Biased usage of synovial immunoglobulin heavy chain variable region 4 by the anti-glucose-6-phosphate isomerase antibody in patients with rheumatoid arthritis

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Abstract. Rheumatoid arthritis (RA) is the most common inflammatory arthritis, characterized by marked infiltration of mononuclear cells including B cells into the inflamed synovium. Anti-glucose-6-phosphate isomerase (GPI) antibody (Ab) is an arthritogenic Ab in K/BxN T cell receptor transgenic mice, and is also present in some patients with RA. To characterize synovial B cells from anti-GPI Ab-positive RA, synovial immunoglobulin (Ig) heavy chain variable regions (VH) were compared with those of negative individuals. Synovial tissues were obtained from six RA patients (three anti-GPI Ab-positive and three anti-GPI Ab-negative). Ig-VH genes were amplified by PCR using family-specific primers and were subsequently sequenced. In synovial B cells from anti-GPI Ab-positive RA patients, VH4 and JH4 were predominantly expressed ($p<0.0001$). The immunoglobulin heavy chain complementarity-determining region 3 (IgH-CDR3) length in the synovium of anti-GPI Ab-positive individuals was shorter than that in anti-GPI Ab-negative individuals ($p=0.0005$). In addition, the IgH-CDR3 of anti-GPI Ab-positive patients was rich in basic-ionized amino acids (arginine, histidine, and lysine) near their central position, suggesting a high affinity. Our results support the notion that Ig-VH4 B cells in RA synovium with anti-GPI Ab are affinity-matured and that anti-GPI Ab might be associated with the skewed IgH-CDR3.

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Abbreviations: -, negative; +, positive; DH, heavy chain diversity regions; FR, framework region; GPI, glucose-6-phosphate isomerase; IgH-CDR3, immunoglobulin heavy chain complementarity determining region 3; JH, heavy chain joining regions; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; VH, heavy chain variable regions

Key words: autoantibodies, rheumatoid arthritis, glucose-6-phosphate isomerase, B cells, synovium

Introduction

Rheumatoid arthritis (RA) is an inflammatory condition characterized by systemic polyarthritis with bone erosion that affects the peripheral joints. The etiology of RA remains unclear with immunological processes including T cell-B cell interactions, innate immunity, and cytokine activity being implicated (1). In RA, the synovium contains many infiltrating mononuclear cells including B cells at various developmental stages, T cells, and macrophages (2).

Treatments for RA include agents that target cytokines such as tumor necrosis factor. These inflammation-neutralizing approaches have achieved good results in reducing not only joint inflammation but also bone erosion. More recently, depletion of B cells from RA patients has also produced significant therapeutic benefits in several clinical trials (3,4). B cells are thought to be crucial in the pathogenesis of RA, through the production of autoantibodies, antigen presentation, cytokine secretion, and costimulatory signaling. In fact, autoantibodies including rheumatoid factor and anti-cyclic citrullinated peptide antibody (Ab) have been used as diagnostic markers of RA. However, most such autoantibodies are not pathogenic. In contrast, anti-glucose-6-phosphate isomerase (GPI) Ab is a candidate arthritogenic Ab, identified using the K/BxN arthritis model (5-7). In this model, disease development was initiated by the activation of B and T cells. In addition, B cell-deficient mice do not develop arthritis (8). Anti-GPI Ab is also detected in some RA patients, with the reported prevalence varying from 5% to 64% of RA patients (9-12). This Ab is associated with extra-articular manifestations, and its titer correlates with the disease activity (10,13). Moreover, anti-GPI Ab is also detected in the inflamed synovium of RA (14-16). Thus, B cells and autoantibodies appear to play important roles in the pathogenesis of RA in anti-GPI Ab-positive patients, especially in the inflamed joint synovium.

Immunoglobulin molecules are composed of two heavy chains and two light chains, and are characterized by the

Table I. Profile of participating patients (RA1-RA6).

