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Gastric Autoantigenic Proteins in *Helicobacter Pylori* Infection

Ji Sook Park,[1] Su-Jin Lee,[1] Tae Hyo Kim,[2] Jeongsuk Yeom,[1] Eun-Sil Park,[1] Ji-Hyun Seo,[1] Jin-Su Jun,[1] Jae-Young Lim,[1] Chan-Hoo Park,[1] Hyang-Ok Woo,[1] Hee-Shang Youn,[1] Gyung-Hyuck Ko,[3] Hyung-Lyun Kang,[4] Seung-Chul Baik,[4] Woo-Kon Lee,[4] Myung-Je Cho,[4] and Kwang-Ho Rhee[4]

Departments of [1]Pediatrics, [2]Internal Medicine, [3]Pathology, and [4]Microbiology, Gyeongsang National University School of Medicine, Gyeongsang Institute of Health Science, Jinju, Korea.

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Corresponding author: Dr. Hee-Shang Youn, Department of Pediatrics, Gyeongsang National University School of Medicine, 79 Gangnam-ro, Jinju 660-702, Korea.
Tel: 82-55-750-8158, Fax: 82-55-752-9339
E-mail: hsyoun@gnu.ac.kr

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Purpose: This study tried to identify novel gastric autoimmune antigens that might be involved in aggravating the atrophic gastritis among patients with *Helicobacter pylori* infection using two-dimensional immunoblotting analysis. **Materials and Methods:** Proteins from gastric mucosal antrectomy specimens and AGS cells (gastric adenocarcinoma cell lines derived from a Caucasian patient who had received no prior therapy) were 2-dimensionally immunoblotted separately with a pool of 300 sera from *H. pylroi*-infected patients at Gyeongsang National University Hospital. **Results:** Thirty-eight autoantigenic proteins including alcohol dehydrogenase [NADP+], alpha enolase, gastrokine-1, gastric triacylglycerol lipase, heat shock 70 kDa protein 1, and peroxiredoxin-2 were identified in the gastric mucosal tissue. Fourteen autoantigenic proteins including programmed cell death 6-interacting protein, serum albumin and T-complex protein 1 subunit gamma were identified in the AGS cells. Albumin, alpha-enolase, annexin A3, cytoplasmic actin 1, heat shock cognate 71 kDa protein and leukocyte elastase inhibitor were commonly observed autoantigenic proteins in both gastric mucosal tissue and AGS cells. Alpha-enolase, glutathione S-transferase P, heat shock cognate 71 kDa protein, heat shock 70 kDa protein 1, human mitochondrial adenosine triphosphate synthase (ATP) subunit beta, mitochondrial 60 kDa heat shock protein, peroxiredoxin-2, 78 kDa glucose-regulated protein precursor, tyrosine-protein phosphatase non-receptor type 11 and Tryptophan-Aspartic acid (WD) repeat-containing protein 1 showed 60% or higher amino acid positivity. **Conclusion:** These newly identified gastric autoimmune antigens might be useful in the control and prevention of gastroduodenal disorders, and might be valuable in breaking the vicious circle that exists in gastroduodenal disorders if their pathophysiological roles could be understood in the progress of chronic atrophic gastritis, gastroduodenal ulcers, intestinal metaplasia, and gastric carcinogenesis.

Key Words: *Helicobacter pylori*, gastric atrophy, autoantigen, 2D immunoblotting

INTRODUCTION

Chronic gastritis is usually divided into type A and B. Type A gastritis (fundal gas-

tritis) is characterized by diffuse atrophic changes, intestinal metaplasia, and inflammatory changes of the gastric body and fundus, and associated with achlorhydria and pernicious anemia. The autoantiboides against parietal cells, proton pump (H^+,K^+-ATPase) and intrinsic factors are considered to play the main pathogenetic roles.[1,2] On the other hand, type B gastritis (antral gastritis) shows mucosal atrophic changes, intestinal metaplasia, and inflammatory changes of gastric antrum. Initially, lesions in the stomach are sparse, with progression to larger lesions of the gastric body through the gastric lesser curvature. Autoantibodies had not been considered as etiologies until recently. *Helicobacter pylori* was identified as an etiologic agent of type B gastritis by Marshall and Warren in 1984.[3] It also became known to play a main role in the pathogenesis of gastric ulcer, duodenal ulcer, gastric carcinoma and gastric mucosa associated lymphoid tissue lymphoma.[4-6] In addition to chronic and active inflammatory responses in gastric lamina propria induced by *H. pylori* infection,[7,8] anti-gastric autoimmune antibodies have also been suggested to play a role in the process from *H. pylori* infection in a young child to gastric carcinoma in a late life through gastric mucosal atrophy and intestinal metaplasia.[9] Anti-parietal cell antibody is documented in 50% of patients with chronic type B gastritis, and the inflammatory responses by the autoimmune reaction are implicated with the development and progression of chronic type B gastritis.[10]

The human immune system recognizes invading bacteria and their derived materials as new antigens and initiates the removal of the antigens using specific or non-specific immune responses. *H. pylori* typically inhabit the gastric mucosa or junctions between epithelial cells, which are regions poorly surveyed by the host immune defense system. Persistence of infections can lead to a host hypersensitivity response that progressively destroys host tissue with time.[11] *H. pylori* infection can produce both humoral and cell-mediated immune reactions, and chronic repetitive infection can stimulate an autoimmune gastritis because of bacterial molecular mimicry with gastric tissue molecules.[12]

Koreans are infected with *H. pylori* from early infancy and most are carriers by 10-years-of-age.[13,14] Therefore, some adult Koreans with *H. pylori* infection might have high titers of anti-*H. pylori* antibodies and its cross-reactive anti-gastric tissue autoantibodies. The presence of an anti-gastric cell immune response in the absence of serum antibodies for Lewis x and Lewis y has been reported,[15,16] and the observed atrophic phenomena are not confined to the parietal cells of gastric body, but are found diffusely and multifocally throughout the gastric mucosa. Therefore, there may be as-yet unidentified antoantigenic proteins in gastric mucosa that play important roles in the pathophysiology of *H. pylori*-induced gastric carcinogenesis. In the present study, we performed proteomics investigations of anti-gastric autoantibody profiles in the sera of 300 *H. pylori* infected Korean adults.

