Identification of streptococcal proteins reacting with sera from Behçet's disease and rheumatic disorders

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ABSTRACT

Objective. *We evaluated the reactivity of sera from Behçet's disease (BD), systemic lupus erythematosus (SLE), dermatomyositis (DM), rheumatoid arthritis (RA), and Takayasu's arteritis (TA) patients against human α-enolase and streptococcal α-enolase, and identified additional streptococcal antigens.*

Methods. *Enzyme-linked immunosorbent assay (ELISA) and immunoblotting were performed using sera from patients with BD, SLE, DM, RA, and TA and healthy volunteers (control) against human α-enolase and streptococcal α-enolase. Immunoblot analysis and matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry were used to identify and recombine other streptococcal antigens.*

Results. *Specific positive signals against recombinant human α-enolase were detected by IgM ELISA of serum samples from 50% of BD, 14.3% of SLE, 57.1% of DM, 42.9% of RA, and 57.1% of TA patients. Specific positive signals against streptococcal α-enolase were detected from 42.9% of BD, 14.3% of DM, and 14.3% of TA patients. No SLE and RA sera reacted against streptococcal α-enolase antigen. Streptococcal proteins reacting with sera were identified as hypothetical protein (HP) for SLE and DM patients, acid phosphatase (AP) for RA patients, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for TA patients.*

Conclusions. *We observed that RA patients did not present serum reactivity against either HP or GAPDH though BD, SLE, DM, and TA patients did. Also, AP reacted with sera from BD, SLE, DM, RA, and TA patients.*

Introduction

Behçet's disease (BD) is a chronic, multisystemic vasculitis that mainly affects small blood vessels, and its pathogenesis remains enigmatic (1). Blood vessels, especially endothelial cells, are believed to be the primary target in this disease, presenting various symptoms of vasculitis and/or thrombosis (1). α-Enolase has been identified as a target antigen of IgM-type anti-endothelial cell antibodies in BD patients (2). Autoantibodies to α-enolase can be interpreted as either an epiphenomenon reflecting epithelial or endothelial cell damage secondary to chronic inflammation, or a pathogenic autoimmune response to α-enolase inducing inflammation (3). The precise role of α-enolase in BD pathogenesis is not clear, and both antibody sensitivity and specificity of against α-enolase remain to be elucidated, as in various other diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Kawasaki disease, and severe asthma (3-7).

The role of streptococci in BD immunopathogenesis has been demonstrated by several reports (8-13). According to our previous study, sera from BD patients that reacts with recombinant human α-enolase also cross-reacts with *Streptococcus sanguis (S. sanguis)* antigen (13). By using proteomic techniques, including 2-dimensional gel electrophoresis and matrix-assisted laser desorption ionisation-time-of-flight (MALDI-TOF) mass spectrometry, the cross-reacting *S. sanguis* antigen was determined to be streptococcal α-enolase (estimated molecular weight (M_r)/p*I*, 47075/4.7; NCBI accession number, gi 15900994; Swiss-Prot accession number, Q935W7) (13).

In this study, we evaluated the reactivity of sera from BD, SLE, dermatomyositis (DM), RA, and Takayasu's arteritis (TA) patients against human α-enolase and streptococcal α-enolase. We also identified and evaluated other streptococcal antigens reacting with the same sera from BD, SLE, DM, RA, and TA.

Materials and methods

Patients

The study was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. We collected serum samples from healthy volunteer donors (n=14), BD patients (n=14), who were diagnosed based on the International Study Group for BD and the revised criteria of the BD Research Committee of Japan (14, 15), and SLE (n=14), DM (n=14), RA (n=14), and TA (n=14) patients after obtaining informed consent. Diagnosis of SLE, DM, RA and TA was made according to the American College of Rheumatology revised criteria for SLE (16), Bohan and Peter criteria for polymyositis and DM (17-19), 1988 revised American Rheumatism Association criteria for classification of RA (20), and American College of Rheumatology 1990 criteria for the classification of TA (21), respectively, by two rheumatologists. Blood samples of each study group were collected at the time point of active disease state. All of the serum samples were stored at -70°C.

