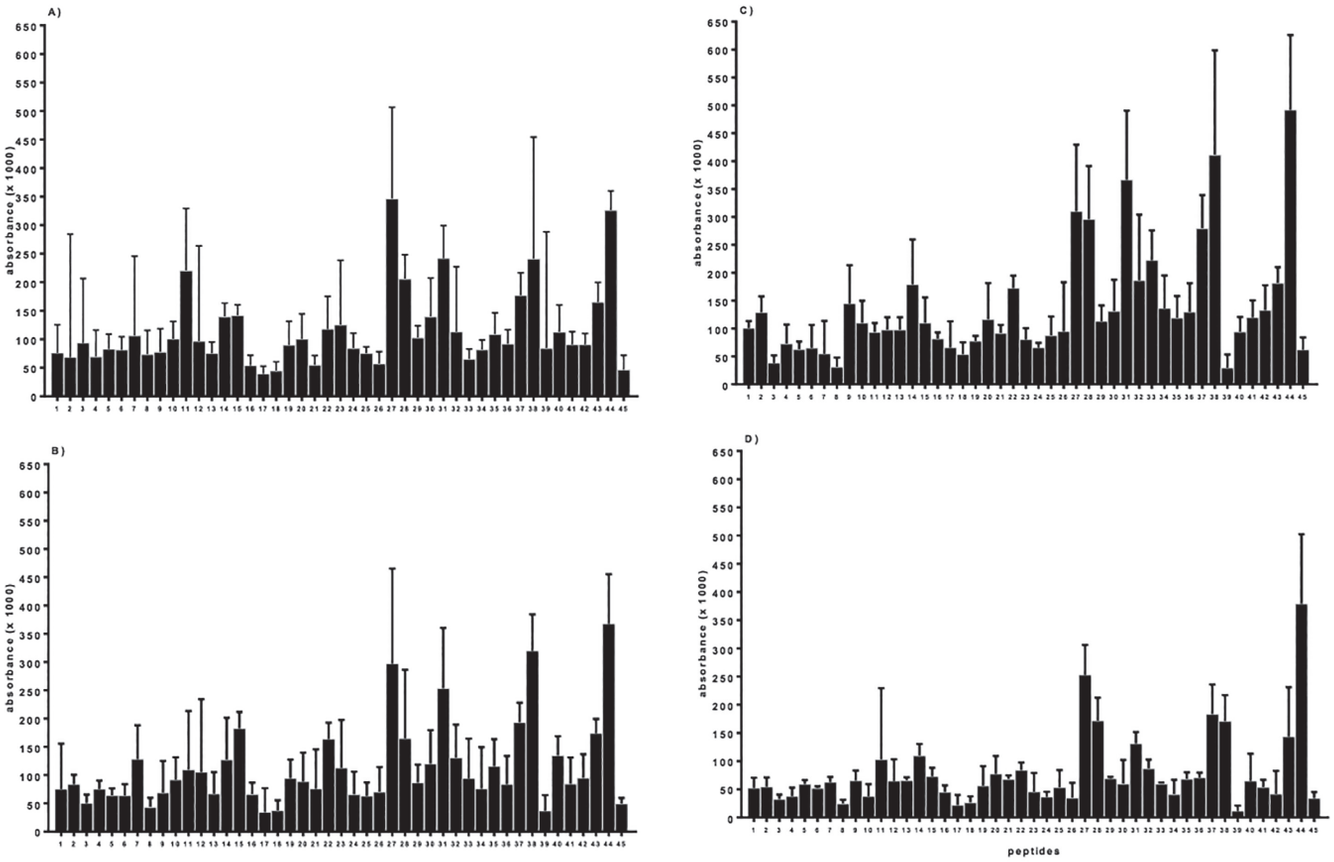

Fig. 1. IgG-reactivity of sera from 14 anti-topo-I-positive (**A**) and 12 -negative SSC-patients (**B**), six patients with other collagen disorders (**C**) and eleven healthy controls (**D**) with 45 peptides spanning the whole length of topoisomerase I (25 aa, 8 aa overlapping). Medians and interquartile ranges are given. Significance levels for differences in the reactivities between the different groups of patients are given in Table II.

