NKT Cells in Human Autoimmune Diseases

Multiple Sclerosis

MS is an autoimmune demyelinating disease of the CNS. Illes Z *et al.* reported a reduction in Vα24Jα18 cells among $Vα24^+$ cells from the peripheral blood of patients with MS compared to healthy subjects using single-strand conformation polymorphism method to detect TCR gene rearrangements [43]. Van der Vliet *et al.* showed a decrease in the number of NKT cells by screening of $Vα24^+Vβ11^+$ cells in the blood [44]. Araki M *et al.* demonstrated that DN NKT cells in the periphery were greatly reduced in remission whereas the reduction of $CD4^+$ NKT cells was marginal [45]. Furthermore $CD4^+$ NKT line cells expanded from MS in remission produced a larger amount of IL-4 than those from healthy subjects or from MS in relapse. Therefore, we speculated that the Th2 bias of $CD4^+$ NKT cells may play a role in the regulation of Th1 type autoantigen reactive T cells. In contrast, Gausilng *et al.* did not find a significant difference in the number of DN $Vα24^+$ NKT cells in PBL between from MS patients and from healthy controls [46]. The basis for the discrepancy between the number of NKT cells among these studies is not clear. Considering that the proportion of Vα24Jα18 T cells in normal individuals varies among studies, it may not be easy to compare these studies.

Type I Diabetes

Studies of the frequency of human NKT cells in PBMCs in patients with T1D have had conflicting results. In initial studies, Wilson B *et al.* studied identical twin/triplet sets discordant for disease, and reported that diabetic siblings have lower frequencies of DN Vα24Jα18 T cells in their peripheral blood than non-diabetic siblings [47]. In addition, Kukreja AG *et al.* showed a reduction in the number of NKT cells in newly diagnosed patients [48]. However, more recent papers reported unaltered or increased NKT cells in recent-onset patients with type I diabetes [49,50]. Wilson B *et al.* also showed that DN Vα24Jα18 T cell clones isolated from diabetics had an impaired ability to produce IL-4 [47]. In contrast, Lee PT *et al.* reported IL-4 production by NKT cells was similar among these groups as assessed by intracytoplasmic staining following short-term PMA and ionomycin stimulation [49]. At this stage, it is hard to interpret the discrepancies between these results, since the methods for detecting NKT cells and the functional assays used differ between these studies.

Systemic Autoimmune Disease

Sumida and colleagues found that αβ+ DN T cells were increased in Scleroderma patients and that there was oligoclonal expansion of $Vα24^+TCR^+$ cell among these cells [51]. Although the invariant Vα24Jα18 T cells were dominant among these cells from healthy donors, invariant Vα24Jα18 T cells were replaced by clones with other Vα24 TCR^+ cells in Scleroderma patients. In addition, Maeda *et al.* have reported the expansion of non-invariant Vα24 TCR^+ cells but not Vα24Jα18 T cells in the synovium of RA patients [52]. Similar to this study, these authors observed the expansion of non-invariant Vα24 TCR^+ clones in patients with active SLE [53, 54]. Furthermore, following predonisolone therapy, Vα24Jα18 T cells increased among Vα24 TCR^+ cells. The recovery of Vα24Jα18 T cells in patients with predonisolone therapy was also observed among patients with MS (Araki M and Yamamura T, unpublished observation). Kojo S *et al.* and other groups investigated the number of NKT cells using Vα24 and Vβ11 mAb to detect NKT cells in patients with several different autoimmune diseases, including SLE, Scleroderma and RA [43, 55]. They found lower numbers of $Vα24^+Vβ11^+$ NKT cells in the peripheral blood than controls. Kojo S *et al.* also showed in this study that half of the patients with autoimmune disease responded to α-GC in culture.

PROSPECTS FOR GLYCOLIPID THERAPY FOR AUTOIMMUNE DISEASES

It remains unclear whether the defect in NKT cells is causal for autoimmune disease or occur as a secondary consequence of the autoimmune process. However, given the efficacy of OCH and α-GC in mouse models, stimulation of NKT cells with glycolipid antigens seems to be an attractive strategy for the treatment of autoimmune diseases. Although several studies have shown that administration of α-GC caused liver damage, the hepatotoxicity was minimal in Phase I trials of α-GC for patients with cancer. Given the lack of severe toxicity in humans, it seems reasonable to use glycolipids for the prevention or therapy of selected human autoimmune disorders. α-GC has been shown to exacerbate EAE, depending on the strain of mouse and stage of disease tested and to have only a marginal effect on CIA. In this situation, treatment with OCH might be preferable to α-GC for Th1-mediated diseases such as MS, type I diabetes and RA, as OCH elicits a predominantly IL-4 response rather than IFN-γ in contrast to α-GC, which might afford greater protection from EAE and MS.

Both rodent and human NKT ells have been reported to recognize α-GC in the context of CD1d. OCH also stimulates human NKT cells, particularly $CD4^+$ NKT cells, and OCH stimulation induces more Th2 cytokine production from NKT cells compared to α-GC stimulation (Araki M and Yamamura T, unpublished observation). The evolutionary conservation and the homogeneous ligand specificity of NKT cells allow us to apply a glycolipid ligand like OCH for the treatment of human disease without considering species barrier or genetic heterogeneity of humans.

CONCLUSION

In this review, we have discussed the supporting data for the role of NKT cells in the regulation of autoimmune diseases. Ligand stimulation of NKT cells is an attractive strategy for prevention or treatment of autoimmune diseases. However, the mechanisms by which NKT cells exert their immunoregulatory functions are still largely unknown and a number of questions require further investigation including the mechanism to recruit NKT cells and control its functions at inflammatory sites and the interaction of other subsets of cells. To clarify the nature of natural ligands for NKT cells is one of the major questions and it could be an interesting natural source of useful ligands for CD1-restricted regulatory cells.

ABBREVIATIONS

NKT = Natural killer T

MHC = Major histocompatibility complex

α-GC = α-galactosylceramide

EAE = Experimental autoimmune encephalomyelitis

CIA = Collagen induced arthritis

Th = T helper

IL = Interleukin

IFN = Interferon

T1D = Type 1 diabetes

MS = Multiple sclerosis

RA = Rheumatoid arthritis

TCR = T cell receptor

DN = Double negative

NF-AT = Nuclear factor of activated T cells

APC = Antigen presenting cell

CNS = Central nervous system

B6 = C57BL/6

MOG = Myelin oligodendrocyte glycoprotein

CFA = Freund's complete adjuvant

NOD = Nonobese diabetic

dsDNA = Double-stranded DNA

REFERENCES

[1] Walker, L.S.K. and Abbas, A.K. (2002) *Nat. Rev. Immunol.*, **2**(1), 11-19.

[2] Hammond, K.J.L. and Godfrey, D.I. (2002) *Tissue Antigens*, **59**(5), 353-363.

[3] Wilson, S.B. and Delovitch, T.L. (2003) *Nat. Rev. Immunol.*, **3**(3), 211-222.

[4] Porcelli, S.A. and Modlin, R.L. (1999) *Annu. Rev. Immunol.*, **17**, 297-329.

[5] Kronenberg, M. and Gapin, L. (2002) *Nat. Rev. Immunol.*, **2**(8), 557-568.

[6] Gumperz, J.E.; Miyake, S.; Yamamura, T. and Brenner, M.B. (2002) *J. Exp. Med.*, **195**(5), 625-636.

[7] van Der Vliet, H.J.; Nishi, N.; de Gruijil, T.D.; von Blomberg, B.M.; van den Eertwegh, A.J.; Pinedo, H.M.; Giaccone, G. and Scheer, R.J. (2000) *Blood*, **95**(7), 2440-2442.

[8] D'Andrea, A.; Goux, D.; De Lalla, C.; Koezuka, Y.; Montagna, D.; Moretta, A.; Dellabona, P.; Casorati, G. and Abrignani, S. (2000) *Eur. J. Immunol.*, **30**(6), 1544-1550.

[9] Park, S.H.; Benlagha, K.; Lee, D.; Balish, E. and Bendelac, A. (2000) *Eur. J. Immunol.*, **30**(2), 620-625.

[10] Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H. and Taniguchi, M. (1998) *Science*, **391**(5343), 177-181.

[11] Brossay, L.; Chioda, M.; Burdin, N.; Koezuka, Y.; Casorati, G.; Dellabona, P. and Kronenberg, M. (1998) *J.Exp. Med.*, **188**(8), 1521-1528.

[12] Spada, F.M.; Koezuka, Y. and Porcellim, S.A. (1998) *J. Exp. Med.*, 188(8), 1529-1534.

[13] Wu, D.Y.; Segal, N.H.; Sidobre, S.; Kronenberg, M. and Champan, P.B. (2003) *J. Exp. Med.*, **198**(1), 173-181.

[14] Stetson, D.B.; Mohrs, M.; Reinhardt, R.L.; Baron, J.L.; Wang, Z-E.; Gapin, L.; Kroneneberg, M. and Locksley, R.M. (2003) *J. Exp. Med.*, **198**(7), 1069-1076.

[15] Ansel, K.M.; Lee, D.U. and Rao, A. (2003) *Nat. Immunol.*, 4(7), 616-623.

[16] Oki, S.; Chiba, A.; Yamamura, T. and Miyake, S. (2004) *J. Clin. Invest.*, **113**(11), 1631-1640.

[17] Shibasaki, F.; Price, ER.; Milan, D. and McKeon, F. (2000) *Nature*, **382**(6589), 370-373.

[18] Owens, T.; Wekerle, H. and Antel, J. (2001) *Nat. Med.*, 7(2), 161-166.