	Age (years)	Sex	Disease duration (years)	Anti-GPI Ab (OD 405 nm)		RF (IU/ml)	CRP (mg/dl)	MMP-3 (ng/ml)
				Human	Rabbit			
RA1	66	F	20	1.78	3.14	156	0.57	363
RA2	70	F	25	2.60	3.47	149	0.42	275
RA3	69	F	16	2.43	2.55	516	3.55	295
RA4	64	F	33	0.72	0.20	78	0.05	ND
RA5	72	F	22	0.62	0.05	119	0.74	215
RA6	74	F	20	0.40	0.36	5	0.56	256

All synovia were from the knees of female patients with rheumatoid arthritis (RA). The cutoff OD was calculated from ELISA reaction of 145 healthy Japanese donors, the mean value + two standard deviation was 1.32 to human recombinant GPI, and 0.94 to rabbit native GPI. Double-positive populations were considered anti-GPI Ab-positive. RA1-3 were anti-glucose-6-phosphate isomerase (GPI) Ab (+), and RA4-6 were anti-GPI Ab (-). Apart from anti-GPI Ab, all other parameters were matched to the utmost extent. GPI, glucose-6-phosphate isomerase; RF, rheumatic factor; CRP, C-reactive protein; MMP-3, matrix metalloproteinase-3; ND, not done.

antigen-binding site sequence and Fc isotype. The antigen-binding site is made up of variable regions and rearranged complementarity-determining regions (CDR) that determine the individual immune properties of any given B cell. The immunoglobulin heavy chain CDR3 (IgH-CDR3) is the most crucial site for antigen binding. H-CDR3 is rearranged by one of 44 variable segments (VH), one or more of 25 diversity segments (DH), and one of six joining segments (JH) (17). In addition, VH genes can be divided into seven sub-families (VH1 to VH7), with overrepresentation of VH4 genes reported in some autoimmune conditions (18). Negative selection of VH4 repertoires is implemented in healthy individuals to avoid autoimmunity (19,20). On the other hand, in RA patients, synovial B cells, especially plasma cells, are also biased to express the VH4 repertoire (21,22), though this was negated in another report (23), and antigen-driven affinity maturation has been reported (23-26).

The present study defined the synovial B cell characteristics of anti-GPI Ab-positive (+) RA patients by analyzing VH regions of synovial B cells from anti-GPI Ab (+) and negative (-) RA patients and compared the rearranged IgH-CDR3 sequences of their VH4 genes. Twenty-seven IgH-VH4 gene sequences from anti-GPI Ab (+) RA patients were compared with thirty-six VH4 gene sequences from anti-GPI Ab (-) patients. In both groups, over 70% of VH4 clones seemed to be undergoing antigen-driven maturation, as evidenced by an R/S ratio of >3 in the CDR and less in the framework region (FR). However, the JH4 gene was more predominant in the synovium of anti-GPI Ab (+) RA patients compared to anti-GPI Ab (-) cases and the lengths of their IgH-CDRs were shorter. In addition, there was no biased usage of VH4 subfamily genes. Together, these findings suggest that B cells from anti-GPI Ab (+) RA synovium are affinity-matured by antigens, with frequent usage of VH4-JH4.

Table II. Specific primers for each VH family.

Internal	
VH1	5'-TCACCATGGACTGCACCTGGA-3'
VH2	5'-CCATGGACACACTTTGCTCCAC-3'
VH3	5'-TCACCATGGAGTTTGGGCTGAGC-3'
VH4	5'-AGAACATGAAACACCTGTGGTTCTT-3'
VH5	5'-ATGGGGTCAACCGCCATCCT-3'
VH6	5'-ACAATGTCTGTCTCCTTCCTCAT-3'
Cγ	5'-CATCGGTCTTCCCCCTGGC-3'
External	
VH1	5'-GAGAAAACCCTGTGAGCACAGCT-3'
VH2	5'-AGTGACTCCTGTGCCCCAC-3'
VH3	5'-GATCAGCACTGAACACAGAGGAC-3'
VH4	5'-GTCATGGACCTCCTGCACAAG-3'
VH5	5'-AGGGCTTCATTTTCTGTCCTCCAC-3'
VH6	5'-GGGGCAGTCACCAGAGCTC-3'
Cγ	5'-GAGCACCTCCGAGAGCACA-3'

Sequences of primers used in nested PCR to detect immunoglobulin heavy chain variable segment (VH) 1-6 family genes.