S. sanguis cultivation and protein extraction

S. sanguis strain ATCC (American Type Culture Collection) 49297 was cultivated as described previously (13). *S. sanguis* colonies were cultured for 48 hours, inactivated by 5% formalin after centrifugation and lysed by adding lysis buffer (10 mmol/L Tris/HCl, pH 7.2, 2% SDS, 158 mmol/L NaCl, and 10 mmol/L dithiothreitol).

Gel electrophoresis and immunoblot analysis of extracted S. sanguis protein

Samples of extracted *S. sanguis* protein were loaded into a 10% polyacrylamide gel, and electrophoresis was performed at a fixed current for 45-90 minutes. The protein was transferred to a nitrocellulose membrane, washed with 0.05% PBST, and incubated overnight at 4°C in blocking buffer. After a second overnight incubation at 4°C (with gentle agitation) in sera from normal controls, BD, SLE, DM, RA, and TA patients, diluted 1:50 with primary antibody dilution buffer, the membrane was washed three times with PBST and incubated with peroxidase-conjugated goat anti-human IgM for two hours. After washing with PBST, diaminobenzidine with 30% H_2O_2 was added, and the membrane was incubated at 37°C for 10 minutes.

Peptide fingerprint by spectrometry and scanning for peptide fingerprints

Samples mixed with saturated α-cyano-4-hydroxycinnamic acid were dropped onto a MALDI plate and dried at room temperature. The mass spectrum was obtained by averaging 40-50 individual laser shots, using a MALDI Reflection TOF instrument (Micromass, Manchester, UK). The autolytic peptide peak (m/z 842.50 and 2211.10) of bovine trypsin was used as an internal standard. Protein identification was accomplished with the Mascot program (available at http://www.matrixscience.com/) using the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) non-redundant protein database (16).

Expression vector construction for human α-enolase and streptococcal α-enolase

The protein-coding regions of full-length and the C-terminal half of human α-enolase were amplified by PCR using the 5'-oligonucleotide primer CGAGCTCCATATGTCTATTCTCAAGATCCATGCCA and CGAGCTCCATATG GATGATC
TCACAGTGACCAACCCAAAG containing the underlined *Nde*I restriction site, respectively, and the 3'-oligonucleotide primers CGGGATCC TTAATGATGATGATGATGCTTGGCCAAG
GGGTTTCTGA containing the underlined *Bam*HI restriction site. The protein-coding region of *S. sanguis* was amplified by PCR with the 5'-oligonucleotide primer CGAGCTCCATATGTCAATTATTACTGATGTTTACGCTCGCGAAGTCCTA containing the underlined *Nde*I restriction site and the 3'-oligonucleotide primer CGGGATCCTTAATGATGATGATGATGTTTTTTCAAGTTATAGAAAG containing the underlined *Eco*RI restriction site. PCR amplification products were gel purified, digested with the appropriate restriction enzymes and cloned into the pRSET A bacterial expression vector (Invitrogen). All constructs were confirmed by DNA sequencing. HP, AP, and GAPDH were synthesised at Peptron, Inc. (Daejeon, Korea).

Bacterial expression and protein purification

All proteins were overexpressed in *Escherichia coli* BL21 and recombinant proteins were purified to apparent homogeneity using Ni-NTA resin according to the manufacturer's instructions (Sigma, St Louis, MO, USA). Protein concentrations were determined with the BCA assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's recommendations, using bovine serum albumin as a protein standard. Protein samples were stored at 30°C until use.

Enzyme-linked immunosorbent assay (ELISA)

IgM ELISA was performed using recombinant human α-enolase antigen and streptococcal antigens. A 96-well microtiter plate (Immuno2, HB, Thermo Scientific, Waltham, MA, USA) was coated with 250 ng recombinant human α-enolase antigen or streptococcal antigens overnight. The plate was washed three times with 0.05% phosphate-buffered saline-Tween 20 (PBST) to block non-specific binding. Then, 100 μL of sera from BD patients, normal controls, and patients with rheumatic disorders, diluted 1:20 in PBST containing 1% bovine serum albumin (Sigma), was added to each well, and the plate was incubated for 1 hour at 37°C.

After washing the plate three times with PBST, antibody binding was quantified colorimetrically by adding a substrate (tetramethylbenzidine, Sigma) to each well. Optical density (OD) of the plates was read spectrophotometrically at 450 nm on an ELISA reader (Dynatech, Alexandria, VA, USA) and positivity was defined as an OD value greater than 3 SD above the mean of normal controls.