[19] Chen, L.Z.; Hochwald, G.M.; Huang, C.; Dakin, G.; Tao, H. Cheng, C.; Simmons, W.J.; Dranoff, G. and Thorbecke, G.J. (1998) *Proc. Natl. Acad. Sci. USA*, **95**(21), 12516-12521.

[20] Croxford, J.L.; Feldman, M.; Chernajovsky, Y. and Baker, D. (2001) *J. Immunol.*, **166**(6), 4124-4130.

[21] Pal, E.; Tabira, T.; kawano, T.; Taniguchi, M.; Miyake, S. and Yamamura, T. (2001) *J. Immunol.*, **166**(1), 662-668.

[22] Miyamoto, K.; Miyake, S. and Yamamura, T. (2000) *Nature*, **413**(6855), 531-534.

[23] Jahng, A.W.; Maricic, I.; Pedersen, B.; Burdin, N. and Naidenko, O. (2001) *J. Exp. Med.*, **194**(12), 1789-1799.

[24] Singh, A.K.; Wilson, M.T.; Hong, S.; Oliveres-Villagomez, D.; Du, C.; Stanic, A.K.; Joyce, S.; Siriam, S.; Koezka, Y. and Van Kaer, L. (2001) *J. Exp. Med.*, **194**(12), 1801-1811.

[25] Furlan, R.; Bergami, A.; Cantarella, D.; Brambilla, E.; Taniguchi, M.; Dellabona, P.; Casorati, G. and Martino G. (2003) *Eur. J. Immunol.*, **33**(7), 1830-1838.

[26] Yoshimoto, T.; Bendelac, A.; Hu-Li, J. and Pau, W.E. (1995) *Proc. Natl. Acad. Sci. USA*, **92**(25), 11931-11934.

[27] Van Roon, J.A. G.; Lafeber, F.P.J.G. and Bijlsma, J.W.J. (2001) *Arthritis. Rheum.*, **44**(1), 3-12.

[28] Chiba, A.; Oki, S.; Miyamoto, K.; Hashimoto, H.; Yamamura, T. and Miyake, S.(2001) *Arthritis. Rheum.*, **50**(1), 305-313.

[29] Delovitch, T.L. and Singh, B. (1997) *Immunity*, **7**(6), 727-738.

[30] Yoshida, K. and Kikutani, H. (2000) *Rev. Immunogenetics.*, **2**(1), 140-146.

[31] Gombert, J-M, Herbelin, A.; Tancrede-Bohin, E.; Dy, M.; Carnaud, C. and Bach, J.-F. (1996) *Eur. J. Immunol.*, **26**(12), 2989-2998.

[32] Shi, Fu-D.; Flodstrom, M.; Balasa, B.; Kim, S.H.; Van Gunst, K.; Strominger, J.L.; Wilson, B. and Sarvetnick N. (2001) *Proc. Natl. Acad. Sci. USA*, **98**(12), 6777-6782.

[33] Wang, B.; Geng, Y.-B. and Wang, C.-R. (2001) *J. Exp. Med.*, **194**(3), 313-319.

[34] Hammond, K.J.L.; Poulton, L.D.; almisano, L.J.; Silveira, P.A.; Godrey, D.I. and Bazter, A.G. (1998) *J. Exp. Med.*, **187**(7), 1047-1056.

[35] Lehuen, A.; Lantz, O.; Beaudoin, L.; Laloux, V.; Carnaud, C.; Bendelac, A.; Bach, J.-F. and Monteriro, R.C. (1998) *J. Exp. Med.*, **188**(9), 1831-1839.

[36] Sharif, S.; Arreaza, G.A.; Zucker, P.; Mi, Q-S.; Sondhi, J.; Naidenko, O.V.; Kronenberg, M.; Koezuka, Y. and Delovitch, T.L.; Gombert, J-M.; Leite-de-Moraes, M.; Gouarin, C.; Zhu, R.; Hameg, A.; Nakayama, T.; Taniguchi, M.; Lepault, F.; Lehuen, A.; Bach. J-F. and Herbelin A. (2001) *Nat. Med.*, **7**(9), 1057-1062.

[37] Hong, S.; Wilson, M.T.; Serizawa, I.; Wu, L.; Singh, N.; Naidenko, O.V.; Miura, T.; Haba, T.; Scherer, D.C.; Wei, J.; Kronenberg, M.; Koezuka, Y. and Van Kaer, L. (2001) *Nat. Med.*, **7**(9), 1052-1056.

[38] Naumov, Y.N.; Bahjat, K.S.; Gausling, R.; Abraham, R.; Exley, M.A.; Koezuka, Y.; Balk, S.B.; Strominger, J.L.; Clare-Salzer, M. and Wilson, S.B. (2001) *Proc. Natl. Acad. Sci. USA*, **98**(24), 13838-13843.

[39] Mieza, M.A.; Itoh, T.; Cui, J.Q.; Makino, Y.; Kawano, T.; Tsuchida, K.; Koike, T.; Shirai, T.; Yagita, H.; Matsuzawa, A.; Koseki H. and Taniguchi, M. (1996) *J. Immunol.*, **156**(10), 4035-4040.

[40] Zeng, D.; Liu, Y.; Sidobre, S.; Kronenberg, M. and Strober, S. (2003) *J. Clin. Invest.*, **112**(8), 1211-1222.

[41] Yang, J.Q.; Singh, A.K.; Wilson, M.T.; Satoh, M.; Stanic, A.K.; Par, J.-J.; Hong, S.; Gadola, S.D.; Mizutani, A.; Kakumanu, S.R.; Reeves, W.; Cerundolo, V.; Joyce, S.; Van Kaer, L. and Singh, R.R. (2003) *J. Immunol.*, **171**(4), 2142-2153.

[42] Yang, J-Q.; Saxena, V.; Xu, H.; Van Kaer, L.; Wang, C.-R. and Singh, R.R. (2003) *J. Immunol.*, **171**(8), 4439-4446.

[43] Illes, Z.; Kondo, T.; Newcombe, J.; Oka, N.; Tabira, T. and Yamamura, T. (2000) *J. Immunol.*, **164**(8), 4375-4381.

[44] Van der Vliet, H.J.J.; von Blomberg, B.M.E.; Nishi, N.; Reijm, M.; Voskuyl, A.E.; van Bodegraven, A.; Polman, C.H.; Rustemeyer, T.;Lips, P.; van den Eertwegh, A.J.M.; Giaccone, G.; Scheper, R.J. and Pinedo, H.M. (2001) *Clin. Immunol.*, **100**(2), 144-148.

[45] Araki, M.; Kondo, T.; Gumperz, J.E.; Brenner, M.B.; Miyake, S. and Yamamura, T. (2003) *Int. Immunol.*, **15**(2), 279-288.

[46] Gausling, R.; Trollmo, C. and Hafler, D.A. (2001) *Clin. Immunol.*, **98**(1), 11-17.

[47] Wilson, S.B.; Kent, S.C.; Patton, K.T.; Orban, T.; Jackdon, R.A.; Exley, M.; Porcelli, S.; Schatz, D.A.; Atkinson, M.A.; Balk, S.P.; Strominger, J.L. and Hafler, D.A. (1998) *Nature*, **391**(6663), 177-181.

[48] Kukreja, A.; Cost, G.; Marker, J.; Zhang, C, Sun, Z.; Lin-Su, K.; Ten, S.; Sanz, M, Exley, M.; Wilson, B, Porcelli, S.; Maclaren, N. (2002) *J. Clin. Invest.*, **109**(1), 131-140.

[49] Lee, P.T.; Putnam, A.; Benlagha, K.; Teyton, L.; Gottlieb, P.A. and Bendelac, A. (2002) *J. Clin. Invest.*, **110**(6), 793-800.

[50] Oikawa, Y.; Shimada, A.; Yamada, S.; Motohashi, Y.; Nakagawa Y.; Irie, J.; Maruyama, T. and Saruta, T. (2002) *Diabet. Care* **25**(10), 1818-1823.

[51] Sumida, T.; Sakamoto, A.; Murata, H.; Makino, Y.; Takahashi, H. Yoshida, H.; Nishioka, K.; Iwamoto, I. and Tanigucni, M. (1995) *J Exp. Med.*, **182**(4), 1163-1168.

[52] Maeda, T.; Keino, H.; Asahara, M.; Taniguchi, M.; Nishioka, K and Sumida, T. (1999) *Rheumatology*, **38**(2), 186-188.

[53] Oishi, Y.; Sumida, T.; Sakamoto, A.; Kita, Y.; Kurasawa, K. Nawata, Y.; Takabayashi, K.; Takahashi, H.; Yoshida, S.; Tanigu chi, M.; Saito, Y. and Iwamoto, I. (2001) *J. Rhuematol.*, **28**(2), 275 283.

[54] Sumida, T.; Maeda, T.; Taniguchi, M.; Nishioka, K. and Stohl, W (1998) *Lupus*, **7**(8), 565-568.

[55] Kojo, S.; Adachi, Y.; Keino, H.; Taniguchi, M. and Sumida, T (2001) *Arthritis. Rheum.*, **44**(5), 1127-1138.

[56] Mizuno, M.; Masumura, M.; Tomi, C.; Chiba, A.; Oki, S.; Yama mura, T.; Miyake, S. (2004) *J. Autoimmune*, **23**, 293-300.

CONCISE REPORT

Association of mannose binding lectin (MBL) gene polymorphism and serum MBL concentration with characteristics and progression of systemic lupus erythematosus

R Takahashi, A Tsutsumi, K Ohtani, Y Muraki, D Goto, I Matsumoto, N Wakamiya, T Sumida

Ann Rheum Dis 2005;**64**:311–314. doi: 10.1136/ard.2003.020172

Objective: To determine whether occurrence, characteristics, and progression of systemic lupus erythematosus (SLE) are associated with polymorphism of the mannose binding lectin (MBL) gene and with serum MBL concentration.