Materials and methods

Subjects. Synovial tissues were obtained from six patients [three were anti-GPI Ab (+) and three were anti-GPI Ab (-)] who satisfied the American College of Rheumatology criteria for RA (1987) (27) (Table I). For selecting anti-GPI Ab-positive patients, enzyme linked immunosorbent assay (ELISA) was performed using two different sources of GPI; a recombinant human GPI (huGPI), and a rabbit muscle GPI (raGPI) (Sigma Chemical Co., St. Louis, MO) which had been described in detail previously (12). Informed consent for using synovial tissues and blood sampling was obtained from all patients at the time of the relevant procedure.

cDNA synthesis. Synovial tissues were minced and homogenized in Isogen (Nippon Gene, Tokyo, Japan) and extracted with chloroform. RNA was precipitated with isopropanol,

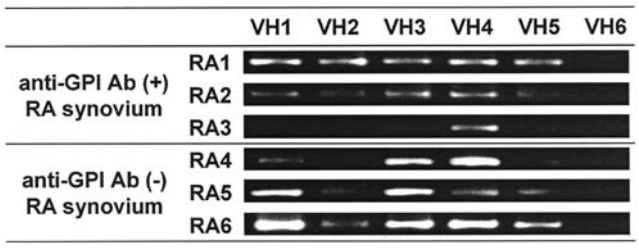

Figure 1. Nested PCR for immunoglobulin heavy chain variable segment (VH) subfamily of rheumatoid synovium. Amplification of VH genes by nested PCR was conducted as shown. Only VH4 genes were amplified in all individuals. VH1 to VH5 were amplified in all cases except RA3.

resuspended in 10 mM Tris·HCl (pH 8.0) and 1 mM EDTA. RevertAid first-strand cDNA synthesis kits (Fermentas, Ontario, Canada) were used for reverse transcription, in accordance with the manufacturer's instructions. Total RNA (5 μg) was used for this reaction.

Amplification of VH genes. Rearranged immunoglobulin VH genes were amplified by PCR. Nested PCR was conducted to obtain sufficient PCR product for electrophoresis. To avoid sequence errors, the first PCR products were subjected to sequencing. Specific oligonucleotides for six different VH families (VH1 to VH6) were used as 5'-primers. Oligonucleotide corresponding to the known Cγ was used as a 3'-primer. For the second PCR, an additional set of primers using the internal sequences was prepared (Table II). One microliter of template was added to 24 μl of a PCR master mix, containing 1.25 U rTaq DNA polymerase (Takara Bio Inc, Shiga, Japan), 2.5 μl of manufacturer's 10X PCR buffer, 2 μl of 2.5 mM dNTPs, and 2.5 μl of each 10-μM primer. The first round of PCR was performed for 25 cycles at 95˚C for 30 sec to denature, 54 or 56˚C for 30 sec to anneal (annealing for VH1, VH2, VH3, VH5 at 56˚C, and for VH4, VH6 at 54˚C), 72˚C for 30 sec to elongate including a previous 5 min of heating at 95˚C to unfold the cDNA, and a final 7-min extension at 72˚C. The second amplification was performed using 1 μl of the first PCR products as templates and the same method as for the first amplification.