21, 23, 24, 27, 32, 37, 38, 40, 43-45 for IgM antibodies (not shown), but there was not always a significant difference to the reactivity observed with sera from patients with anti-topo-I-negative SSc, other collagen disorders or healthy controls (Fig. 1B-D; Table II). Especially with the peptides 27, 28, 36, 37, and 44 also sera from healthy individuals showed strong reactivity (Fig. 1D).

Anti-topo-I-positive SSc sera showed significantly higher IgG-reactivity to 34 of the 45 peptides as compared to healthy controls (no differences were observed for peptides 2, 5, 9, 16, 21, 25, 27, 28, 33, 37, 43, 44; Table II). However, also sera from patients with other collagen disorders showed a significantly increased reactivity to 32 of the 45 peptides, as compared to healthy individuals being negative for the whole topo-I protein. A significant difference in reactivity between anti-topo-I-positive SSc sera and all other groups including the anti-topo-I-negative SSc sera was only observed for peptides 3 (aa35-59), 11 (aa171-195), and 39 (aa647-671).

There was no correlation between ANA-reactivity in the IFT and reactivity towards any peptide. Also correlating the reactivity to the topo-I protein with anti-peptide-reactivity, a significant association was observed only between antibody reactivity towards topo-I and peptides 21 and 41 but not against any of the other peptides (r=0.63 and 0.59, respectively, $p<0.05$; Supplementary material, Table S3).

However, there were quite strong correlations between reactivities towards completely unrelated epitopes as for instance towards peptide 20 and peptides 1, 2, and 5 (r=0.93, r=0.92, and r=0.98, respectively), or the peptides 15 and 16 and peptides 7, 23, 24, 30 and 31 (for all $p<0.01$; Supplementary material, Table S3).

Prevalence of antibodies to the 45 peptides

Based on the reactivity of sera from healthy controls cut off values were calculated for each peptide (see 'methods'). Patients' sera with an absorption above this cut off were defined as positive. As shown in Table III, in anti-topo-I-positive SSc sera a prevalence of ≥50% of IgG-antibodies was observed for peptides 3, 8, 10, and 39, with peptide 24 even 79% were positive. Patients' sera recognised 7-71% of the 45 peptides (median 21%).

However, also sera from patients with anti-topo-I-negative SSc and other collagen disorders reacted with most of these peptides, especially with peptides 10 and 24. Anti-topo-I-negative SSc sera recognised 2-51% of the 45 peptides (median 23%). Significant differences in prevalences of anti-peptide antibodies between anti-topo-I- positive and negative SSc-sera were observed for peptides 3, 5, 8, 39 (Table III).

IgM-antibodies reacted predominantly with peptides 5, 7, 10, 11, 13, 16, 32, and 44 (not shown).

Table II. Significance levels comparing the reactivities of patients with different disorders towards 45 peptides of topo-I.