Methods: Codon 54 MBL gene polymorphism of 147 patients with SLE and 160 healthy controls was determined by polymerase chain reaction-restriction fragment length polymorphism. Serum concentration of MBL was measured by enzyme immunoassay. Fluctuations of serum MBL were analysed with respect to disease characteristics and activity.

Results: Frequency of homozygosity for codon 54 minority allele was 6% (9/147) in patients with SLE, and significantly higher than in controls ($p=0.0294$, Fisher's exact test). MBL polymorphism in patients with SLE was not significantly associated with disease characteristics or immunological phenotypes. Patients homozygous for the B allele tended to have a higher risk of infection during treatment. Levels of C3 and CH_{50} were slightly, but significantly, associated with serum MBL concentration in patients with SLE homozygous for the majority allele. During the course of SLE, serum MBL concentration increased in 6/14 patients, and decreased in 7 after initiation of immunosuppressive treatment.

Conclusions: MBL gene polymorphism influences susceptibility to SLE, but has no direct effect on disease characteristics. Serum MBL levels fluctuate during the course of SLE in individual patients. MBL genotyping may be useful in assessing the risk of infection during treatment of SLE.

Mannose binding lectin (MBL) is a molecule that shares many features with C1q. MBL comprises a trimer of three identical polypeptides, and several trimers further combine to form a bouquet-like structure.[1] MBL mediates lectin dependent activation of the complement pathway,[1] and has an important role in host defence against micro-organisms. People lacking this protein could develop severe episodes of bacterial infections from early life.[2] Several polymorphisms have been reported for the MBL gene, and a large interindividual difference in serum MBL concentration is caused by the possession of variant alleles. Codon 52, 54, and 57 polymorphisms are all on exon 1 of the MBL gene, and the presence of any of the minority alleles significantly reduces serum MBL concentration. Furthermore, homozygosity for minority alleles results in almost complete deficiency of serum MBL.[3] This has been attributed to increased degradation of the mutated protein.[4]

Recently, several studies have suggested that possession of MBL minority alleles may be associated with occurrence of systemic lupus erythematosus (SLE).[5 6] It is known that C1q deficiency is associated with severe symptoms of SLE.[7] Two possible explanations for associations between MBL or C1q deficiency and occurrence of SLE can be proposed: (*a*) MBL and C1q can bind to and initiate uptake of apoptotic cells into macrophages,[8 9] and abnormal clearance of apoptotic cells caused by MBL or C1q deficiency may result in overexpression of autoantigens; (*b*) viral infection is believed to be one of the causes of SLE,[10] and MBL or C1q deficiency may lead to more frequent infections.

This study was conducted on the premise that occurrence, characteristics, and progression of SLE are associated with polymorphism of the MBL gene and with serum MBL concentration. To our knowledge, this is the first study that has measured serum MBL concentration before and after immunosuppressive treatment in patients with newly diagnosed SLE.

PATIENTS AND METHODS

Samples from 147 Japanese patients with SLE followed up at our hospital, were used for the study. All patients fulfilled the 1997 American College of Rheumatology Classification Criteria for SLE. Samples from 160 Japanese healthy volunteers served as controls.

Genomic DNA was purified from peripheral blood leucocytes using the DNAQuick DNA purification kit (Dainippon Pharmaceuticals, Osaka, Japan), and stored at −30°C. Typing of the MBL gene allele was performed by polymerase chain reaction-restriction fragment length polymorphism, according to the method of Madsen *et al*.[3] The wild-type allele was designated allele A, and codon 54 substitution (glycine to aspartic acid) was designated allele B. Previous studies have shown that codon 52 and 57 polymorphisms are not present or extremely rare in the Japanese population.[11] Serum concentration of MBL was measured by a specific enzyme immunoassay using two rabbit polyclonal anti-MBL antibodies as described previously.[12]

Table 1 Codon 54 genotypes of the MBL gene in patients with SLE and healthy controls

	SLE	Healthy controls	p Value
AA + AB	138 (AA; 84, AB; 54)	158 (AA; 101, AB; 57)	0.0294
BB	9	2	
Total	147	160	

Allele A, codon 54 wild type majority allele; allele B, codon 54 variant minority allele.
p Value by Fisher's exact test.

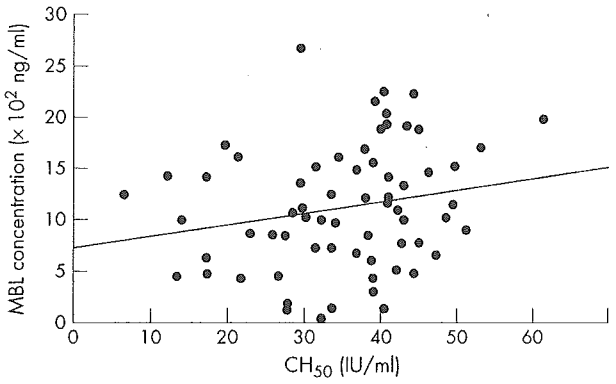

Figure 1 Relationship between serum MBL concentration and CH_{50} in genotype AA patients with SLE. $r_s = 0.253$, $p = 0.0412$ by Spearman's rank correlation test.

Fisher's exact test was used to compare the frequencies of genotypes AA/AB and BB, between disease and control groups, and to compare clinical characteristics between patients with genotypes AA/AB and those with BB. Mann-Whitney's U test was used to compare ages at diagnosis of SLE between patients with genotypes AA/AB and those with BB, and to compare serum MBL concentration between patients and controls of the same genotype. Spearman's rank correlation test was used to compare serum MBL concentration and the levels of anti-DNA antibody, C3, C4, and CH_{50}. Values of $p \leqslant 0.05$ were considered significant.

RESULTS

MBL gene genotypes were studied in patients with SLE and healthy controls (table 1). Among 147 patients with SLE, 9 were homozygous for allele B, which was significantly increased compared with controls ($p = 0.0294$).

We analysed the difference in disease characteristics among patients with SLE categorised by MBL genotypes. Ages (mean (SD)) at diagnosis of SLE tended to be younger in patients with allele B (AA: 32.5 (14.8); AB: 30.7 (15.2); BB: 23.4 (13.3)), but no significant differences were seen ($p = 0.0681$). Clinical characteristics, serological, and immunological measures did not significantly differ between genotype BB patients and other patients with SLE. This is

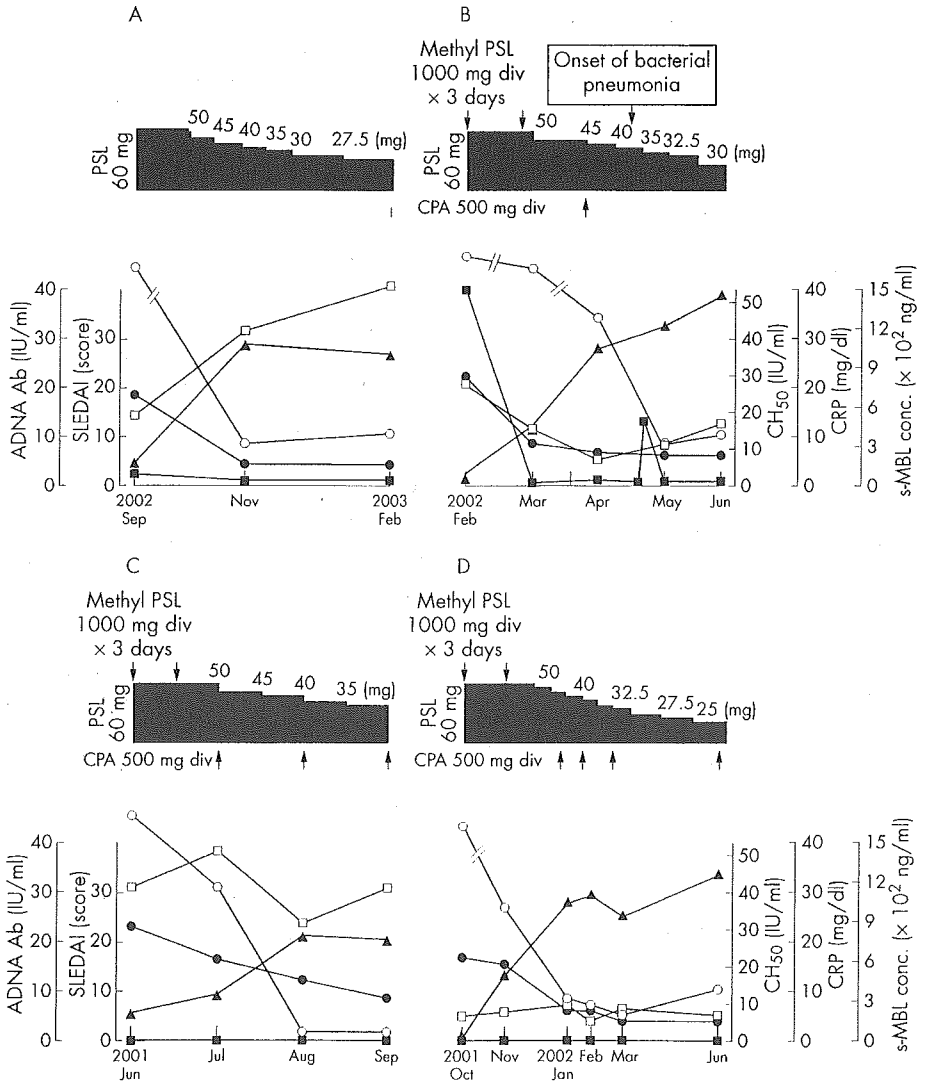

Figure 2 Fluctuation of serum MBL concentration and clinical variables during immunosuppressive treatment in patients with newly diagnosed SLE. Open squares, serum MBL concentrations (s-MBL); closed squares, C reactive protein (CRP); open circles, anti-DNA antibody (ADNA Ab); closed circles, SLE Disease Activity Index (SLEDAI); closed triangles: CH_{50}. PSL, prednisolone; CPA, cyclophosphamide.

most probably because of the small size of the BB cohort. However, incidence of infections requiring admission to hospital was significantly higher in patients with genotype BB than in other patients (genotype AA +AB; 35/132 patients, BB; 5/8 patients, $p = 0.0287$).