Sequence analysis. Aliquots of the first PCR products were separated by electrophoresis using a 1.5% agarose gel, and DNA bands in the range of 400 bp were purified from the agarose gel using a MinElute gel extraction kit (Qiagen, Hilden, Germany). Purified PCR products were cloned into the TA cloning vector (Invitrogen, San Diego, CA). Randomly picked clones were screened for inserts of 400 bp. Positive clones were subjected to cycle sequencing using a BigDye terminator cycle sequence kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. The sequences were determined by capillary sequencer (Applied Biosystems 310 genetic analyzer). BioEdit (Ibis Therapeutics, Carlsbad, NM) was used for sequence comparison, and JoinSolver [National Institutes of Health, National Institute of Arthritis and Musculosketelal and Skin Diseases (NIAMS) and the Center for Information Technology (CIT)] (28) was used to identify putative Ig-VDJ germline sequences and to clarify the ratio of mutations leading to amino acid replacement to silent mutations (R/S ratio) in the CDR and FR. The IMGT database (Marie-Paule Lefranc, Montpellier, France) was used to confirm the putative Ig-VDJ germline sequences. EMBL Nucleotide Sequence Submissions (European Bioinformatics Institute, Wellcome Trust Genome Campus, Cambridge, UK), and GenBank (National Institutes of Health, Bethesda, Maryland) were also used.

Statistical analysis. The Mann-Whitney U test was used to compare the IgH-CDR3 length of VH between anti-GPI Ab (+) and (-) patients. The two-tailed Fisher exact test was used to determine significant differences in distributions of JH gene usage. Data are expressed as mean ± SD. A p value <0.05 was considered statistically significant.

Results

Amplification of VH family genes. Only VH4 genes were identified from all patients by nested PCR (Fig. 1). VH1 to VH5 genes were also identified except for RA3. In contrast, the amplification of VH6 genes was not sufficient for detection.

R/S ratio. After the first PCR, the VH4 gene products were purified and sequenced. Twenty-seven (17, 8, and 2, respectively) individually rearranged VH4 genes were characterized by sequence analysis in the synovium of anti-GPI Ab (+) RA patients (Table III), and 36 (15, 19, and 2, respectively) were identified in the synovium of anti-GPI Ab (-) individuals (Table III). There was no difference in R/S ratio in the CDR of both groups of VH4 clones [70% (19/27) in anti-GPI Ab (+) and 75% (27/36) in anti-GPI Ab (-)]; the R/S ratio in the CDR was >3, indicating antigen-driven maturation.

VH4 subfamily. Sequences were analyzed by using JoinSolver software to determine the implicated VDJ usage. In the synovium of anti-GPI Ab (+) RA patients, the most frequent VH4 subfamily gene was VH4-59 (12 products) followed by VH4-4 (5 products), VH4-39 (4 products), VH4-31 (3 products), VH4-61 (2 products), and finally, VH4-34 (1 product). In anti-GPI Ab (-) individuals, the dominant detected VH4 subfamily gene was VH4-59 (12 products) followed by VH4-39 (10 products), VH4-31 and VH4-61 (5 products each), and VH4-4 and VH4-34 (2 products each) (Fig. 2a). VH4-39 was therefore relatively less frequent in synovial B cells of anti-GPI Ab (+) RA patients, although the statistical significance was not clear.

JH region. In the synovium of anti-GPI Ab (+) RA patients, the most frequent JH gene was JH4 (19 products) followed by JH3 (3 products), JH6 (2 products), and JH1, JH2, and JH5 (1 product each). In anti-GPI Ab (-) individuals, the most frequent synovial JH gene was JH5 (10 products) followed by JH6 (9 products), JH3 and JH4 (7 products each), and JH2 (3 products) (Fig. 2b). Thus, JH4 usage in synovial B cells of anti-GPI Ab (+) RA patients was 70%, and showed a significantly higher frequency compared to 19% usage in anti-GPI Ab (-) individuals ($p<0.0001$).