Peptide no.	Anti-topo-I-pos SSc patients *vs*. anti-topo-I-neg SSc patients	Anti-topo-I-pos SSc *vs*. other collagen disorders	Anti-topo I-pos SSc *vs*. healthy individuals	Anti-topo-I-neg SSc *vs*. other collagen-disorders	Anti-topo-I-neg SSc *vs*. healthy individuals	Other collagen disorders *vs*. healthy individuals
N-terminal domain						
1	0.938	0.592	**0.033***	0.673	0.166	**0.004****
2	0.817	0.265	0.112	0.092	0.074	**0.021***
3	**0.050***	**0.007****	**0.000*****	0.111	**0.016***	0.291
4	0.589	0.710	**0.001****	0.574	**0.003****	**0.003****
5	0.064	0.265	0.106	0.672	0.666	0.688
6	0.076	0.363	**0.000*****	0.425	0.096	**0.009****
7	1.000	**0.026***	**0.003****	0.061	**0.001****	0.841
8	0.068	**0.019***	**0.000*****	0.206	**0.002****	0.063
9	0.396	**0.029***	0.101	**0.039***	0.268	**0.003****
10	0.258	0.804	**0.000*****	0.260	**0.010***	**0.003****
11	**0.040***	**0.005****	**0.029***	0.241	0.712	0.615
12	0.837	0.836	**0.018****	0.640	**0.039***	0.070
Core subdomain I/II						
13	0.237	0.076	**0.030***	0.083	0.559	**0.003****
14	0.837	**0.043***	**0.027***	0.092	0.096	**0.004****
15	0.368	0.302	**0.001****	0.206	**0.003****	**0.027***
16	0.410	**0.039***	0.139	0.261	**0.039***	**0.003****
17	0.699	**0.015***	**0.031***	**0.049***	**0.045***	**0.002****
18	0.381	0.283	**0.021***	0.134	0.157	**0.009****
19	1.000	0.343	**0.033***	0.134	**0.039***	0.269
20	0.247	0.564	**0.046***	0.223	0.460	0.088
21	0.247	**0.001**	0.125	0.512	0.758	**0.004****
22	0.396	0.265	**0.006****	0.963	**0.003****	**0.004****
23	0.456	**0.008****	**0.000*****	0.134	**0.004****	**0.031***
24	0.303	**0.029***	**0.000*****	0.512	**0.002****	**0.007****
25	0.303	0.161	**0.080***	0.075	0.442	**0.027***
Core subdomain III						
26	0.520	**0.019***	**0.023***	0.325	**0.039***	**0.003****
27	0.719	0.741	0.063	0.925	0.124	0.108
28	0.877	**0.008****	0.352	**0.044***	0.580	**0.003****
29	0.157	0.741	**0.000*****	0.174	0.131	**0.002****
30	0.471	1.000	**0.004****	0.639	**0.010****	**0.007****
31	0.681	0.083	**0.000*****	0.160	**0.000*****	**0.001****
32	0.537	0.117	**0.021***	0.083	**0.023***	**0.007****
33	0.181	**0.001****	0.250	**0.031***	0.074	**0.002****
34	0.607	**0.004****	**0.010***	0.061	**0.012***	**0.001****
35	0.857	0.773	**0.002****	0.925	**0.031**	**0.012***
36	0.328	0.058	**0.001****	0.061	0.218	**0.007****
37	0.758	**0.005**	0.827	**0.017***	0.951	**0.027***
Linker domain						
38	0.837	0.216	**0.004****	0.190	**0.031***	**0.005****
39	**0.007****	**0.012***	**0.000*****	0.426	**0.005****	0.107
40	0.487	0.322	**0.025***	0.092	**0.014***	0.229
41	0.837	0.201	**0.025***	0.206	0.097	**0.012***
42	0.877	**0.032***	**0.003****	0.160	**0.027****	**0.001****
C-terminal domain						
43	0.681	0.409	0.622	0.640	1.000	0.688
44	0.136	**0.002****	0.784	0.075	0.758	0.108
45	0.316	0.343	**0.016****	0.067	0.124	**0.005****

Significanve levels: *p<0.05; **p<0.01; ***p<0.001

Reactivity of sera from SSc-patients with peptides of topo-I after aSCT and correlation with the clinical outcome after aSCT

Anti-topo-I-reactivity significantly decreased after aSCT (before aSCT median [absorbance x1,000] 885, >18 months after aSCT median [absorbance x1,000] 607, p<0.01) in accordance with our previous data (24). To 17 of the 45 peptides antibody reactivity significantly decreased (shown for IgG antibodies in Fig. 2a-d); not or only hardly affected were the regions between peptides 12-26 (aa 198-450), 33-37 (aa 576-673), 40-42 (aa 702-727) for IgG- (Fig. 2a-d) and the regions 25-27 (aa 432-493), 32-35 (aa 558-637), 41-44 (aa 710-745) for IgM antibodies (not shown).