MATERIALS AND METHODS

Gastric mucosa, AGS cells, and serum samples

This study was approved by the Gyeongsang National University Hospital Institutional Review Board (GNUHIRB-5413).

The gastric antrectomy surgical specimen was obtained from a 29-year-old man with a gastric perforation because of gastric ulcer. The specimen was moderately infected with *H. pylori* and showed severe chronic gastritis with mild activity supported by many lymphoid follicles and neutrophil infiltrations on pathologic examination. There was no intestinal metaplasia. The specimen had been kept deeply frozen in the Gyeongsang National University Hospital (GNUH) Biobank of Korea. Following specimen retrieval, the muscular layer was cut off of the specimen using a knife. The AGS human adenocarcinoma epithelial cell line (CRL 1739; American Type Culture Collection, Manassas, VA, USA) was obtained from Korean Cell Line Bank. The cells were grown in RPMI 1640 medium (Lonza, Walkersyille, MD, USA) with 10% fetal bovine serum (Lonza) and 100 units/mL of gentamicin at 37°C and 5% CO_2. A pool of 300 sera obtained from patients, confirmed *H. pylori*-infected by immunoblotting at GNUH, was also taken from the same biobank. The serum samples were collected from healthy candidates who visited GNUH for medical check-up and 300 *H. pylori*-infected sera of the collected serum samples were pooled.

2-DE and immunoblotting

To homogenize the gastric mucosa, 0.15 g of the tissue was solubilized in 1 mL of 50 mM Tris-HCL (pH 7.2), containing 10 μL of protease inhibitor and 10 μL of 100 mM ethylenediaminetetraacetic acid (EDTA). Homogenized gastric mucosa and collected AGS cells were separately lysed in solubilization buffer, composed of 8 M urea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 100 mM dithiothreitol (DTT), and 40 mM Tris-HCl (pH 7.2).

Proteins derived from the extracts of gastric mucosa and AGS cells were separated by two-dimensional-PAGE (2-DE). In brief, isoelectrofocusing was performed on 17-cm pH 5-8 IPG strips (Bio-Rad, Hercules, CA, USA). The strips were rehydrated for 13 hours in a 300 μL of solution consisting of 7 M urea, 2 M thiourea, 2% CHAPS, 100 mM DTT, and 0.5% ampholyte (Bio-rad) containing 60 μg of the protein sample. Strips were electrofocused at 60000 Vh, with a maximum of 10000 V at 20°C using the Protean IEF cell (Bio-rad). Prior to the second dimension, strips were incubated in equilibration buffer (375 mM Tris-HCl, pH 8.8, 6 M urea, 2% SDS, 30% glycerol) with 2% DTT for 10 min and then with 2.5% iodoacetamide in the same buffer without DTT for 10 min. The separation was performed on 7.5-17.5% SDS-PAGE gradient gels using a PROTEAN II xi 2-D cell (Bio-rad).

The separated proteins on other gels were transferred to nitrocellulose membranes. Following blocking with 1% BSA in TBST buffer (Tris-buffered saline containing 0.1% Tween 20) for 1 hour at 37°C, the membranes were subjected to IgG immunoblotting using the 1 : 10 diluted mixed sera of *H. pylori* infected patients for 45 minutes at 37°C. After three times washes with TBST (Tris-Buffered Saline and Tween 20) buffer, the membranes were incubated with alkaline phosphatase-conjugated rabbit anti-human IgG as a secondary antibody (gamma chain specific; DAKO A/S, Glostrup, Denmark) at a 1 : 1000 dilution for 30 minutes at 37°C. After five times washes, the membranes were processed using an enzyme reaction with 5-bromo-4-chloro-3-indolyl phosphate, p-toluidine salt and nitro blue tetrazolium chloride in alkaline phosphate buffer. The protein patterns in some gels were visualized directly by silver[17] or Coomassie blue staining.

Image analysis

Gel images were obtained by scanning the silver or Coomassie blue stained gels with the Fluor-S Multi Image system (Bio-rad) incorporating the PDQUEST program.

Spot identification

The excised silver stained protein spots were destained with the chemical reducers [30 mM $K_3Fe(CN)_6$, 100 mM $Na_2S_2O_3 \cdot 5H_2O$] and in-gel digested.[18] Protein identification was repeated three times using spots from different gels. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was carried out using a Voyager Biospectrometry Workstation (PE Biosystem, Foster City, CA, USA) with the following parameters: 20 kV accelerating voltage, 75% grid voltage, 0.02% guide wire voltage, 150 ns delay, and a mass gate from 800-3500. Peptide mass fingerprints were searched using the Protein Prospector Package program MS-FIT (http://prospector.ucsf.edu/ucsfhtml/msfit.htm; ProteinPtospector V 5.8.0) and NCBI database (SwissProt. 2011.01.11). In addition, the amino acid sequences of the peptides were deduced and searched for homogeneity with *H. pylori* peptides using the BLASTp program of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