Serum MBL concentration reflected the MBL genotype of the individual subject, in accordance with previous reports[5] (data not shown). Among subjects with the same genotype, patients with SLE tended to have a higher MBL concentration than controls, but without statistical significance. The level of CH_{50} was weakly but significantly associated with serum MBL concentration in patients with SLE with genotype AA ($p = 0.0412$) (fig 1). In genotype AA patients, C3 was also associated with serum MBL concentration, although C4 was not (C3; $p = 0.0494$, C4; $p = 0.4265$). No significant relationship between anti-DNA antibody titre and serum MBL was found. In patients with other genotypes, no significant association was seen between serum MBL concentration and levels of anti-DNA antibody or complement components (data not shown).

We studied fluctuation of serum MBL concentration during immunosuppressive treatment in patients with newly diagnosed SLE (fig 2). In patient 1 with genotype AA (fig 2A), serum MBL increased in parallel with CH_{50} after initiation of methylprednisolone treatment, while the SLE Disease Activity Index (SLEDAI) and anti-DNA antibody decreased. In patient 2 with genotype AA (fig 2B), serum MBL concentration decreased after initiation of methylprednisolone pulse therapy, while CH_{50} increased. After CRP decreased to normal levels, MBL gradually increased in parallel with CH_{50}. In patient 3 with genotype AA (fig 2C), serum MBL did not show a clear trend, although disease activity steadily decreased. In patient 4 with genotype AB (fig 2D), serum MBL was low throughout, reflecting the MBL genotype. When the serum MBL concentration before and after immunosuppressive treatment was compared in 14 newly diagnosed patients, it increased in 6/14 patients (genotype AA: 2, AB: 4), and decreased in 7 patients (genotype AA: 5, AB: 2). There was no significant association between increase or decrease of serum MBL concentration and genotypes and clinical phenotypes in patients with SLE (data not shown).

DISCUSSION

Several studies have indicated that MBL gene polymorphism influences susceptibility to SLE.[5 6] When the components of the classical pathway of complement (C1q, C1r, C1s, C4, or C2) are deficient, it has been suggested that abnormal clearance of not only immune complexes[13] but also apoptotic cells[8] contributes to the occurrence of SLE. It has been indicated that inappropriate levels of apoptotic nuclei may be a major source of autoantigens in SLE.[14] Recently, it was reported that MBL can bind to apoptotic cells and initiate their uptake by macrophages,[9] and thus, abnormal clearance of apoptotic cells due to MBL deficiency may provide a source of autoantigens in SLE. However, deficiency of MBL is not an extremely high risk factor, in contrast with deficiencies of other complement molecules such as C1q.[7] The precise consequences of MBL deficiency for the onset and progression of SLE remain unclear. The lag time between occurrence of the first symptom attributable to SLE and diagnosis of definite SLE was reported to be significantly shorter for variant allele carriers than in those with genotype AA.[6] Therefore, the MBL gene may be a disease modifier locus rather than a true SLE susceptibility locus. Although no significant correlation between disease characteristics and MBL genotypes was seen, genotype BB was significantly associated with occurrence of infection in our patients, in accord with a previous report.[6] MBL genotyping may help in assessment of the risk of opportunistic infections in patients with SLE.

The balance of MBL production and consumption determines serum MBL levels. As the presence of MBL deposits in tissues of autoimmune patients has been demonstrated,[15 16] we expected that MBL would be consumed during active disease, and that serum MBL concentration might reflect disease activity and pathological features of SLE in individual patients. To test this hypothesis, we measured serum MBL concentration during immunosuppressive treatment in patients with newly diagnosed disease. As shown in fig 2, serum MBL concentration did fluctuate during the course of immunosuppressive treatment in patients with SLE, especially in genotype AA patients. In patients 1 and 2, the increasing phase of serum MBL concentration may reflect the decreased consumption of MBL while SLE activity gradually decreased, and the decreasing phase may reflect reduced production of MBL because MBL is an acute phase inflammatory protein.[17] Thus, MBL levels appear to reflect disease activity in some patients. The weak but significant association between serum MBL concentration and serum C3 or CH_{50} levels supports this view.

In conclusion, frequency of homozygosity for a minority allele of the MBL gene was increased in patients with SLE compared with controls, confirming previous studies. MBL gene polymorphism may have no direct effect on disease characteristics, but patients homozygous for the minority allele had significantly more frequent episodes of infections. Serum MBL levels did fluctuate during the course of SLE in individual patients, although the mechanism of their fluctuation and their consequences in SLE are unclear. The value of serum MBL monitoring in clinical practice should be determined in future studies.

Authors' affiliations
R Takahashi, A Tsutsumi, Y Muraki, D Goto, I Matsumoto, T Sumida, Division of Rheumatology, Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, Japan
K Ohtani, N Wakamiya, Department of Microbiology, Asahikawa Medical College, Japan

Correspondence to: Dr A Tsutsumi, 1-1-1 Tennodai Tsukuba-city, Ibaraki 305-8575, Japan; atsutsum@md.tsukuba.ac.jp

Accepted 9 May 2004

REFERENCES

1 **Holmskov U**, Malhotra R, Sim RB, Jensenius JC. Collectins: collagenous C-type lectins of the innate immune defense system. *Immunol Today* 1994;**15**:67–74.
2 **Summerfield JA**, Ryder S, Sumiya M, Thursz M, Gorchein A, Monteil MA, *et al.* Mannose binding protein gene mutations associated with unusual and severe infections in adults. *Lancet* 1995;**345**:886–9.
3 **Madsen HO**, Garred P, Kurtzhals JA, Lamm LU, Ryder LP, Thiel S, *et al.* A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics* 1994;**40**:37–44.
4 **Sumiya M**, Super M, Tabona P, Levinsky RJ, Arai T, Turner MW, *et al.* Molecular basis of opsonic defect in immunodeficient children. *Lancet* 1991;**29**:1569–70.
5 **Davies EJ**, Snowden N, Hillarby MC, Carthy D, Grennan DM, Thomson W, *et al.* Mannose-binding protein gene polymorphism in systemic lupus erythematosus. *Arthritis Rheum* 1995;**38**:110–14.
6 **Garred P**, Voss A, Madsen HO, Junker P. Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. *Genes Immun* 2001;**2**:442–50.
7 **Walport MJ**, Davies KA, Botto M. C1q and systemic lupus erythematosus. *Immunobiology* 1998;**199**:265–85.
8 **Korb LC**, Ahearn JM. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. *J Immunol* 1997;**158**:4525–8.
9 **Ogden CA**, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, *et al.* C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J Exp Med* 2001;**194**:781–95.

10 **Okada M**, Ogasawara H, Kaneko H, Hishikawa T, Sekigawa I, Hashimoto H, *et al*. Role of DNA methylation in transcription of human endogenous retrovirus in the pathogenesis of systemic lupus erythematosus. *J Rheumatol* 2002;**29**:1678–82.
11 **Sasaki K**, Tsutsumi A, Wakamiya N, Ohtani K, Suzuki Y, Watanabe Y, *et al*. Mannose-binding lectin polymorphisms in patients with hepatitis C virus infection. *Scand J Gastroenterol* 2000;**35**:960–5.
12 **Ohtani K**, Suzuki Y, Eda S, Kawai T, Kase T, Keshi H, *et al*. High-level and effective production of human mannan-binding lectin (MBL) in Chinese hamster ovary (CHO) cells. *J Immunol Methods* 1999;**222**:135–44.
13 **Atkinson JP**. Complement deficiency: predisposing factor to autoimmune syndromes. *Clin Exp Rheumatol* 1989;**7**:S95–101.
14 **Mevorach D**, Zhou JL, Song X, Elkon KB. Systemic exposure to irradiated apoptotic cells induces autoantibody production. *J Exp Med* 1998;**188**:387–92.
15 **Hisano S**, Matsushita M, Fujita T, Endo Y, Takebayashi S. Mesangial IgA2 deposits and lectin pathway-mediated complement activation in IgA glomerulonephritis. *Am J Kidney Dis* 2001;**38**:1082–8.
16 **Steinfeld S**, Penaloza A, Ribai P, Decaestecker C, Danguy A, Gabius HJ, *et al*. D-mannose and N-acetylglucosamine moieties and their respective binding sites in salivary glands of Sjögren's syndrome. *J Rheumatol* 1999;**26**:833–41.
17 **Thiel S**, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. *Clin Exp Immunol* 1992;**90**:31–5.