Also the number of peptides recognised by the SSc sera before and after aSCT significantly decreased (before aSCT median 21% of the 45 peptides, time point 3 median 5%, p<0.05).

In a further step we analysed whether patients with good response (group 1) and those with relapse or no response (group 2) differed in their reactivity towards the peptides *before* aSCT. It became evident that only for a few peptides antibody reactivity was higher in group 2- as compared to group 1-patients. The most prominent differences were observed for peptides 3, 5, and 39 (Fig. 3) but the differences were not statistically significant because of low numbers of patients in each group and large standard deviations.

Also *after* aSCT antibody reactivity towards the peptides did not differ significantly comparing the patients of group 1 and 2; but again, statistically analysis may be hampered by the low number of patients in each group.

Sera from patients of group 1 reacted with 11–27% of the 45 peptides (median 20%), sera from patients of group 2 with 7–71% (median 31%; difference between both groups not significant). The number of peptides recognised by sera from patients of both groups significantly decreased after aSCT (from 20% of the 45 peptides recognised before aSCT to 4% at timepoint 3 in group 1 and from 31% to 4% in group 2).

Table III. Prevalence of IgG-antibodies to 45 peptides of topoisomerase I in 14 anti-topo-I-positive SSc patients as compared to controls.

Peptide No	Aa no.	SSc anti-topo-I-positive (n=14)	SSc anti-topo-I-negative (n=12)	Other connective tissue diseases (n=6)	Healthy controls (n=11)
		Number (%) anti-peptide positive			
Topofl	1-765	**14 (100)**	0	0	0
N-terminal domain					
1	1-25	4 (29)	5 (42)	**3 (50)**	0
2	18-42	4 (29)	2 (17)	0	0
3	35-59	**9 (64)**	2 (17)	0	0
4	52-76	5 (36)	3 (25)	2 (33)	0
5	69-93	5 (36)	0	0	0
6	86-110	6 (43)	2 (17)	1 (17)	0
7	103-127	6 (43)	5 (42)	1 (17)	0
8	120-144	**9 (64)**	1 (8)	1 (17)	0
9	137-161	1 (7)	1 (8)	2 (33)	0
10	154-178	**7 (50)**	**6 (50)**	**3 (50)**	0
11	171-195	3 (21)	0	0	0
12	188-212	4 (29)	4 (33)	1 (17)	0
Core subdomain I/II					
13	205-229	3 (21)	3 (25)	**3 (50)**	0
14	222-246	0	3 (25)	2 (33)	0
15	239-263	6 (43)	7 (58)	2 (33)	0
16	256-280	1 (7)	2 (17)	2 (33)	0
17	273-297	3 (31)	4 (33)	**3 (50)**	0
18	290-314	3 (31)	2 (17)	2 (33)	0
19	307-331	1 (7)	2 (17)	0	0
20	324-348	2 (14)	2 (17)	2 (33)	0
21	341-365	0	2 (17)	2 (33)	0
22	358-382	4 (29)	5 (42)	**4 (67)**	0
23	375-399	5 (36)	5 (42)	0	0
24	392-416	**11 (79)**	5 (42)	**3 (50)**	0
25	409-433	1 (7)	2 (17)	1 (17)	0
Core subdomain III					
26	426-450	2 (14)	4 (33)	**3 (50)**	0
27	443-467	3 (21)	2 (17)	0	0
28	460-484	0	2 (17)	2 (33)	0
29	477-501	5 (36)	5 (42)	**4 (67)**	0
30	494-518	5 (36)	3 (25)	2 (33)	0
31	511-535	6 (43)	5 (42)	**5 (83)**	0
32	528-552	5 (36)	4 (33)	**5 (83)**	1 (9)
33	545-569	0	1 (8)	**4 (67)**	0
34	562-586	3 (21)	5 (42)	**4 (67)**	0
35	579-603	3 (21)	3 (25)	2 (33)	0
36	596-620	1 (7)	0	1 (17)	1 (9)
37	613-637	0 1 (8)	1 (17) 1 (9)		
Linker domain					
38	630-654	4 (29)	2 (17)	**4 (67)**	1 (9)
39	647-671	**7 (50)**	1 (8)	0	1 (9)
40	664-688	2 (14)	2 (17)	0	0
41	681-705	1 (7)	2 (17)	1 (17)	1 (9)
42	698-722	2 (14)	5 (42)	**4 (67)**	0
C-terminal domain					
43	715-739	1 (7)	0	0	0
44	*732-756*	*0*	*1 (8)*	*1 (17)*	*0*
45	741-765	5 (36)	3 (25)	**4 (67)**	0