Immunoblot analysis

After separately mixing purified recombinant human α-enolase and strepto-

coccal antigens with the same amount of sample buffer, samples (3 μg) were loaded into a 10% polyacrylamide gel and subjected to electrophoresis at 100V. Protein was transferred to nitrocellulose membrane at 150 mA 10-15V, and the membrane was washed with PBST and incubated for one to three hours at room temperature in blocking buffer (5% non-fat dry milk, 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20). Each membrane was incubated with gentle agitation for one hour at room temperature with sera from normal controls and BD or rheumatic disorder patients, including SLE, DM, RA, and TA, diluted 1:20 with primary antibody dilution buffer. Goat anti-human α-enolase antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as a positive control at 1:100. The membrane was washed three times with PBST and incubated at room temperature for two hours with peroxidase-conjugated goat anti-human IgM antibody. 3,3-diaminobenzidine tetrahydrochloride (Sigma) containing 30% H_2O_2 was added after washing three times with PBST, and the membrane was incubated at 37°C for 10 minutes.

Statistical analysis

A two-sample proportion test with chi-square (χ^2) test (Fisher's exact test) was used to analyse differences in serum reactivity against human α-enolase, and streptococcal α-enolase, HP, AP, and GAPDH depending on each disease. All analyses were performed using Statistical Package for the Social Sciences version 11.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant when an error probability (*P*) was less than 0.05.

Results

Serum reactivity against recombinant human α-enolase

The mean ELISA results for sera from BD, SLE, DM, RA, and TA patients detecting anti-human and streptococcal α-enolase antibodies are summarised in Table I. The mean optical density (OD) ± 1 SD for human α-enolase of 14 healthy controls was 0.065±0.029. A result greater than three SD above the mean was defined as indicating positive reactivity. Serum samples from 7 of 14 BD patients (50%) gave specific positive signals against recombinant human α-enolase (Table II and Fig. 1). Two of 14 SLE patients (14.3%), eight of 14 DM (57.1%), six of 14 RA (42.9%), and eight of 14 TA patients (57.1%) also showed positive results for anti-α-enolase antibodies. Differences in the percentage of positive sera were not statistically significant between BD and other rheumatic disorders (each $p>0.05$). Sera that gave a positive ELISA result recognised specific bands by using immunoblot analysis, while no reactivity was seen in sera that gave negative ELISA results or normal controls.

Table I. The mean optical densities of ELISA for anti-human and streptococcal α-enolase antibodies, streptococcal conserved HP, AP, and GAPDH in sera from BD, SLE, DM, RA, and TA patients.

Group	Mean optical density*				
	Human α-enolase	Streptococcal α-enolase	HP	AP	GAPDH
Normal control subjects	0.065 ± 0.029	0.171 ± 0.072	0.109 ± 0.029	0.120 ± 0.042	0.122 ± 0.077
BD patients	0.232 ± 0.167	0.331 ± 0.143	0.443 ± 0.248	0.487 ± 0.166	0.440 ± 0.224
SLE patients	0.123 ± 0.112	0.156 ± 0.068	0.213 ± 0.154	0.218 ± 0.127	0.289 ± 0.133
DM patients	0.174 ± 0.086	0.250 ± 0.094	0.296 ± 0.153	0.282 ± 0.077	0.344 ± 0.138
RA patients	0.178 ± 0.122	0.274 ± 0.068	0.128 ± 0.059	0.160 ± 0.070	0.152 ± 0.068
TA patients	0.188 ± 0.121	0.276 ± 0.082	0.374 ± 0.130	0.346 ± 0.102	0.404 ± 0.092

*Mean optical density is presented as mean±SD.

Table II. Reactivity of anti-human and streptococcal α-enolase antibodies, streptococcal conserved HP, AP, and GAPDH obtained from normal control subjects, BD, other rheumatic disease patients.