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Autoimmunity Reviews 4 (2005) 475–478

AUTOIMMUNITY REVIEWS

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Immunoglobulin G from anti-glucose-6-phosphate isomerase antibodies positive patient with rheumatoid arthritis induces synovitis in cynomolgus monkeys

Takeshi Suzuki[a], Yoshifumi Muraki[a], Takanori Yasukochi[a,b], Hua Zhang[a,b], Yuko Kori[a], Ei Wakamatsu[a], Taichi Hayashi[a], Daisuke Goto[a], Satoshi Ito[a], Akito Tsutsumi[a], Hiroshi Sumichika[c], Takayuki Sumida[a], Isao Matsumoto[a,b,*]

[a]*Clinical Immunology, Major of Advanced Biomedical Applications, Graduate of Comprehensive Human Sciences, University of Tsukuba, University of Tsukuba, 1-1-1 Tenodai, Ibaraki 305-8575, Japan*

[b]*PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama, Japan*

[c]*Mitubishi Pharma Corporation, 1000, Kamoshida-cho, Aoba-ku, Yokohama, Japan*

Received 14 April 2005; accepted 7 May 2005
Available online 31 May 2005

Abstract

Anti-glucose-6-phosphate isomerase (GPI) antibodies (Abs) solely induce arthritis in mice. High titers of anti-GPI Abs are found in some patients with rheumatoid arthritis (RA), but their pathogenic role remains elusive. The aim of this study was to evaluate the pathogenic role of anti-GPI Abs in cynomolgus monkeys. IgG fractions were separated from sera of anti-GPI Abs-positive RA patients and healthy subjects and directly injected into the metacarpophalangeal joints of 4 cynomolgus monkeys. At day 16, the joints were harvested and examined histologically and immunohistochemically. The expression of C5a receptor (C5aR) molecule in the synovium was quantified by real-time PCR using cDNA from monkey joints. In monkey joints, IgG including anti-GPI Abs resulted in recruitment of granulocytes and mononuclear cells, strong deposition of human IgG on the articular surface, and overexpression of C5aR, but no joint swelling. No infiltrated cells or IgG deposition were observed in monkeys injected with IgGs from healthy subjects. Our results suggest that IgG fraction from RA patients including anti-GPI Abs may play a crucial role in the generation of synovitis in monkeys, although the pathogenesis of anti-GPI Abs in RA patients is still uncertain.

Keywords: Rheumatoid arthritis; Autoantibodies; Glucose-6-phosphate-isomerase; Pathogenicity

* Corresponding author. Clinical Immunology, Major of Advanced Biomedical Applications, Graduate of Comprehensive Human Sciences, University of Tsukuba , 1-1-1 Tennodai, Tsukuba 305-8575, Japan. Tel./fax: +81 29 853 3186.
E-mail address: ismatsu@md.tsukuba.ac.jp (I. Matsumoto).

1568-9972/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.autrev.2005.05.005

Contents

The K/BxN T cell receptor transgenic mouse model was spontaneous model of inflammatory arthritis characterized by arthritic manifestations similar to those of rheumatoid arthritis (RA). [1] Arthritis in this model could be almost entirely provoked by autoantibodies to the ubiquitous self antigen glucose-6-phosphate isomerase (GPI) [2]. However, once the pathogenic antibodies (Abs) have been produced by the autoimmune reaction, there is no further requirement for lymphoid cells. [3] The effector mechanisms of anti-GPI Abs have been confirmed by the requirement of innate immune system players (e.g., complement cascade, Fcgamma receptor, neutrophils, mast cells). [4–7] Recently, immunization with human GPI was reported to provoke arthritis in DBA/1 mouse, supporting the notion that autoimmunity to GPI directly play some role of arthritis in genetically unaltered mice. [8] These results also indicated that ubiquitous autoantigens might be the targets of arthritogenic Abs.

The first report on anti-GPI Abs in human showed a high frequency of such Abs in the sera of RA patients [9], though their frequency is still debated. [10–14] Our anti-GPI Abs assay [10] seemed to be highly specific, because its employs two different GPIs; a recombinant human GPI and rabbit native GPI. Our results showed that only 15% of RA patients had high titers of anti-GPI Abs, although the severity of arthritis correlated with serum levels of anti-GPI Abs. [10] Others have shown that extra-articular complications in RA were associated with serum levels of anti-GPI Abs. [15] However, the arthritogenic role of these Abs remains unclear.

To investigate whether human anti-GPI Abs present in the serum have arthritogenic properties, we injected serum anti-GPI Abs obtained from patients with RA into several strains of mice. However, this did not produce swelling of the joints in mice (unpublished data). The results of these preliminary experiments suggested that human GPI Abs did not solely induce arthritis, although we could not confirm the differences in the innate immune system between human and mice. In such experiments, it is better to use monkeys because they are genetically closer to human than mice. Moreover, recent studies reported that orally administered C5aR antagonist is only effective in human and cynomolgus monkey. [16] Based on these properties, we used cynomolgus monkeys in this study.

1. Intra-articular injection of human IgGs in cynomolgus monkey induce IgG deposition on the articular surface and induce minor synovitis

To examine the arthritogenic effects of serum IgG including anti-GPI Abs from RA, human IgGs containing anti-GPI Abs purified from serum of RA1 patient (clinical data and anti-GPI Abs were summarized in Table 1) or serum IgGs from healthy subjectswere injected four times at days 0, 3, 6, 9<IgG fractions (0.15 mg × 4 times)>directly into MP joints of cynomolgus monkeys. Joint swelling and blood tests were monitored and at day 16 the joints were harvested, stained with H and E and examined his-

Table 1
Clinical data and anti-GPI titer in a patient with RA

	Age/Sex	Disease duration (years)	RF	CRP (mg/dl)	Rheumatoid nodule	Hu-GPI Abs OD405	Ra-GPI Abs OD405	Anti GPI-Abs
RA1	68/F	23	+	4.01	–	2.43	2.55	+

Cutoff values; Hu-GPI, 1.32 OD, Ra-GPI, 0.94 OD.
RF: rheumatoid factor,+: positive,–: negative.

tologically and immunohistochemically. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Tsukuba University. Recruitment of infiltrated cells into the joints and strong deposition of human IgG onto the articular surface were clearly evident in monkeys that received intraarticular injection of RA IgG (Fig. 1A and C), although no finger joint swelling was noted. In contrast, neither infiltrated cells nor IgG deposition were observed in monkeys that received IgGs from healthy subjects (Fig. 1B and D). These results indicate that serum IgG including anti-GPI Abs from RA patients deposit preferentially on the articular surface and might recruit effector cells via C5aR or FcγR.

2. Overexpression of C5aR in synovia of monkeys treated with IgG anti-GPI Abs

Previous studies showed that injection of anti-GPI Abs did not induce arthritis in C5aR-deficient K/BxN mice [4], implying that C5a/C5aR interaction in the joint is important for the development of arthritis. To determine the role of C5aR in our study, we quantified the expression level of C5aR in the MP synovia using Taq Man real-time PCR. As a positive control, we used neutrophils from cynomolgus monkey. C5aR mRNA was highly expressed in the synovia of monkeys injected with IgG from anti-GPI Abs-positive RA, but not in those injected with IgG from healthy subjects ($p=0.0039$) (Fig. 1E). These find-

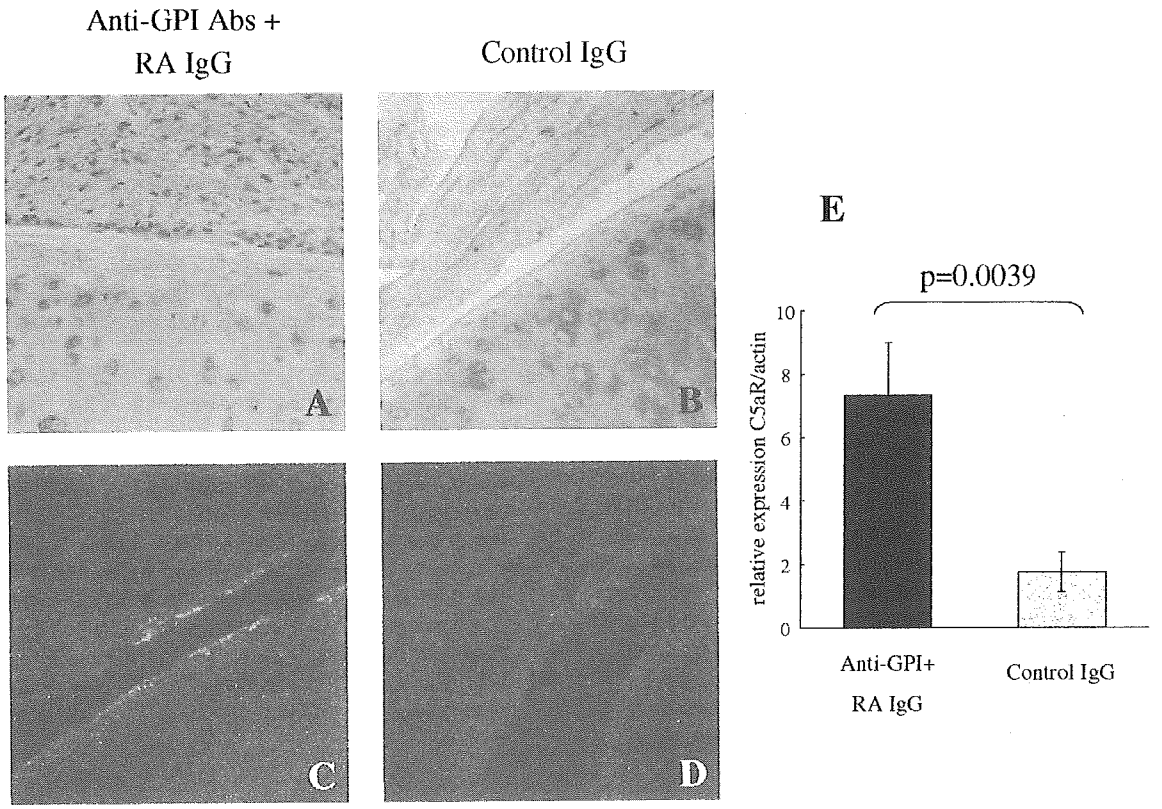

Fig. 1. Serum IgG containing anti-GPI Abs from RA patients formed immunocomplexes on the articular surface and provoked minor synovitis via C5aR. Cryostat sections of MP joints of cynomolgus monkeys (A, B, C, D) injected 4 times with IgGs from anti-GPI Abs-positive RA patients or IgGs from healthy subjects. H and E staining of joints injected with IgGs containing anti-GPI Abs from RA (A) and IgGs from healthy subjects (B). Note that infiltration of effector cell occurred only in response to anti-GPI Abs. Immunohistochemical studies using anti-human IgG (red) with anti-GPI Abs from RA patient (C) or IgG from healthy subjects (D). Note the strong deposition of human IgG on the articular surface by IgG anti-GPI Abs. Magnification A-D × 200. Six MP joints synovium (three for IgG-anti GPI Abs from RA patients, and three for IgG from healthy subjects) were harvested and used in this study. Data are mean ± SD of relative expression of the gene in each joints (E). Differences in C5aR expression between RA-GPI positive vs. HS were compared and analyzed with the nonparametric Mann–Whitney *U* test. *P* values less than 0.05 with 95% confidence interval were considered significant.

ings suggest that immunocomplex deposition by human RA IgG including anti-GPI Abs might induce complement cascade activation via C5aR-bearing cells. Possible explanation of the incomplete perturbation might be due to differences in innate immune systems between human and monkey.