Moreover, there was no change in peptide specificity recognised by patients' sera after aSCT.

Discussion

To our knowledge, this is the first study analysing autoantibody reactivity towards peptides spanning the whole length of topo-I in patients with SSc who underwent aSCT. Several conclusions can be drawn: i) Peptide 39 (aa647-671) was the only peptide which was able to differentiate between anti-topo-I positive SSC and patients with other disorders including the anti-topo-I-negative SSc. Further reactions were obtained with peptides in the regions aa18-229, aa307-399, aa613-671, and aa664-756. ii) Up to 50% of patients' sera reacted with peptides (peptides 29-34) which are within the previously defined immunodominant region of aa 451-573, but also sera from healthy individuals and anti-topo-I-negative patients were positive as already reported (17). iii) Except for peptide 21 and 42, there was no correlation between antibody reactivity to topo*fl* and distinct peptides. iv) Antibody reactivity towards several peptides decreased after aSCT but still remained positive in most instances. v) There was no change in peptide/epitope specificity of the anti-topo-I-antibodies after aSCT; vi) Reactivity towards peptide 39 and to less extend to peptides 3 and 5 was lower in patients with good response to aSCT than in patients with relapse or no response.

Recently, the peptide 489-573 of topo-I has been described to be an immunodominant epitope in SSc (16), and we also found a prevalence of antibodies to this peptide in 71% of anti-topo-I-positive SSc sera (24). However, this reactivity was not confined to this group of patients, because also six of the 12 patients with anti-topo-I-negative SSc and 3 of the six patients with other collagen disorders were positive (data not shown). Furthermore, peptides within this region (peptides 29-34) were also recognised by sera from patients with other collagen disorders in up to 83% as shown in the present study. This is in line with previous data using the somewhat longer 451-593aa fragment of topo-I which was also recognised by sera from SSc-patients, healthy individuals, and from patients with other rheumatic diseases (17); authors, therefore, postulated, that these antibodies may belong to the naturally occurring antibodies.