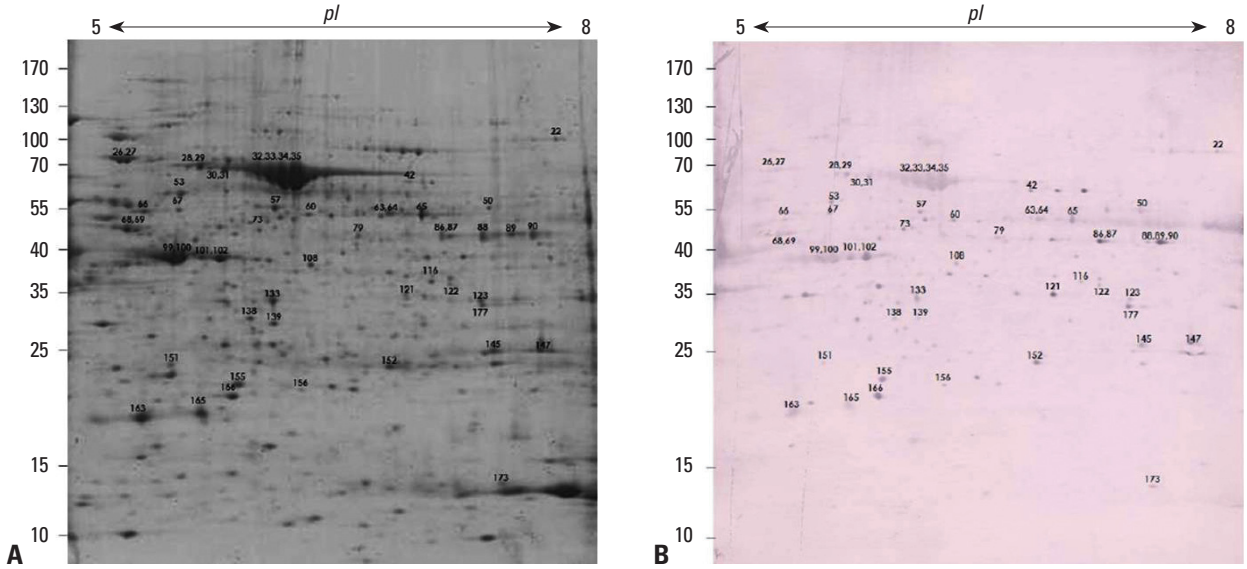

Fig. 1. Autoantigenic proteins visualized by 2-D immunoblotting derived from human gastric mucosa. (A) 2-DE of total protein extracts from human gastric mucosal tissue. (B) 2-D immunoblot of human gastric mucosal tissue by a pool of 300 sera obtained from *Helicobacter pylori*-seropositive patients. 2-DE, two-dimensional-PAGE.

Table 1. Mass Spectrometric Identification of Autoantigens of Human Gastric Antrectomy Mucosal Tissue by a Pool of 300 Sera Obtained from *Helicobacter Pylori*-Seropositive Patients

Spot No.	Coverage (%)	Protein MW (Da)/PI	Protein name	Accession No.
22	31.4	85426/7.4	Aconitate hydratase, mitochondrial	Q99798
26, 27	28.7	72334/5.1	78 kDa glucose-regulated protein	P11021
28, 29	16.9	70899/5.4	Heat shock cognate 71 kDa protein	P11142
30, 31	28.2	70053/5.5	Heat shock 70 kDa protein 1	P08107
32-35	27.4	69367/5.9	Serum albumin	P02768
42	26.6	66194/6.2	WD repeat-containing protein 1	O75083
50	24.2	54151/7.6	Dihydrolipoyl dehydrogenase, mitochondrial	P09622
53	30	61055/5.7	60 kDa heat shock protein, mitochondrial	P10809
57	38.6	56783/6.0	Protein disulfide-isomerase A3	P30101
60	27.8	52391/5.9	Selenium-binding protein 1	Q13228
63	27.1	54862/6.3	Retinal dehydrogenase 1	P00352
64	26.7	54862/6.3	Retinal dehydrogenase 1	P00352
65	39.9	54862/6.3	Retinal dehydrogenase 1	P00352
66	26.1	46737/5.4	Alpha-1-antitrypsin	P01009
67	27	52964/5.4	Vitamin D-binding protein	P02774
68, 69	41.6	56560/5.3	ATP synthase subunit beta, mitochondrial	P06576
73	27.3	47372/5.6	Actin-related protein 3	P61158
79	35.5	50664/6.1	Rab GDP dissociation inhibitor beta	P50395
86	17.1	45238/6.8	Gastric triacylglycerol lipase	P07098
87	40.8	47169/7.0	Alpha-enolase	P06733
88	17.1	45238/6.8	Gastric triacylglycerol lipase	P07098
89	11.3	47169/7.0	Alpha-enolase	P06733
90	21.9	45238/6.8	Gastric triacylglycerol lipase	P07098
99, 100	55.7	41737/5.3	Actin, cytoplasmic 1	P60709
101, 102	51.7	42645/5.3	Creatine kinase B-type	P12277
108	21.9	42742/5.9	Leukocyte elastase inhibitor	P30740
116	31.4	36573/6.3	Alcohol dehydrogenase [NADP+]	P14550
121	24.7	38282/6.3	UDP-glucose 4-epimerase	Q14376
122	21	36815/6.5	Fructose-1,6-bisphosphatase 1	P09467
123	39.3	37207/6.7	Aflatoxin B1 aldehyde reductase member 3	O95154
133	40.1	36639/5.7	L-lactate dehydrogenase B chain	P07195
138	44.3	36376/5.6	Annexin A3	P12429
139	49.8	35883/5.8	Annexin A4	P09525
145	28.4	28870/6.6	Carbonic anhydrase 1	P00915
147	41.9	29246/6.9	Carbonic anhydrase 2	P00918
151	47.6	26560/5.4	Glutathione S-transferase Mu 3	P21266
152	41.1	25035/6.0	Peroxiredoxin-6	P30041
155	40	23356/5.4	Glutathione S-transferase P	P09211
156	32.4	22346/5.9	Abhydrolase domain-containing protein 14B	Q96IU4
163	23.8	20331/5.6	Gastrokine-1	Q9NS71
165	23.8	20331/5.6	Gastrokine-1	Q9NS71
166	42.4	21892/5.7	Peroxiredoxin-2	P32119
173	55.1	15999/6.7	Hemoglobin subunit beta	P68871
177	31.1	36426/6.9	Malate dehydrogenase, cytoplasmic	P40925