Group (No. of subjects)	No. (%) of subjects positive for anti-α-enolase and streptococcal antigen antibodies				
	Human α-enolase	Streptococcal α-enolase	HP	AP	GAPDH
Normal control subjects (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BD patients (14)	7 (50)	6 (42.9)	8 (57.1)	12 (85.7)	10 (71.4)
SLE patients (14)	2 (14.3)	0 (0)	8 (57.1)	5 (35.7)	2 (14.3)
DM patients (14)	8 (57.1)	2 (14.3)	10 (71.4)	9 (64.3)	6 (42.9)
RA patients (14)	6 (42.9)	0 (0)	0 (0)	6 (42.9)	0 (0)
TA patients (14)	8 (57.1)	2 (14.3)	9 (64.3)	12 (85.7)	10 (71.4)

Serum reactivity against recombinant streptococcal α-enolase

The 14 healthy controls had a mean OD ± SD of 0.171±0.072 against streptococcal α-enolase (Table I). Six of 14 BD patients (42.9%, Table II and Figure 1) were positive against streptococcal α-enolase by ELISA. Two of 14 DM (14.3%) and 2 of 14 TA patients (14.3%) also tested positive for anti-streptococcal α-enolase antibodies. No SLE or RA patients reacted against streptococcal α-enolase antigen. A significantly higher percentage of serum samples from BD patients tested positive compared to SLE and RA patients (each $p=0.016$), but not compared to DM and TA patients (each $p>0.05$). Sera that reacted against streptococcal α-enolase also reacted against human α-enolase and against specific bands by using immunoblot analysis, while sera with negative reactivity and from normal controls showed no reactivity by immunoblot.

Isolation and identification of streptococcal antigens reacting with sera from BD, SLE, DM, RA, and TA patients

Immunoblotting using whole *S. sanguis* extract was performed with sera from BD, SLE, DM, RA, and TA patients that reacted with recombinant human α-enolase (Fig. 2). The protein