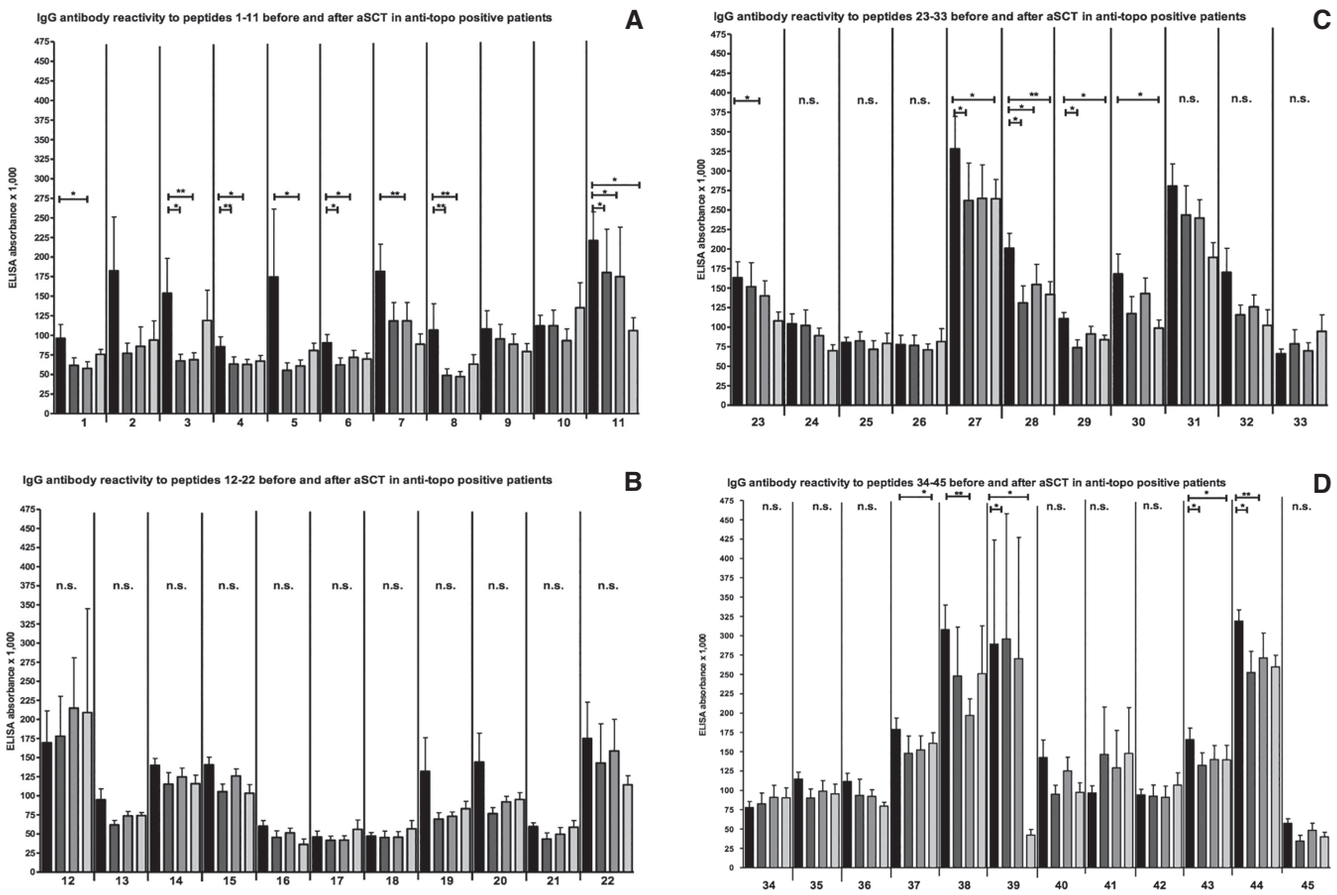

Fig. 2. IgG-reactivity to peptides 1-45 (a-d) spanning the whole length of topoisome4,-rase I (25 aa, 8 aa overlapping) testing sera from anti-topo positive sera before and after aSCT. Mean and standard deviations are given. * significant as compared to time point 0 ($p<0.05$).
= before aSCT (n=14) = patients followed for 1-9 months after aSCT (n=9), = patients followed for 10-18 months (n=13), = patients followed for ≥19 months (n=8).

Topo-I contains five distinct regions. There are several studies using different recombinant topo-I-proteins/peptides for B-cell epitope mapping in SSc-patients, but results were inconsistent (9, 10, 14, 15, 26). In the present study we found positive results with peptides in the N-terminal region (aa1-215), especially peptides 3-5 (aa35-93), the subdomain I/II (aa216-435) (especially peptides 8, 10, 24), with aa647-671 (peptide 39) in the linker domain (aa637-713), and peptide 44 (aa732-756) in the C-terminal domain (aa714-765) being in accordance to other studies (14, 15, 27-29). Interestingly, antibodies to peptide 44 were also found in healthy individuals, and they were predominantly of the IgM-type; since this peptide contains six sequential amino acids (EKIYNK) that are identical to a sequence present in the group-specific antigen ($p30^{gag}$) of some mammalian retroviruses (15), one could speculate that an infectious process might have been involved in the induction of these antibodies. For the peptides 3, 11, and 39 blast analyses revealed no significant correlation with infectious antigens.