MW, molecular weight; PI, isoelectric point; WD, Tryptophan-Aspartic acid; ATP, adenosine triphosphate; GDP, Guanosine Diphophate; UDP, Uridine Diphosphate.

RESULTS

Forty-four autogantigenic protein spots were identified from gastric mucosa by 2-DE and immunoblotting (Fig. 1, Table 1). Of these, retinal dehydrogenase 1, gastric lipase, gastrokine-1, and alpha enolase had several overlapping spots. Thirty-eight novel proteins were identified by immunoblotting: ahydrolase domain-containing protein 14B, actin-related protein 3, aflatoxin B1 aldehyde reductase member 3, alcohol dehydrogenase [NADP+], alpha enolase, alpha-1-antitrypsin, annexin A3, annexin A4, carbonic anhydrase 1, carbonic anhydrase 2, creatine kinase B-type, cytoplasmic actin 1, cytoplasmic malate dehydrogenase, fructose-1,6-bisphosphatase 1, gastrokine-1, gastric triacylglycerol lipase, glutathione S-transferase Mu 3, glutathione S-transferase P, heat shock cognate 71 kDa protein, heat shock 70 kDa protein 1, hemoglobin subunit beta, leukocyte elastase inhibitor, L-lactate dehydrogenase B chain, mitochondrial aconitate hydratase, mitochondrial ATP synthase subunit beta, mitochondrial dihydrolipoyl dehydrogenase, mitochondrial 60 kDa heat shock protein, peroxiredoxin-2, peroxiredoxin-6, protein disulfide-isomerase A3, Rab GDP dissociation inhibitor beta, retinal dehydrogenase 1, selenium-binding protein 1, serum albumin, 78 kDa glucose-regulated protein, Uridine Diphophate (UDP)-glucose 4-epimerase, vitamin D-binding protein, and Tryptophan-Aspartic acid (WD) repeat-containing protein 1.

From AGS cells, 19 autoantigenic protein spots were present in 2-D immunoblotting. Three of them (actin cytoplasmic 1, T-complex protein 1 subunit gamma, and album) had several spots. From AGS cells, 14 unique antigens were identified: alpha-enolase, annexin A3, cytoplasmic actin 1, cytoplasmic isocitrate dehydrogenase [NADP], heat shock cognate 71 kDa protein, leukocyte elastase inhibitor, mitochondrial glycine amidinotransferase, programmed cell death 6-interacting protein, serum albumin, T-complex protein 1 subunit gamma, T-complex protein 1 subunit alpha, tyrosine-protein phosphatase non-receptor type 11, T-complex protein 1 subunit theta and T-complex protein 1 subunit epsilon (Fig. 2, Table 2). Of these, tyrosine-protein phosphatase non-receptor type 11, T-complex protein 1 subunit alpha, T-complex protein 1 subunit gamma, T-complex protein 1 subunit theta, T-complex protein 1 subunit epsilon, cytoplasmic NADP, mitochondrial glycine amidinotransferase, and programmed cell death 6-interacting protein were noted only in gels of AGS cells.

Heat shock cognate 71 kDa protein, cytoplasmic actin 1, alpha-enolase, annexin A3, albumin, and leukocyte elastase inhibitor were present in both gastric antral mucosa tissue and AGS cells (Fig. 3).

Amino acid identity and positivity between autoantigens derived from gastric mucosa and AGS cells and proteins derived from *H. pylori* were identified. Thirty-seven proteins had some identity and positivity of amino acid sequences (Table 3). Of these proteins, mitochondrial ATP synthase subunit beta (*H. pylori* 26695; F0F1 ATP synthase subunit beta), WD repeat-containing protein 1 (DNA-directed RNA polymerase subunit beta), mitochondrial 60 kDa heat shock protein (chaperonin GroEL), tyrosine-protein phosphatase