IgH-CDR3 characteristics: amino acid composition and IgH-CDR3 length. There was no statistically significant difference

Table III. Synovial immunoglobulin VH4 repertoire of anti-GPI Ab (+) and (-) RA patients.

	VH	DH	H-CDR3	bp	JH	R/S ratio within CDR
Anti-GPI Ab (+) RA patients						
RA1	4-04	2-21	NMAGDVIGFFDY	14	4	2/0
	4-04	4-23	S**R**NPIDYPLGYFDY	16	4	2/1
	4-04	5-05	GYSYGLFDV	11	4	12/2
	4-04	6-25	Q**RHRR**GDFDI	12	3	2/0
	4-31	3-10	EEL**RR**I**R**GPFFDY	15	4	9/3
	4-34	6-06R	GEQDE**H**QVSS**R**FFFYYYIDV	22	6	9/2
	4-39	2-15	QGYCSGGTCQDFDY	16	4	3/0
	4-39	3-10	QGA**R**QWFGEFGAFDY	17	4	6/0
	4-59	3-09	LSPGGNFDFDL	13	4	6/1
	4-59	3-10	DGEGGSYYFDY	13	4	9/1
	4-59	3-10R	**H**NNTW**H**PFDY	12	4	8/1
	4-59	3-16R	LPP**R**GNY**R**LDS	13	4	5/1
	4-59	6-13	VPGFSSTWFEVDY	15	4	6/2
	4-59	6-13	FSGSFYGWFDP	13	5	7/0
	4-59	IR	VSTQTDY	9	4	5/1
	4-59	1-07	APPPWL**RR**VSTGTWL	17	2	5/3
	4-61	2-02R	GRQPDYYYAMDV	14	6	8/4
RA2	4-04	5-12R	SPDN**R**NTLDI	12	3	7/2
	4-31	3-10	GYYYGPGSY**H**PFET	16	4	3/1
	4-31	6-13	D**R**DAAAG**R**WVDY	14	4	3/0
	4-39	1-26	PVVGA**R**DPAPFDL	15	3	4/2
	4-59	3-03	**R**GGPTE**H**	9	1	4/1
	4-59	3-09	D**R**GQEYGIDS	12	4	4/2
	4-59	IR1R	LGQLGD**H**	9	4	12/2
	4-61	1-20	VSLLGY**KR**NDG**K**Y**H**FDY	19	4	6/2
RA3	4-39	3-10	YI**R**GV**R**SGGYFDY	15	4	4/2
	4-59	1-26	**H**GVDSGSFYAFDY	15	4	3/0
Anti-GPI Ab (-) RA patients						
RA4	4-31	3-10	D**H**GSGSSYFFSPNYGMDV	20	6	3/1
	4-34	5-12	GNSGNGYYFYNYMDV	17	6	11/3
	4-39	2/OR15-2R	FTITLF**R**G**K**EGNY	15	4	5/0
	4-39	3/OR15-3	QNGLQS**R**VDYFDF	15	4	7/1
	4-39	IRR	GGGVNLGSGAFYDE	16	4	18/7
	4-59	1-01	GGGFSSNWSLAPFAFDI	19	3	3/2
	4-59	2-15	DVDCVGGSCYSSDWFDP	19	5	5/1
	4-59	3-22	LWGSSGLYGENWFDP	17	5	5/1
	4-59	4/OR15-4	DVTSVQTTMVPAFDY	17	4	9/2
	4-59	5-05	DI**R**GYGYGYFDL	14	2	13/1
	4-59	6-19	DT**H**TAVPGDDYFES	16	4	7/3
	4-61	1-26	ESL**K**VGSTCFDP	14	5	9/3
	4-61	3-10	A**R**PDGSESFY**R**YLDL	17	2	4/1
	4-61	3-10	EQTGL**R**GQNM	12	3	7/2
	4-61	4-23	EGDYGGSYYYYYMDL	17	6	11/0
RA5	4-04	2-15	AGGGDCSGATCYSYYYGMDV	22	6	5/0
	4-31	2-21	GFGSSVIAMAYYFDY	17	4	3/1
	4-31	4-04	L**H**AE**R**ALGFWFDP	15	5	17/3
	4-31	4/OR15-4	VAPGAMPDDASEI	15	3	8/1
	4-34	3-09	MANLTGTPGLGI	14	3	7/2
	4-39	2/OR15-2R	DYITIFGVAPFDP	15	5	4/1

Table III. Continued.