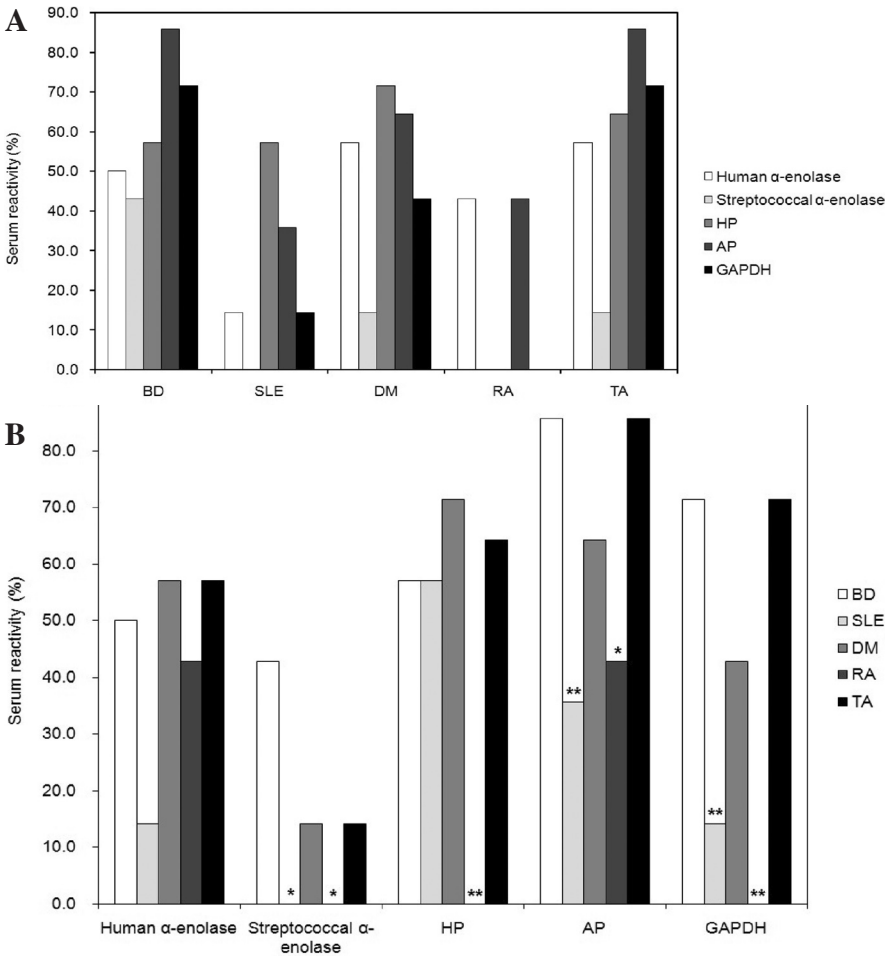

Fig. 1. A-B. Frequency of anti-human and streptococcal α-enolase antibodies, anti-streptococcal hypothetical protein (HP), acid phosphatase (AP), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies in Behçet's disease (BD), systemic lupus erythematosus (SLE), dermatomyositis (DM), rheumatoid arthritis (RA), and Takayasu's arteritis (TA) patient sera.

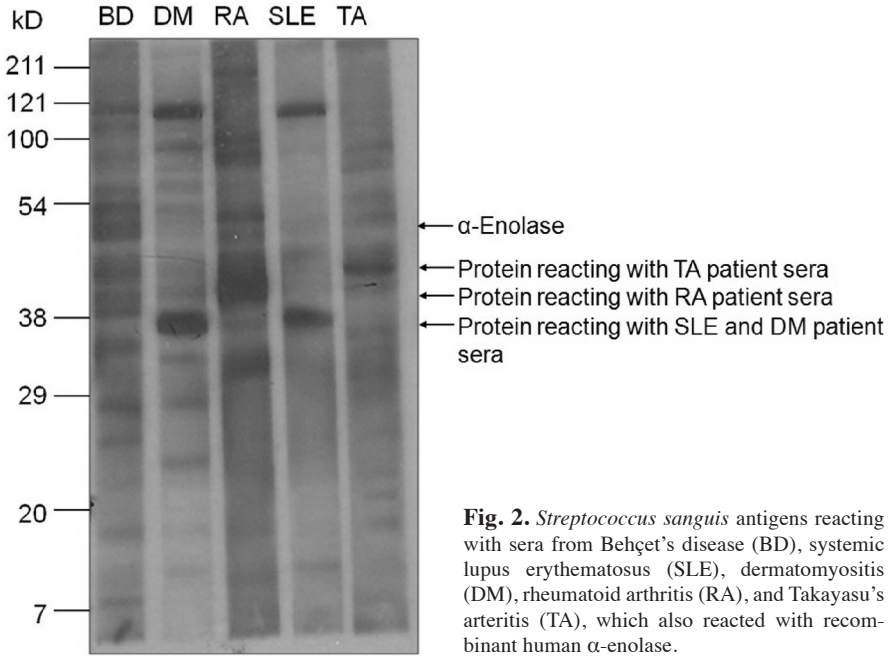

Fig. 2. *Streptococcus sanguis* antigens reacting with sera from Behçet's disease (BD), systemic lupus erythematosus (SLE), dermatomyositis (DM), rheumatoid arthritis (RA), and Takayasu's arteritis (TA), which also reacted with recombinant human α-enolase.

bands were excised from a polyacrylamide gel, digested with trypsin, and the resulting peptide fragments were analysed by mass spectrometry. Sera from SLE and DM patients reacted with the streptococcal protein identified as hypothetical protein (HP) by MALDI-TOF mass spectrometry (Fig. 3a, Table III), RA sera reacted with acid phosphatase (AP; Fig. 3b), and TA sera reacted with glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Fig. 3c).

BD, SLE, DM, RA, and TA sera reactivities with recombinant streptococcal HP

The mean OD ± SD of 14 healthy controls was 0.109±0.029 for an ELISA against streptococcal HP (Table I). Eight of the 14 BD patients (57.1%, Table II and Fig. 1) had a positive signal against streptococcal HP by IgM ELISA. Eight of 14 SLE patients (57.1%), 10 of 14 DM patients (71.4%), and 9 of 14 TA (64.3%) patients also showed anti-streptococcal HP antibodies by IgM ELISA. No RA patients reacted against streptococcal HP. Differences in the percentage of positive sera were not statistically significant between BD and other rheumatic disorders (each $p>0.05$), except RA ($p=0.002$).