In conclusion, these findings suggest that RA IgG including anti-GPI Abs present in serum preferentially form on the articular surface immunocomplexes that might induce complement activation via C5aR-bearing cells, resulting in minor synovitis. However direct pathogenic role of IgG from human RA is still uncertain.

Take-home messages

- Antibodies against glucose-6-phosphate isomerase (GPI) are detectable in some RA patients.
- The pathogenesis of human anti-GPI Abs are still unraveled.
- Intra-articular injection of human IgGs in cynomolgus monkey induce IgG deposition on the articular surface and induce infiltrated cells.
- Overexpression of C5aR in synovia of monkeys treated with IgG from anti-GPI Abs positive RA patients.

References

[1] Kouskoff V, Korganow A-S, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoreactivity. Cell 1996;87:811–22.
[2] Matsumoto I, Staub A, Benoist C, Mathis D. Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. Science 1999;286:1732–5.
[3] Korganow A-S, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, et al. From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. Immunity 1999;10:451–61.
[4] Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, et al. Arthritis critically dependent on innate immune system players. Immunity 2001;16:157–68.
[5] Ji H, Pettit A, Ohmura K, Ortiz-Lopez A, Duchatelle V, Degott C, et al. Critical roles for interleukin 1 and tumor necrosis factor alpha in antibody-induced arthritis. J Exp Med 2002; 196:77–85.
[6] Wipke BT, Allen PM. Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. J Immunol 2001;167:1601–8.
[7] Lee DM, Friend DS, Gurish MF, Benoist C, Mathis D, Brenner M. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. Science 2002;297:1689–92.
[8] Schubert D, Maier B, Morawietz L, Krenn V, Kamradt T. Immunization with glucose-6-phosphate isomerase induces T cell-dependent peripheral polyarthritis in genetically unaltered mice. J Immunol 2004;172:4503–9.
[9] Schaller M, Burton DR, Ditzel H. Autoantibodies to GPI in rheumatoid arthritis: linkage between an animal model and human disease. Nat Immunol 2001;2:746–53.
[10] Matsumoto I, Lee DM, Goldbach-Mansky R, Sumida T, Hitchon CA, Schur PH, et al. Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and spectrum of other chronic autoimmune disorders. Arthritis Rheum 2003;48:944–54.
[11] Schaller M, Benoit VM, Ditzel HJ. Correspondence: Response. Nat Immunol 2002;3:412–3.
[12] Kassahn D, Kolb C, Solomon S, Bochtler P, Illges H. Few human autoimmune sera detect GPI. Nat Immunol 2002;3: 411–2.
[13] Schubert D, Schmidt M, Zaiss D, Jungblut PR, Kamradt T. Autoantibodies to GPI and creatine kinase in RA. Nat Immunol 2002;3:411.
[14] Herve CA, Wait R, Venables PJ. Glucose-6-phosphate isomerase is not a specific autoantigen in rheumatoid arthritis. Rheumatology (Oxford) 2003;42:645–51.
[15] Van Gaalen FA, Toes RE, Ditzel HJ, Schaller M, Breedveld FC, Verweij CL, et al. Association of autoantibodies to glucose-6-phosphate isomerase with extraarticular complications in rheumatoid arthritis. Arthritis Rheum 2004;50:395–9.
[16] Sumichika H, Sakata K, Sato N, Takeshita S, Ishibuchi S, Nakamura M, et al. Identification of a potent and orally active non-peptide C5a receptor antagonist. J Biol Chem 2002;277: 49403–7.

Mod Rheumatol (2005) 15:258–263
DOI 10.1007/s10165-005-0405-y

ORIGINAL ARTICLE

Taichi Hayashi · Isao Matsumoto · Yoshifumi Muraki
Reiko Takahashi · Yusuke Chino · Daisuke Goto
Satoshi Ito · Akito Tsutsumi · Takayuki Sumida

Clinical characteristics of anti-glucose-6-phosphate isomerase antibody-positive Japanese patients with rheumatoid arthritis

Received: January 11, 2005 / Accepted: May 12, 2005

Abstract Anti-glucose-6-phosphate isomerase (GPI) antibodies (Abs) are known to be arthritogenic in mice. These Abs are elevated in several forms of arthritic condition in humans, although their prevalence in rheumatoid arthritis (RA) patients is still in debate. Some RA patients have increased levels of anti-GPI Abs, but their clinical manifestation and relevance to other Abs are not clearly elucidated. The aims of this study were to explore the clinical and hematological characteristics of RA with anti-GPI Abs, and to compare their prevalence in RA patients, systemic lupus erythematosus (SLE) patients, and healthy subjects (HS) in a Japanese population. Anti-GPI Abs were positive in 16 patients with RA (12%, $n = 137$), in 10 patients with SLE (8%, $n = 131$), and in 6 HS (4%, $n = 139$). C-reactive protein (CRP), immunoglobulin G, and the antinuclear antibody titer were higher in anti-GPI-positive patients than in those who were negative ($P = 0.049$, $P = 0.0003$, and $P = 0.002$, respectively). Moreover, the positivity of anti-GPI Abs was correlated with CRP more than with rheumatoid factor in RA patients. It is unclear whether anti-GPI Abs can predict the progress of disease, but the prevalence of these Abs was higher in active RA patients with severe arthritis, suggesting that anti-GPI Abs may be related to the pathogenesis of severe forms of arthritis.

Key words Auto-antibody · Clinical parameters · Glucose-6-phosphate isomerase (GPI) · Rheumatoid arthritis (RA) · Systemic lupus erythematosus (SLE)

T. Hayashi · I. Matsumoto (✉) · Y. Muraki · R. Takahashi · Y. Chino · D. Goto · S. Ito · A. Tsutsumi · T. Sumida
Clinical Immunology, Major of Advanced Biomedical Applications, Graduate School of Comprehensive Human Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan
Tel. +81-29-853-3186; Fax +81-29-853-3186
e-mail: ismatsu@md.tsukuba.ac.jp

I. Matsumoto
PRESTO, Japan Science and Technology Agency, Kawaguchi, Japan

Introduction

Rheumatoid arthritis (RA) is the most common type of inflammatory polyarthritis. This disease is considered to be mainly an autoimmune disorder, although the detailed etiology is still unknown.[1] Several autoantibodies have been identified [e.g., rheumatoid factor, anti-cyclic citrullinated peptide (CCP) antibodies (Abs), and anti-fillagrin Abs] in the serum of RA patients, and many of them are useful markers for diagnosis, but they have not been proven to be pathogenic.

Anti-glucose-6-phosphate isomerase (GPI) Abs detected in the serum of K/BxN T-cell receptor transgenic mice[2,3] have been confirmed to have arthritogenic potential.[4] One research group reported a high prevalence (64%) of these Abs in RA patients,[5] although this observation could not be reproduced.[6–8] Because anti-GPI Abs are one of the major candidates for arthritogenic antibodies,[9] their relationship with certain clinical parameters should be elucidated in detail. The aims of this study were to find the characteristic hematological and clinical features of RA patients with anti-GPI Abs, and to clarify their prevalence in human RA patients and in a control group of Japanese subjects.

In anti-GPI Ab-positive RA patients, CRP was higher than in RA patients negative for these Abs ($P = 0.049$), suggesting that anti-GPI Abs are correlated with disease activity. Moreover, immunoglobulin G and antinuclear antibody titers were also higher in anti-GPI Ab-positive than in -negative patients ($P = 0.0003$ and $P = 0.002$, respectively), so it is possible that anti-GPI Ab production is relevant to production of some types of autoantibody. These findings suggest that anti-GPI Abs are correlated with severe arthritis in RA patients and might be a useful arthritic marker in some RA patients.

Materials and methods

Patients

Serum samples were obtained from 137 patients with RA (23 men, 114 women) in Tsukuba University Hospital. All RA patients satisfied the classification criteria of the American College of Rheumatology (1987).[10] Their mean age was 56.8 years (range, 15–85), and mean disease duration was 12.6 years (range, 1–46 years). At the time of this study, 133 patients were receiving medication (50 were receiving methotrexate, and 99 were receiving oral prednisolone).

Serum samples were also obtained from 131 patients with systemic lupus erythematosus (SLE) in Tsukuba University Hospital, and from 139 healthy subjects (HS). Their mean ages were 42.0 years (range, 18–75) and 32 years (range, 20–63), respectively. All SLE patients satisfied the 1997 revised American College of Rheumatology criteria for SLE.[11]

At the time of routine venepuncture, informed consent for drawing blood was obtained from all patients and participating HS.