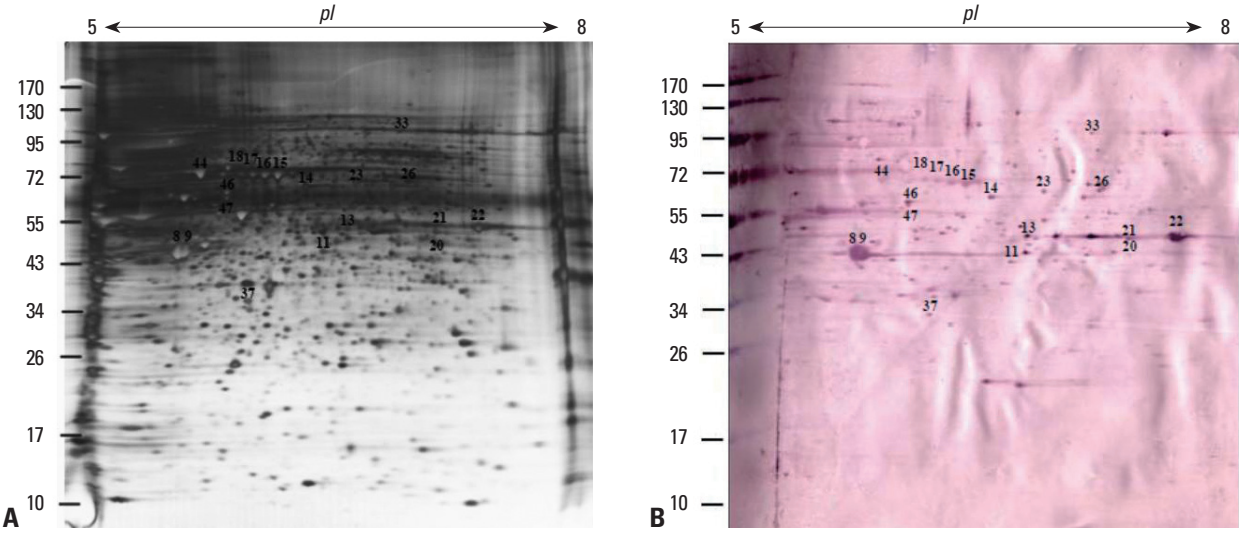

Fig. 2. Autoantigenic proteins visualized by 2-D immunoblotting derived from AGS cells. (A) 2-DE of total protein extracts from AGS cells. (B) 2-D immunoblot of total proteins from AGS cells by a pool of 300 sera obtained from *Helicobacter pylori*-seropositive patients. 2-DE, two-dimensional-PAGE; AGS, gastric adenocarcinoma cell lines derived from a Caucasian patient who had received no prior therapy.

Table 2. Mass Spectrometric Identification of Autoantigenic Proteins of AGS by a Pool of 300 Sera Obtained from *Helicobacter Pylori*-Seropositive Patients

Spot No.	Coverage (%)	Protein MW (Da)/PI	Protein name	Accession No.
8	30.7	41737/5.3	Actin, cytoplasmic 1	P60709
9	30.7	41737/5.3	Actin, cytoplasmic 1	P60709
11	21.4	42742/5.9	Leukocyte elastase inhibitor	P30740
13	27	60534/6.1	T-complex protein 1 subunit gamma	P49368
14	22.7	60344/5.8	T-complex protein 1 subunit alpha	P17987
15	13.6	69367/5.9	Serum albumin	P02768
16	13.6	69367/5.9	Serum albumin	P02768
17	13.6	69367/5.9	Serum albumin	P02768
18	13.6	69367/5.9	Serum albumin	P02768
20	19.8	46660/6.5	Isocitrate dehydrogenase [NADP] cytoplasmic	O75874
21	21.5	48298/8.0	Glycine amidinotransferase, mitochondrial	P50440
22	34.3	47169/7.0	Alpha-enolase	P06733
23	28.3	60534/6.1	T-complex protein 1 subunit gamma	P49368
26	19.3	68461/6.9	Tyrosine-protein phosphatase non-receptor type 11	Q06124
33	18.8	96024/6.1	Programmed cell death 6-interacting protein	Q8WUM4
37	38.1	36376/5.6	Annexin A3	P12429
44	16.4	70872/5.4	Heat shock cognate 71 kDa protein	P11142
46	20.8	59621/5.4	T-complex protein 1 subunit theta	P50990
47	27.2	59672/5.5	T-complex protein 1 subunit epsilon	P48643

MW, molecular weight; PI, isoelectric point; NADP, Nicotinamide Adenine Dinucleotide Phosphate.