BD, SLE, DM, RA, and TA sera reactivities with recombinant streptococcal AP

The mean OD ± SD of 14 healthy controls was 0.120±0.042 for an IgM ELISA against streptococcal AP (Table I). Twelve of 14 BD patients (85.7%, Table II and Fig. 1) showed reactivity against streptococcal AP by IgM ELISA. Five of 14 SLE (35.7%), nine of 14 DM patients (64.3%), and six of 14 RA (42.9%), and 12 of 14 TA patients (85.7%) also tested positive for anti-streptococcal AP antibodies. A significantly higher percentage of serum samples from BD patients tested positive compared to SLE and RA patients ($p=0.007$ and $p=0.018$, respectively), but not compared to DM and TA patients (each $p>0.05$).

BD, SLE, DM, RA, and TA sera reactivities with recombinant streptococcal GAPDH

The mean OD ± SD of the 14 healthy

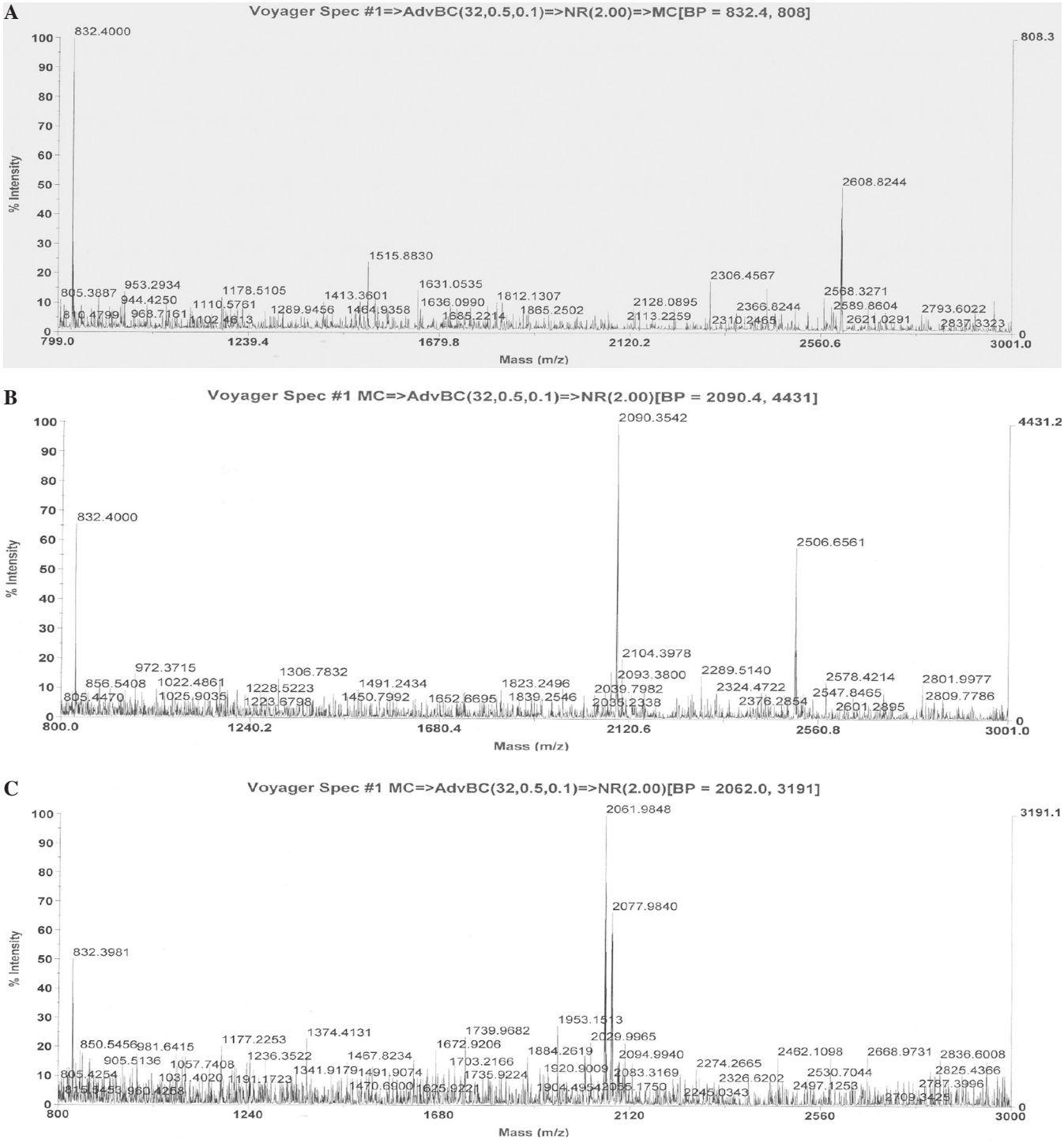

Fig. 3. (**A**) Streptococcal protein reacting with systemic lupus erythematosus and dermatomyositis patient sera identified as hypothetical protein by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry; (**B**) streptococcal protein reacting with rheumatoid arthritis patient sera revealed as acid phosphatase; (**C**) streptococcal protein reacting with Takayasu's arteritis patient sera identified as glyceraldehyde-3-phosphate dehydrogenase.

controls was 0.122±0.077 for an IgM ELISA against streptococcal GAPDH (Table I). Ten of 14 BD patients (71.4%, Table II and Fig. 1) reacted against streptococcal GAPDH by IgM ELISA. Two of 14 SLE (14.3%), six of 14 DM patients (42.9%), and 10 of 14 TA patients (71.4%) tested positive for anti-streptococcal GAPDH antibodies. No RA patients reacted against streptococcal GAPDH. A significantly higher percentage of serum samples from BD patients tested positive compared to SLE and RA patients (p=0.002 and p<0.0001, respectively), but not compared to DM and TA patients (each p>0.05).

Discussion

BD patients have significantly more *S. sanguis* as oral bacterial flora than

Table III. Identified streptococcal conserved HP, AP, and GAPDH by MALDI-TOF mass spectrometry.

Spot No.	Protein name	Gene symbol	NCBI accession No.	Swiss-Prot accession No.	Sequence coverage (%)	no. of unique peptides matched	Estimated molecular weight (M_r)/p*I*
1	HP	spr1391	Gi 15459041	-	26	6	28331/5.70
2	AP	lppc	Gi 71854195	Q99Y38	23	4	32555/8.64
3	GAPDH	gap	Gi 77171633	Q3D164	23	7	35980/5.17