Enzyme-linked immunosorbent assay for detecting anti-GPI antibody

Blood samples were centrifuged, and the serum obtained was divided into aliquots. To select anti-GPI-positive patients, we used recombinant human GPI (huGPI), which has been described in detail previously,[6] or rabbit muscle GPI (raGPI) (Sigma, St Louis, MO, USA). Both antigens were used at 5 μg/ml (diluted in phosphate-buffered saline, PBS) to coat microtiter plates (Sumilon S, Sumitomo Bakelite, Tokyo, Japan) (12 h, 4°C). After the plates were washed three times with washing buffer (0.05% Tween 20 in PBS), Block Ace (diluted 1/4 in 1×PBS, Dainippon Pharmaceuticals, Osaka, Japan) was used for saturation (30 min at 37°C). After two washes, sera (diluted 1/50) were added and the plates incubated for 12 h at 4°C. After washing, alkaline phosphatase (AP)-conjugated anti-human IgG (Fc-fragment specific, American Qualex, San Clemente, CA, USA) was added to the plate (dilution: 1/500, 1 h, room temperature). After three washes, color was developed with AP reaction solution (containing 9.6% diethanol amine and 0.25 mM $MgCl_2$, pH 9.8) with AP substrate tablets (Sigma; one AP tablet per 5 ml of AP reaction solution). Plates were incubated for 1 h at room temperature, and the optical density (OD) was measured by plate spectrophotometry at 405 nm. Determinations were performed in triplicate and standardized between experiments by reference to a highly positive human anti-GPI serum. The primary reading was processed by subtracting OD readings of control wells (coated with gluthathione-S-transferase (GST) and Block Ace for huGPI-GST and raGPI, respectively). The cutoff OD was calculated from the enzyme-linked immunosorbent assay (ELISA) reaction of 137 healthy control Japanese donors. The mean value plus one standard deviation was 0.98 to human recombinant GPI, and 0.63 to rabbit native GPI. Double-positive populations were considered anti-GPI Abs-positive.

Rheumatoid factor, antinuclear antibodies, and immunoglobulin G (IgG) concentrations

Rheumatoid factor (RF) was determined by a nephelometric commercial test (RFII; Tina-quant, Nissui, Tokyo, Japan). Antinuclear antibodies (ANAs) were determined by a standard indirect immunofluorescence technique on HEp-2 cells. Serum ANA titer at >1/40 was considered positive.

The immunoglobulin G (IgG) concentration was evaluated by a sandwich ELISA.

Statistical analysis

Patient groups were compared using the χ-squared test for proportions. Differences were considered significant whenever $P < 0.05$. Statistical analysis was performed using StatView for Macintosh statistical software (StatView Software; SAS Institute, Cary, NC, USA).

Results

ELISA for detecting anti-GPI antibody

As discussed in length previously,[6] we utilized two different sources of GPI, mainly to avoid cross-reactivity or contamination during preparation; in particular, contaminated protein in native rabbit GPI has been reported previously.[7] The recombinant form of human GPI is 100% identical to human GPI protein (confirmed by sequencing), but it may not have the same conformation or posttranslational modifications as the natural enzyme. Thus, we also used native GPI from rabbit liver, which is commercially available. While native rabbit GPI is not strictly identical to the human GPI protein (93% identity), it provides the native conformation of the enzyme with normal posttranslational modifications. Double positivity for human and rabbit GPI Abs was clearly correlated with the positive Western blot results.[6] When discriminated in this way, positivity of anti-GPI Abs in the serum was 12% (16/137) in patients with RA, 8% (10/131) in those with SLE, and 4% (6/139) in HS. The distribution of anti-GPI Abs, especially those showing independent positivity to huGPI or raGPI in RA and SLE patients and HS, are summarized in Fig. 1. Statistical analysis showed no significant difference in the anti-GPI Ab positivity between RA and SLE patients ($\chi^2 = 1.251$ with 2 degrees of freedom, $P = 0.3618$), although a significant difference was found between RA patients and HS ($\chi^2 = 5.098$ with 2 degrees of freedom, $P = 0.0418$).

Comparison of clinical features of anti-GPI-positive and -negative RA patients

To analyze the differences in clinical manifestations between anti-GPI Ab-positive and -negative RA patients, we compared mean age, disease duration, sex, and X-ray stage. Mean age was 56.0 (range, 40–70) versus 56.8 (range, 15–85) years, and mean disease duration was 9.8 (range, 1–25) versus 12.6 (range, 1–46) years for positive and negative patients, respectively. All 16 (100%) anti-GPI Ab-positive RA patients were women, but 23 (19%) anti-GPI Ab-negative RA patients were men ($P = 0.0559$) (Table 1). In terms of X-ray stage, we divided the patients into two groups, those in stage I or II and those in stage III or IV. Nine (56%) patients were stage III or IV and 7 (44%) were stage I or II among anti-GPI Ab-positive RA patients. Among anti-GPI Ab-negative RA patients, 71 (61%) were stage III or IV and 45 (39%) were stage I or II ($P = 0.705$) (Table 1).

Table 1. Clinical features and antinuclear antibodies (ANAs) of anti-glucose-6-phosphate isomerase (GPI) antibody (Ab)-positive and -negative rheumatoid arthritis patients

	Anti-GPI Abs		*P*
	Positive	Negative	
Mean age ($n = 137$)	56	56.8	*0.9000*
Disease duration (years) ($n = 137$)	9.8	12.6	*0.2336*
Sex (no. of patients) ($n = 137$)			
Male	0	23	*0.0559*
Female	16	98	
Disease stage ($n = 132$)			
I or II	7	45	*0.7050*
III or IV	9	71	
ANAs (no. of patients) ($n = 94$)			
Positive	8	7	*0.0003**
Negative	8	71	

*Statistically significant

This analysis showed that anti-GPI Abs were expressed predominantly in female patients, but the disease progression was not clearly different between anti-GPI Ab-positive and -negative RA patients.

Comparison of laboratory data for anti-GPI-positive and -negative RA patients

To investigate whether anti-GPI Abs were relevant with respect to inflammation markers, we compared C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) between anti-GPI Ab-positive and -negative RA patients. The CRP concentration in anti-GPI Ab-positive RA patients (mean, 2.56mg/dl; range, 0.11–10.30) was higher ($P = 0.049$) than that in anti-GPI Ab-negative RA patients (mean, 1.47mg/dl; range, 0.02–8.71) (Fig. 2A). In contrast, ESR was comparable; mean ESR was 48.1mm/h (range, 6–126) versus 42.3mm/h (range, 2–146) ($P = 0.467$) in positive and negative patients, respectively (Fig. 2B). We also analyzed the maximum CRP concentration throughout the disease course of RA; the mean maximum CRP was 5.71 (range, 0.82–11.83) in positive patients, versus 3.91 (range, 0.03–14.74) in negative patients ($P = 0.106$). These findings suggest that RA patients with anti-GPI Abs had an active form of arthritis.

We also investigated the relationship between the Abs IgG, RF, and ANA between anti-GPI Ab-positive and -negative RA patients. The mean IgG concentration was 1819mg/dl (range, 763–3308) in positive patients versus 1440mg/dl (range, 576–2095) in negative patients ($P = 0.0003$) (Fig. 2C). IgA and IgM titers, by contrast, were comparable between positive and negative patients (data not shown). The mean RF titer at the time of collection was 189U/ml (range, 6–992) in positive patients versus 138U/ml (range, 2–2120) in negative patients ($P = 0.5372$) (Fig. 2D). To discriminate whether RF positivity had any connection to anti-GPI Abs positivity, we screened RF positivity

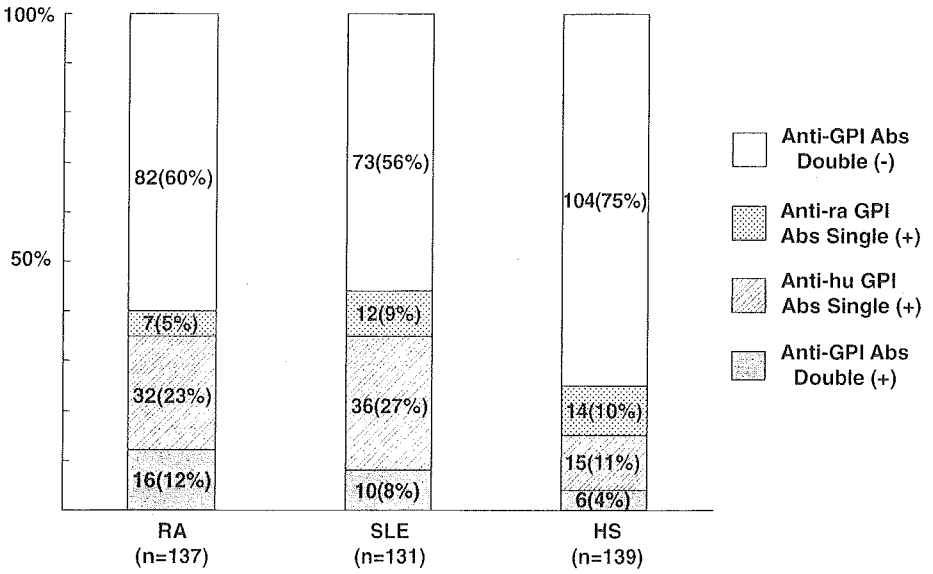

Fig. 1. Positivity against recombinant human (hu) and native rabbit (ra) glucose-6-phosphate isomerase (*GPI*) in rheumatoid arthritis (*RA*) and systemic lupus erythematosus (*SLE*) patients and healthy subjects (*HS*). This figure shows the distribution of the double-negative, single-positive (hu or ra), and double-positive population in patients with RA or SLE and in HS. *Ab*, antibody