	VH	DH	H-CDR3	bp	JH	R/S ratio within CDR
	4-39	3-03	**H**VNFEVVIGRWFD**H**	16	5	13/3
	V39	3-03	LGALFGADSYYGMDV	17	6	6/1
	V39	4-23	**K**DYADYEGFAY	13	5	6/0
	V39	5-12	YISATMEDF	11	3	11/2
	V39	6-13	DAGYSSS**RH**PVGFDP	17	5	8/3
	V39	6-19R/3-16	**H**A**R**IGA**H**YTYGSF**R**LFDAFDV	23	3	5/1
	V59	3-03	D**K**SGYYTPGGYYYYYGMDV	21	6	3/2
	V59	3-03	APYWSGYVYGLDV	15	6	7/1
	V59	3-10	ETYYSASGSYYSGQYYFEY	21	4	6/1
	V59	4/OR15-4	**H**GGLYPYYYFAMDV	16	6	5/0
	V59	6-19	**R**TDDYS**R**GWYWYFDP	17	2	6/1
	V59	6-19R	**H**AI**HR**FSTAFPNWFDP	18	5	3/2
	V61	4-17	DASLLYGDYVSWFDP	17	5	8/5
RA6	V04	1-14R	DP**R**TV**K**TMDV	12	6	6/4
	V59	3-22	GP**H**DTMTNYYGLNAFDI	19	3	7/4

The characters of immunoglobulin sequences using VH4 family genes are shown. Bold characters represent based-ionized amino acids (R, arginine; H, histidine; K, lysine). Underline indicates R/S ratio >3.