healthy and other disease controls (23-25). The BD patients show strong delayed cutaneous hypersensitivity reactions as well as oral aphthous ulcerations against streptococcal antigens by skin injection or oral prick with streptococcal antigens (25). Cytosolic proteins localised on the streptococcal surface include glutamine synthetase, Hsp70, α-enolase, glucose-6-phosphate isomerase, ornithine carbamoyltransferase, and GAPDH (26-29). A previous report identified citrullinated α-enolase as a potential autoantigen in patients with anti-CCP-positive RA (6, 30). According to the previous report, antibodies against citrullinated α-enolase were detected in 46% of the 52 RA patients, whereas only 13% reacted with the non-citrullinated form of α-enolase (6). Sera from RA patients presenting reactivity against citrullinated α-enolase peptide 1 cross-reacted with citrullinated recombinant *Porphyromonas gingivalis* enolase (30).

In BD, our study group identified α-enolase as a target antigen of IgM-type anti-endothelial cell antibody in BD patients, which cross-reacts with an *S. sanguis* antigen subsequently identified as streptococcal α-enolase (2,13). This study also found that streptococcal GAPDH, AP, and HP react with sera from BD patients. The clinical significance of detecting various autoantibodies against several streptococcal surface antigens in a single patient remains unclear. Possible interpretations include an epiphenomenon reflecting epithelial or endothelial cell damage secondary to chronic inflammation, or a pathogenic autoimmune response to α-enolase and GAPDH that induces inflammation. We also postulate that cell damage from direct human endothelial cell invasion by viridans group streptococci and subsequent bacterial division may induce antibody formation leading to cross-reactivity against simultaneously exposed human cytoplasmic antigens and streptococcal surface antigens with structural mimicry.

The precise role of α-enolase in BD pathogenesis is not clear, and antibody sensitivity and specificity against α-enolase in various diseases remain to be elucidated. Enolase on the surface of various cells functions as the plasminogen receptor and plays a crucial role in fibrinolysis by binding plasminogen. α-enolase, especially expressed on the endothelial cell surface and reacting with plasminogen, has been suggested to have an important role in initiating the disease process by modulating the pericellular and intravascular fibrinolytic system (2, 4). Our group recently demonstrated the reactivities of serum anti-α-enolase antibodies in BD and other rheumatic diseases with moderate specificity and found that serum anti-α-enolase antibodies in BD can be associated with vascular system involvement (31).