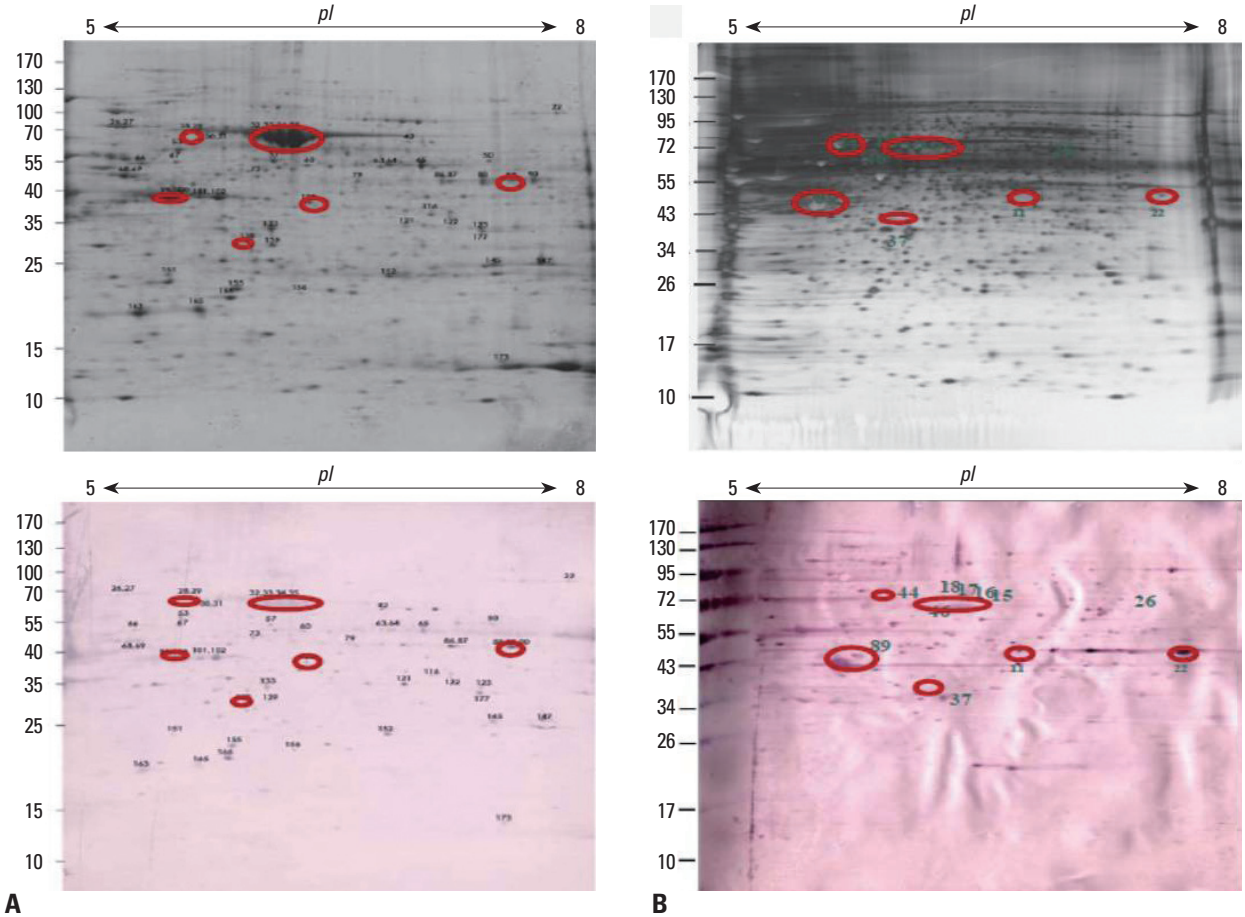

Fig. 3. Common autoantigenic proteins are indicated with red circles on 2-DE silver staining and immunoblotting of gastric mucosal tissue (A) and AGS cells (B). 2-DE, two-dimensional-PAGE.

Table 3. Amino Acid Sequences Homologies between Autoantigenic Proteins of Human Gastric Mucosal Tissue and AGS Cells and Proteins of *Helicobacter Pylori*

Homo sapiens		*Helicobcter pylori* 26695 (J99)		Amino acid identiy (%)	
Protein name	Gene ID	Protein name	Hp ID	Identity	Positivity
ATP synthase subunit beta, mitochondrial	506	F0F1 ATP synthase subunit beta	HP1132	68	80
WD repeat-containing protein 1	9948	DNA-directed RNA polymerase subunit beta	HP1198	48	76
60 kDa heat shock protein, mitochondrial	3329	Chaperonin GroEL	HP0010	53	73
Tyrosine-protein phosphatase non-receptor type 11	5781	Glutamine synthetase (J99)	JHP0461	53	73
78 kDa glucose-regulated protein	3309	Molecular chaperone DnaK	HP0109	53	71
Heat shock cognate 71 kDa protein*	3312	Molecular chaperone DnaK	HP0109	48	68
Heat shock 70 kDa protein 1	3303	Molecular chaperone DnaK	HP0109	48	67
Peroxiredoxin-2	7001	Alkyl hydroperoxide reductase (tsaA)	HP0136	48	66
Alpha-enolase*	2023	Phosphopyruvate hydratase	HP0154	46	65
Glutathione S-transferase P	2950	Putative type II DNA modification enzyme	JHP0045	32	65
Vitamin D-binding protein precursor	2638	Biopolymer transport protein (exbD)	HP1446	41	59
Programmed cell death 6-interacting protein	10015	Hypothetical protein	HP0460	39	57
Glutathione S-transferase Mu 3	2947	Leucyl-tRNA synthetase	JHP1452	41	56
Peroxiredoxin-6	9588	Bacterioferritin comigratory protein (bcp)	HP0136	33	53
Leukocyte elastase inhibitor	1992	Iron-regulated outer membrane protein	HP0876	32	52
Annexin A4	307	Dihydrodipicolinate synthase (J99)	JHP0410	33	51
Actin-related protein 3	10096	VirB4 homolog (virB4)	HP0017	32	50
UDP-glucose 4-epimerase	2582	UDP-glucose 4-epimerase	HP0360	30	49
Actin, cytoplasmic 1*	60	Flagellar assembly protein H	HP0353	28	49
T-complex protein 1 subunit gamma	7203	Chaperonin GroEL	HP0010	25	48
Malate dehydrogenase, cytoplasmic	4190	Cytochrome c oxidase, diheme subunit, membrane-bound (fixP)	HP0147	35	47
Protein disulfide-isomerase A3 precursor	2923	Thioredoxin	HP1458	29	47
T-complex protein 1 subunit epsilon	22948	Chaperonin GroEL	HP0010	27	47
Retinal dehydrogenase 1	216	Delta-1-pyrroline-5-carboxylate dehydrogenase	HP0056	25	46
Serum albumin*	213	D-amino acid dehydrogenase (dadA)	HP0943	27	44
Fructose-1,6-bisphosphatase 1	2203	Fructose-1,6-bisphosphatase	HP1385	29	43
Carbonic anhydrase 2	760	Carbonic anhydrase (J99)	JHP1112	28	42
Annexin A3*	306	Hypothetical protein (J99)	JHP1425	19	42
Glycine amidinotransferase, mitochondrial	2628	Vacuolating cytotoxin	HP0887	31	41
T-complex protein 1 subunit alpha	6950	Chaperonin GroEL	HP0010	25	41
T-complex protein 1 subunit theta	10694	Chaperonin GroEL	HP0010	22	41
Creatine kinase B-type	1152	Dipeptide ABC transporter, periplasmic dipeptide-binding protein (dppA)	HP0298	24	40
Isocitrate dehydrogenase [NADP] cytoplasmic	3417	Isocitrate dehydrogenase (icd)	HP0027	20	40
Aflatoxin B1 aldehyde reductase member 3	22977	Aldo-keto reductase, putative	HP1193	26	39
Aconitate hydratase	50	Bifunctional aconitate hydratase 2/2-methylisocitrate	HP0779	23	39
Alcohol dehydrogenase [NADP+]	10327	Aldo-keto reductase, putative	HP1193	22	39
Carbonic anhydrase 1	759	Carbonic anhydrase	HP1186	22	39