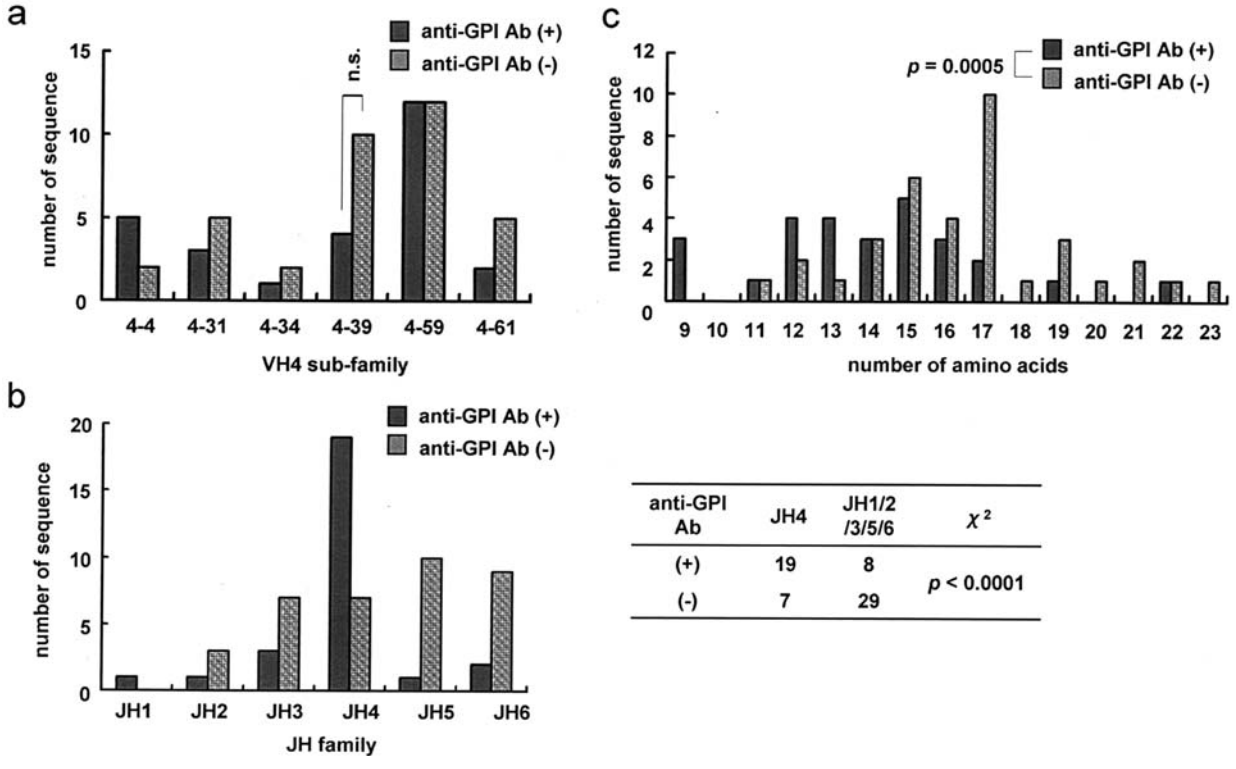

Figure 2. Comparison of synovial VH4 B cells from rheumatoid arthritis (RA) patients with or without anti-glucose-6-phosphate isomerase (GPI) Ab. (a) Usage of VH4 subfamily frequencies of VH4 gene usage in synovial B cells from RA patients are shown. In anti-GPI Ab (+) patients, VH4-59 (12 products), VH4-4 (5 products), VH4-39 (4 products), VH4-31 (3 products), VH4-61 (2 products), and VH4-34 (1 products) were identified. In anti-GPI Ab (-) patients, VH4-59 (12 products), VH4-39 (10 products), VH4-31 and VH4-61 (5 products each), and VH4-4 and VH4-34 (2 products each) were identified. VH4-39 showed a relatively low frequency in synovial B cells of anti-GPI Ab (+) RA patients, although the comparison is statistically not significant. (b) The usage of immunoglobulin heavy chain joining segment (JH) family. The frequencies of JH gene usage of synovial VH4 B cells from RA patients are shown. JH4 (19 products) was the most frequent gene used in anti-GPI Ab (+) RA patients, although this gene was not predominant in anti-GPI Ab (-) individuals ($p<0.0001$ by two-tailed Fisher exact test between JH4 and others). (c) The number of amino acids in the immunoglobulin heavy chain complementarity-determining region 3 (IgH-CDR3). The IgH-CDR3 lengths of VH4 B cells are shown. Lengths varied between 9 and 23 (mean, 15.46 ± 3.09) amino acids. In anti-GPI Ab (+) RA patients, the IgH-CDR3 lengths (14.00±2.96 amino acids) of synovial VH4 B cells were significantly shorter than those of anti-GPI Ab (-) individuals (16.56±2.75 amino acids) ($p=0.0005$ by Mann-Whitney's U test).

between the groups in terms of amino acids usage of IgH-CDR3, although this region in anti-GPI Ab (+) RA patients was rich in basic-ionized amino acids (arginine, histidine, and lysine) in their near central position, compared to the composition in anti-GPI Ab (-) individuals (Table III).

The IgH-CDR3 amino acid lengths varied from 9 to 23 amino acids (mean, 15.46±3.09 amino acids). In anti-GPI Ab (+) RA patients, the IgH-CDR3 length of synovial immunoglobulins using VH4 was significantly shorter than the length of those in anti-GPI Ab (-) individuals (p=0.0005, Fig. 2c). These findings suggest the prevalence of affinity-matured VH4 B cells in the synovium of anti-GPI Ab (+) RA patients.