There were only three peptides, namely peptide 3 (aa35-59), peptide 11 (aa35-59) and 39 (aa647-671) against which reactivity was significantly higher in anti-topo-I-positive sera than in anti-topo-I-negative SSc sera, sera from patients with other collagen disorders, and healthy controls. However, peptide 11 was recognised by only 3 of the 14 anti-topo-I-positive SSc-sera (21%), while with peptides 3 and 39 64% and 50%, respectively were positive. These two peptides might represent two further immunodominant epitopes in anti-topo-I-positive SSc.

All these data confirm previous observations that reactivity towards linear epitopes of topo-I is rather heterogeneous (28, 30); the fact that there was a strong correlation in antibody reactivity between unrelated peptides indicate an important role of antibodies to conformational epitopes. In this respect it is surprising that antibody reactivity to the whole enzyme topo*fl* strongly correlated with only one peptide (peptide 24, aa392-416). Performing blast analyses, there was an identity of this peptide of 80-85% with topoisomerase from several nematodes, but no bacterial protein, and this was also observed for peptide 44. Considering the immunological similarities between SSc and parasitic diseases, *i.e.* eosinophilic reactions, predominance of T helper 2-reactions, enhanced production of pro-fibrotic mediators (31, 32) – the hypothesis of an involvement of nematodes in the pathogenesis of SSc is tempting.

Introduction of aSCT as treatment for progressive SSC (22, 23, 33, 34) – as also for other autoimmune disorders –