ATP, Adenosine Triphosphate; WD, Tryptophan-Aspartic acid; UDP, Uridine Diphosphate; NADP, Nicotinamide Adenine Dinucleotide Phosphate.
*Proteins identified in both human mucosal tissue and AGS cells.

non-receptor type 11 (glutamine synthetase; J99), 78 kDa glucose-regulated protein (molecular chaperone DnaK), heat shock cognate 71 kDa protein (molecular chaperone DnaK), heat shock 70 kDa protein 1 (molecular chaperone DnaK), peroxiredoxin-2 (alkyl hydroperoxide reductase), alpha-enolase (phosphopyruvate hydratase), and glutathione S-transferase P (putative type II DNA modification enzyme) had over 60% amino acid positivity, compared with amino acid sequences of proteins from *H. pylori*. Amino acid sequence of mitochontrial ATP synthase subunit beta had the highest identity (68%) and positivity (80%), compared with that of F0F1 ATP synthase subunit beta from *H. pylori* 26695.

Ten common antigens derived from both gastric mucosa and AGS cells had amino acid identity and positivity with proteins of *H. pylori*. Gastric lipase, gastrokine-1, selenium-binding protein 1, alpha-1-antitrypsin precursor, Rab GDP dissociation inhibitor beta, L-lactate dehydrogenase B chain, abhydrolase domain-containing protein 14B, and hemoglobin subunit beta had no amino acid identity or positivity from proteins of *H. pylori*.

DISCUSSION

This study examined the involvement of autoimmune antigenic proteins in the course of gastric mucosal changes from normal gastric epithelium to intestinal metaplasia through atrophic gastritis after *H. pylori* infection using 2-DE and immunoblotting. 2-DE is a useful tool to detect disease-specific proteins and to analyze the detected proteins via mass spectrometry. Auto-antibodies in autoimmune diseases are commonly used in clinical practice as biomarkers, however, novel autoantigens that have been or would be found via 2-D immunoblotting could have a potential to be used in clinical practice as diagnostic biomarkers and therapeutic tools.

Autoimmune diseases associated with *H. pylori* infection have been reported globally. Even though autoantigens have low or no homogeneity with *H. pylori* proteins, these studies have clarified that they can induce autoimmune reactions if they have similar conformational epitope with *H. pylori*. Ko, et al.[19] reported that 25 (35.2%) of 71 monoclonal antibodies made by cleaved whole cells of *H. pylori* cross reacted with gastric epithelial cells in a patient with *H. pylori* infection, 23 (32.5%) with fetal gastric epithelial cells, 15 (21.1%) with smooth muscle cells, 11 (15.5%) with renal tubular cells, 11 (15.5%) with ductal cells in salivary glands, eight (11.3%) with duodenal epithelial cells, five (7.0%) with inflammatory cells, three (4.2%) with follicular cells in a thyroid gland, and one (1.4%) with colonic epithelial cells. As the aforementioned monoclonal antibodies could react immunologically with fetal gastric epithelial cells that have never been exposed to *H. pylori* and diverse human cells, *H. pylori* might harbor antigens capable of cross-reaction with various human tissue antigens. The results suggest that an autoimmune reaction might be involved in the pathogenesis of not only *H. pylori*-associated gastrointestinal disorders, but also extra-intestinal autoimmune disorders. Autoantigens reported to be involved in diverse autoimmune disorders associated with *H. pylori* infection include carbonic anhydrase II similar alpha carbonic anhydrase of *H. pylori* in autoimmune pancreatitis,[20] anti-CagA antibody, anti-HSP 65 antibody, and anti-HSP 60 antibody in artherosclerosis,[21-23] anti-CagA antibody in Graves disease,[24] anti-platelet glycoprotein antibody in immune thrombocytopenic purpura,[25] proteins from endothelial or smooth muscle cells against CagA in hypertension,[26] and HSP 60 of *H. pylori* associated with Sjogren syndrome.[27] A healthy human body has immune regulatory systems including T_{reg} cells. However, prolonged stimuli to Th1 cells and individual genetic factors can provoke autoimmune reactions.