Discussion

Anti-GPI Ab is frequently detected in patients with aggressive forms of RA (12,13), and its level correlates significantly with extra-articular manifestations such as rheumatoid nodules, rheumatoid vasculitis, and Felty's syndrome (10). We reported previously that serum IgG from anti-GPI Ab (+) RA patients preferentially attached to the articular surface of the meta-carpophalangeal joints of the monkey, inducing recruitment of granulocytes and mononuclear cells into the synovium (29). These results indicated that human serum immunoglobulins from RA patients include autoantibodies to specific protein(s) expressed in the joint cavity. Furthermore, human GPI protein is expressed on the cartilage and synovial surface in RA (7) and anti-GPI Ab is present in the synovial fluid, suggesting that the local production of such autoantibodies might be associated with arthritis. To address this hypothesis, we focused on the synovial B cells of anti-GPI Ab (+) patients.

In the present study, VH4 genes were detected in the synovium of all patients with RA. In some autoimmune diseases such as systemic lupus erythematosus (SLE) (30,31), VH4 genes are overrepresented in peripheral B cells (18), although negative selection of VH4 genes occurs in healthy individuals (19,20). These observations implicate VH4 genes as a self-reactive gene family. The frequency of VH4 genes in peripheral B cells from RA patients was not different from that of healthy individuals (32), however VH4 genes were highly expressed in the rheumatoid synovial B cells (21,22). In addition, antigen-driven immune maturation of B cells is characterized by an R/S ratio >3 within the CDR (33). Our study demonstrated that VH4 (+) synovial B cells in patients with RA are affinity-matured, because immunoglobulins with a high R/S ratio were dominant.

A skewed VH4 subfamily in RA synovium was not identified in this study, but when we compared anti-GPI Ab (+) with (-) patients, VH4 subfamily usages of synovial B cells from anti-GPI Ab (+) RA patients were less frequent for VH4-39. In peripheral blood of SLE, VH4-34 (V4.21) was overexpressed and correlated with some autoantibodies (30,31), but no specific subfamily repertoire has been identified in the synovium of RA patients. We do not know whether these skewed VH4 subfamilies are related to arthritogenicity, however, there are reports that some autoantibodies with VH4-34 (V4.21) segments are related to the pathogenicity of SLE (34,35). Since anti-GPI Ab is a candidate arthritogenic antibody, it would be interesting to identify the skewed VH4 subfamilies by increasing numbers of the sequence.

Our sequence analysis noted that synovial IgH-CDR3 from anti-GPI Ab (+) RA patients was enriched in basic-ionized amino acids. The ionized side-chains of arginine in the CDRs contribute to higher binding affinity for some antigens such as DNA, cardiolipin (36,37), and TAG72 (38). A previous study found that arginine in IgH-CDR3 of human and murine anti-dsDNA was most likely to be generated during V-D-J rearrangement in B cells, and the higher frequency of arginine in the IgH-CDR might similarly be due to the clonal expansion of B cells (38). In addition, the precise location of arginine is important for the binding (37).

IgH-CDR3 length and amino acid composition is the major contributor to antigen specificity and affinity (39-41). Matured immunoglobulins have shorter CDR3s than non-matured ones in both mice and humans (42,43). In the present anti-GPI Ab (+) RA patients, the CDR3 length of synovial immunoglobulins using VH4 was significantly shorter and the JH4 usage was significantly higher than those of anti-GPI Ab (-) individuals. These data support the notion that synovial B cells of anti-GPI Ab (+) patients are affinity-matured with higher affinity to a particular antigen.

In conclusion, our findings on synovial B cells in RA patients positive for anti-GPI Ab clearly demonstrated a high frequency of VH4-JH4 subfamily genes rich in basic amino acids and shorter CDR3 length, indicating affinity-matured B cells, reactive to autoantigens such as GPI. Future studies using anti-GPI Ab-producing B cell hybridomas should shed light on the functional role of anti-GPI Ab in the pathogenesis of RA.

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