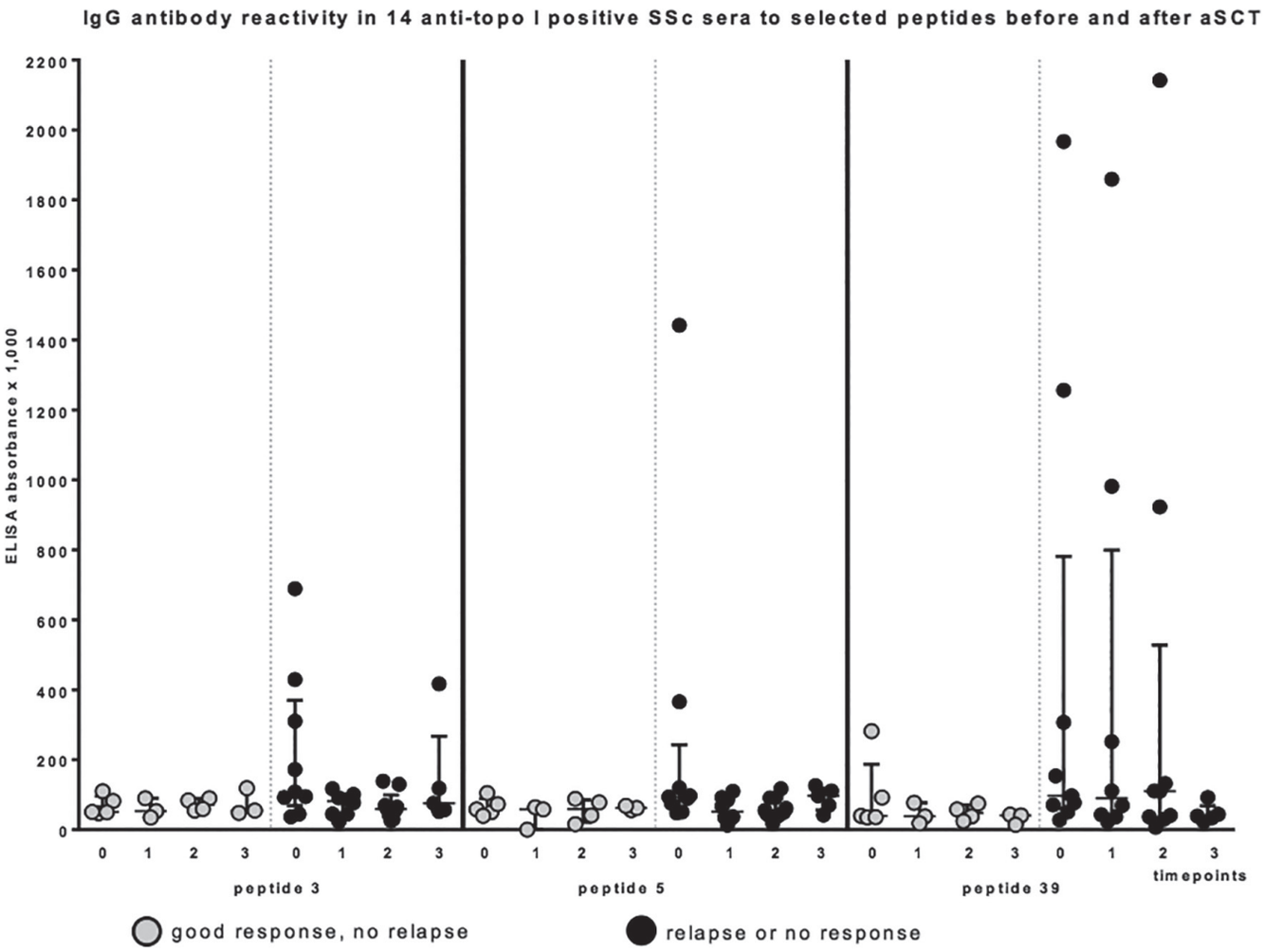

Fig. 3. Antibody reactivity to selected peptides of topoisomerase I in 14 patients with SSc according to response to aSCT. Group 1: good response, no relapse; group 2: relapse or no response. time point 0 = before aSCT (n=5 group 1, n=9 group 2), 1: patients followed for 1-9 months after aSCT (group 1: n=3; group 2 n=9), 2: patients followed for 10-18 months (group 1: n=6; group 2 n=8), 3: patients followed for > 19 months (group 1: n=4, group 2: n=5). Individual values are given and median as well as interquartile ranges are indicated.

was based on the concept that it may lead to an 'immune reset' by eradication of auto-reactive lymphocytes by immunoablative conditioning, and/or the correction of dysregulated immune balance by newly developed (regulatory?) lymphocytes derived from transplanted haematopoietic stem cells (20, 35). However, Storek *et al*. (36) showed that the level of anti-Scl70 antibodies remained abnormally high throughout 24 months after aSCT, which was in accordance with our own data (24). As shown in the present study, also peptide reactivity was altered only in a few patients, and especially peptide specificity did not change. This may either indicate that aSCT did not lead to re-programming the immune system at least in these patients, that the $CD34^+$ progenitor cells reinfused may be prone to differentiate again into autoreactive cells directed against topo-I antigens, or that the kind of treatment was inefficient to eliminate the autoreactive cells including long-living plasma cells still producing autoantibodies. This is in contrast to a dramatic clinical response to the treatment in most of the patients. It would have been of interest to analyse the IgG-subclasses of anti-peptide antibodies and whether there might be a switch of the IgG-subclass specificity after aSCT; however we had not enough serum amounts left for these analyses.

With respect to our previous finding that antibodies to the immunodominant epitope topo489-573 were preferentially found in patients who did not respond to aSCT or developed a relapse we also analysed, whether the presence of antibodies to distinct peptides of topo-I, their persistence or their reappearance may correlate with the outcome after aSCT. However, these analyses were hampered by the low number of patients which allowed no statistical evaluation. Nevertheless, there was evidence that patients of group 2 with no response or relapse after aSCT had higher antibody titers against peptide 39 than patients with good response.

In conclusion we showed that antibodies to topo-I are rather heterogeneous reacting with different epitopes, but the clinically – especially diagnostically - relevant antibodies may be directed mainly against conformational epitopes. There was no significant change in epitope-reactivity and – specificity after aSCT. However, there was some evidence that the presence of

antibodies to peptide 39 (aa647-671) in the linker domain before aSCT was associated with a worse prognosis after aSCT, but this has to be proven in a larger group of patients.

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