In 1989, Negrini, et al.[28] reported cross reactions between 15 of 21 monoclonal antibodies against *H. pylori* and different immunohistochemical stains of foveolar cells, pyloric gland, red blood cells, or white blood cells against 21 monoclonal antibodies. They subsequently reported the cross-reaction of gastric epithelial cells with monoclonal antibodies in 84% of 82 patients with *H. pylori* infection,[29] and strong cross-reaction of anti-Lewis monoclonal antibody from the lipopolysaccharide of *H. pylori* with human and mouse gastric mucosa.[30] Uibo, et al.[31] also reported the presence of homologous amino acid sequences (72% in 25 amino acid overlap) between H^+,K^+-ATPase in parietal cells and the urease B subunit of *H. pylori*. Ma, et al.[32] reported that serum auto-antibodies in a patient with type A gastritis could react with H^+,K^+-ATPase of swine and *H. pylori*. These reports suggest that anti-H^+,K^+-ATPase antibodies produced after *H. pylori* infection could react with parietal cells. However, we could not find H^+,K^+-ATPase in autoantigenic protein profiles. This might be due to the loss of proton pump proteins during protein extraction process or due to intrinsic problems in our antrectomy specimen in which most parietal cells are intact and most epithelial cells are mucus secreting ones.

We also used AGS cells to discover autoantigens against

gastric cells. Although cancer-related antigens are associated with cancer cells, they are also present as autoantigens from normal gastric cells. This was the reason why 2-DE was performed using AGS cells. Because AGS cells are from a cancer cell line, the expected autoantigens were cancer/testis antigen like MAGE-3, differentiation antigen like gp100, tumor specific antigen like mutated p53, mutated p21/ras, overexpressed self antigen like HPV E6/E7, viral antigen like hepatitis C virus, and oncofetal antigen like carcin-embryonic antigen and alpha fetoprotein.[33] Clinically, autoantigens in cancer patients are hardly used for diagnosis and severity assessment of their disease, because the autoantigens associated with cancers are also expressed in healthy adults. As an example, one study documented that alpha-enolase or heterogeneous nuclear ribonucleprotein I was expressed in more than 50% of healthy Chinese and annexin II, F-actin capping protein beta subunit and calreticulin were also expressed in more than 20% of healthy Chinese.[34] The present detection of alpha-enolase, heat shock 70 kDa protein, annexin II, and preoxiredoxin 6 corroborates the results of the previous study.

We compared the amino acid sequences of the novel gastric mucosal tissue proteins and AGS cells with those of *H. pylori* to estimate molecular mimicry between human tissue and *H. pylori* proteins. Homology in amino acid sequences of mitochondrial ATP synthase subunit beta, WD repeat-containing protein 1, mitochondrial 60 kDa heat shock protein, tyrosine-protein phosphatase non-receptor type 11, 78 kDa glucose-regulated protein precursor, heat shock cognate 71 kDa protein, heat shock 70 kDa protein 1, peroxiredoxin-2, alpha-enolase, and glutathione S-transferase P was identified.

If these 10 homologous proteins are similar to the conformational epitope of *H. pylori*, they might cause gastric mucosal atrophy through an autoimmune mechanism, such as H^+,K^+-ATPase. However, the amino acid sequence homology differed from the conformational epitope. The approach was limited by the failure to detect H^+,K^+-ATPase even after three experiments using gastric mucosal tissue. Therefore, we cannot be sure whether our results are restricted to only human gastric mucosal proteins. The present findings implicate gastric lipase and gastrokine 1 as gastric cell-specific autoantigens. Chief cells on fundus secrets gastric lipases, known as acidic lipasese, which have their optimal pH of 5 and control approximately 25% of lipid hydrolysis in adults.[35] Further studies are needed to determine whether the autoimmune reaction against gastric lipase is related with the destruction of chief cells on the gastric fundus. Gastrokine 1 is abundant in normal gastric cells, however, it is decreased in *H. pylori*-infected gastric cells, and the decrease is associated with the delayed recovery of damaged gastric cells.[36] Further studies are also warranted as to whether autoimmune reaction against gastrokine 1 is related with the destruction of gastric cells. The absence of homology between gastric lipase, gastrokine 1, and any protein of *H. pylori* indicates that an autoimmune reaction against gastric lipase and gastrokine 1 might not be directly related to *H. pylori* infection. However, this speculation is tentative, since the conformational epitopes of the proteins differ from homology of amino acid sequences.

An acknowledged limitation was that our study was an expansive autoantigenic study. Gastric mucosal tissue harbors diversely heterogeneous cells, such as smooth muscle cells, vessels, nerves, and inflammatory cells, besides gastric diverse epithelial cell lines. It is also possible that the results of AGS cells reflect the cancer-specific proteins more importantly presented during carcinogenesis and in cancer cells instead of normal gastric epithelial cell specific antigens. Some autoantbodies against antigenic proteins which we found might have not been involved in *H. pylori*-derived gastric mucosal alteration, but frequently identified in the healthy population because we used the pooled sera obtained from patients with various disease backgrounds.[34] Aforementioned limits should be considered when interpreting our results as autoantigens.

Despite the limitations, Heat shock congnate 70 kDa protein, cytoplasmic actin 1, annexin A3, albumin, alpha-enoase, and leukocyte elastase were detected in both gastric mucosal tissue and AGS cells. Mitochondrial ATP synthase subunit beta, WD repeat-containing protein 1, mitochondrial 60 kDa heat shock protein, tyrosine-protein phosphatase non-receptor type 11, 78 kDa glucose-regulated protein precursor, heat shock cognate 71 kDa protein, heat shock 70 kDa protein 1, peroxiredoxin-2, alpha-enolase, and glutathione S-transferase P showed homology in amino acid sequences with proteins derived from *H. pylori*.

In summary, we tentatively suggest that newly identified gastric proteins from gastric mucosal tissue and AGS cells might provide tools of control and prevention of gastrointestinal disorders associated with *H. pylori* infection. These autoantigens might break the vicious cycle in gastroduodenal disorders if their pathophysiological roles in the progress of chronic atrophic gastritis, gastroduodenal ulcers, intestinal metaplasia, and gastric cancer could clearly be understood.

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