Because α-enolase has identified as a target antigen of IgM-type anti-endothelial cell antibody (AECA) in BD patients (2), several mechanisms that describe the pathophysiologic role of AECAs in inflammatory diseases can also be associated with BD development (32-34). AECA binding to endothelial cells may result in endothelial cell activation, which may be associated with increased chemoattractant and/or cytokine secretion as well as prostacyclin secretion or inhibition (32, 33). AECAs could also trigger the inflammatory processes by complement dependent cytotoxicity and/or antibody dependent cellular toxicity (34).

In this study, we identified streptococcal GAPDH as a target antigen reacting with the sera from patients with BD or other rheumatic disorders, except RA. The exact role of GAPDH in BD and other rheumatic disorder pathogenesis remains to be elucidated. Inflammatory diseases of the blood vessel wall, including BD, Kawasaki's disease, TA, and giant cell arteritis, characteristically demonstrate endothelial proliferation, fibrosis and thrombus formation, which eventually result in tissue ischemia. In this study, BD and TA sera showed relatively high reactivity against GAPDH. Group A streptococcus surface-localised GAPDH binds a number of human proteins, including plasmin, plasminogen, lysozyme, myosin, actin, and fibronectin (26, 35, 36). Therefore, GAPDH reacting with plasmin or plasminogen may have a crucial role in initiating the disease process through the fibrinolytic system as seen in cell surface-localised α-enolase.

Viridans group streptococci, such as *S. gordonii*, *S. sanguis*, *S. mutans*, *S. mitis*, *S. oralis*, and *S. salivarius*, can be found in the mouth, throat, colon, and female genital tract. Also, upon reaching the blood stream as a result of trauma, colonisation of the cardiovascular endothelium by viridians group streptococci can be associated with various cardiovascular disorders. Viridans group streptococci also have the ability to invade human umbilical vein and coronary artery endothelial cells (37, 38). Esgleas *et al*. (39) demonstrated that streptococcal surface α-enolase has a high affinity for plasminogen as well as a similarly high affinity for fibronectin. The authors showed that streptococcal surface α-enolase participates in bacterial adhesion to and invasion of endothelial cells by binding fibronectin on human endothelial cells (39). Also, streptococcal surface GAPDH can bind different host proteins such as plasminogen and albumin (40, 41). The precise pathogenic role of streptococcal surface α-enolase and GAPDH by direct invasion of human dermal microvascular endothelial cells in BD patients and the role of human surface-localised α-enolase and GAPDH as a plasmin or plasminogen receptor remain to be elucidated.

In addition to streptococcal α-enolase and GAPDH, we identified streptococ-

cal HP and AP as target antigens reacting with sera from patients with BD and other rheumatic disorders. HPs are predicted proteins, characterised by low identity to annotated proteins (42). Many HPs have been presented without sequence similarity, severely impeding the assignment of function through sequence-based (43). Also, streptococcal AP function and pathogenic role has not been described.

Streptococcal enolase has been reported as a novel cross-reactive antigen, which is suggested to play a major role in the induction of autoimmune processes in streptococcal infection related disorders (44). However, according to the previous report, anti-human and streptococcal enolase antibodies were detected only in 8.3% and 11.3% of 11 SLE patients, respectively, and in 10.6% and 14.7% of 10 RA patients, respectively (44). In the present study, differences in the percentage of positive sera against human α-enolase were not statistically significant between BD and other rheumatic disorders. We observed that RA patients did not present serum reactivity against either HP or GAPDH though BD, SLE, DM, and TA patients did. Also, streptococcal α-enolase did not react with sera from SLE and RA patients, whereas, AP reacted with sera from BD, SLE, DM, RA, and TA patients. However, our results do not constitute a conclusive comparison among the study groups because of the small study sample, and further studies are necessary to determine the diagnostic value and pathogenic role of serum reactivity against human α-enolase, streptococcal α-enolase, HP, AP, and GAPDH in a larger study population.

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