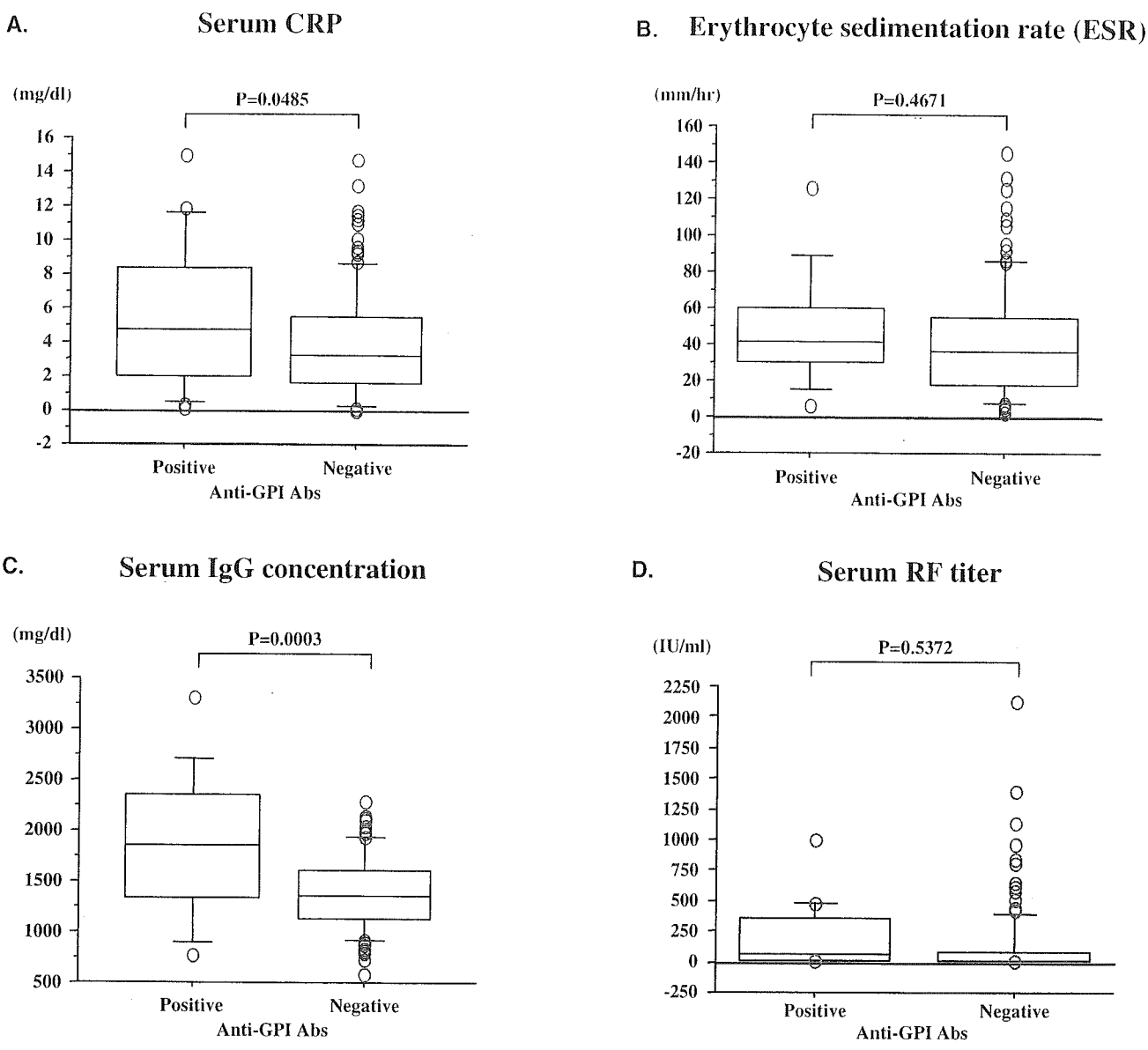

Fig. 2A–D. Comparison of laboratory data for anti-GPI-positive and -negative RA patients. **A** C-reactive protein (*CRP*); **B** erythrocyte sedimentation rate (*ESR*); **C** serum immunoglobulin G (*IgG*) concentration; and **D** the rheumatoid factor (*RF*) titer are compared. Each box represents statistical values. The intermediate line within the box marks the median, and the upper boundary of the box indicates the 75th percentile. The whiskers above and below the box indicate the 90th and 10th percentiles, respectively. The significance is expressed by the *P* values

throughout the disease course. Among anti-GPI Ab-positive RA patients, 13 (81%) were RF positive and 3 (19%) were negative. Among anti-GPI Ab-negative RA patients, 79 (66%) were RF positive and 40 (44%) were RF negative (P = 0.360). Because ANAs were not checked in all RA patients, we compared ANA positivity only in some of the RA patients. Among anti-GPI Ab-positive RA patients, ANAs were positive in eight (50%) and negative in eight (50%). In contrast, among anti-GPI Ab-negative RA patients, ANAs were positive in 7 (9%) and negative in 71 (91%) (P = 0.0003) (Table 1). The pattern of distribution of ANAs was not distinctive, but the prevalence was different between the two groups. In summary, anti-GPI Ab-positive RA patients had higher IgG and ANA positivity than negative patient, but the relationship between anti-GPI Ab positivity and RF was statistically unclear.

Consecutive follow-up study of anti-GPI Abs, RF, and CRP in an anti-GPI Ab-positive patient

To investigate the changes in hematological parameters in an anti-GPI Ab-positive patient, we checked anti-GPI Abs, RF, and CRP from the onset of arthritis. In 2000, at the disease onset, the patient was already positive for anti-GPI Abs, although completely negative for RF. In 2001, when

the disease was almost controlled, anti-GPI Abs and CRP had decreased, but RF had increased. In this patient, fluctuations of anti-GPI Abs were similar to those of CRP, suggesting that anti-GPI Abs might be a good marker for arthritis in some RA patients.

Discussion

The etiology of RA is multifactorial. To understand RA, autoAbs have been used as diagnostic tools and indicators of disease activity. Some autoAbs are elevated in RA patients, but a specific marker is not available.[12] Only few autoAbs have been identified as disease-specific Abs, and anti-GPI Abs are one of these candidate autoAbs identified has having arthritogenicity.[9] Here, we identified some anti-GPI Ab-positive RA patients in a Japanese cohort. Our ELISA assay was highly specific because we used both recombinant bacterial human GPI and native rabbit GPI; double positivity for the two antigens correlated significantly with the results of Western blotting for GPI.[6] However, the prevalence of these Abs in RA patients was only 12%; thus, their sensitivity is very low. In previous studies, when only native rabbit GPI was used, the prevalence of anti-GPI Abs in RA patients was 64%,[5] 45%,[13] or 23%.[14] Several research groups also stated that contaminant proteins introduced during rabbit GPI purification enhanced the reactivity.[6–8,14] On the other hand, can anti-GPI Ab-positive patients be accurately identified if we use only human recombinant GPI? In our previous study,[6] some cross-reactivity also occurred with human recombinant GPI, as confirmed by visualization of a band that was not identical to that of human recombinant GPI. This artifact was probably due to the lack of conformational structure and the glycosylation status of bacterial human recombinant GPI; the latter role was substituted for by native rabbit GPI in this study. Therefore, we utilized two different sources of GPI antigen to discriminate true-positive patients.

The prevalence of anti-GPI Abs was not high compared with that of anti-CCP Abs or RF. As long ago as several decades, RF was utilized as a useful diagnostic marker for RA. RF is elevated in 70%–80% of RA patients, but its pathogenic role is still uncertain.[15] As shown by our consecutive follow-up study of an RA patient, anti-GPI Abs were more highly correlated with disease activity than with RF, suggesting that anti-GPI Ab positivity is a good marker for discriminating the activity of RA in some patients (Fig. 3). Recently, anti-CCP Abs have been recognized as a better marker for the diagnosis of RA,[16] because of their early appearance[17] and their ability to predict the course of the disease.[18,19] We could not identify any difference in radiographic progression between anti-GPI Ab-positive and -negative RA patients, but almost all of the anti-GPI Ab-positive RA patients had elevated levels of anti-CCP Abs (unpublished observation, Yasukochi et al.). It is possible that GPI is also citrullinated and cross-reacts with CCP to some extent, so further analysis needs to be done.

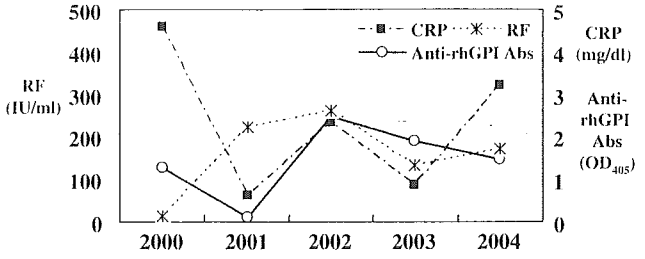

Fig. 3. Consecutive follow-up of hematological parameters in an anti-GPI Ab-positive patient. The figure shows changes in the hematological parameters (anti-rh GPI Abs, CRP concentration, RF titer) in the disease course of an anti-GPI Ab-positive RA patient. The anti-GPI Ab titer was more highly correlated with the CRP concentration than with the RF titer. This tendency was also detected in some other anti-GPI Ab-positive RA patients (data not shown). *OD*, optical density

Anti-GPI Ab-positive patients tend to have a high CRP concentration. Regarding the relationship between disease activity and anti-GPI Abs, several groups have reported that anti-GPI Ab positivity is correlated with disease activity and severity, in particular, extraarticular manifestations;[5,6,20] Felty's syndrome patients were highly represented in the first report.[5] Therefore, we also checked for the occurrence of vasculitis with RA (in Japanese, we termed this type of malignant RA "MRA"); only two MRA patients were part of the study, and one was anti-GPI Ab-positive and the other was negative. Unfortunately, there were no Felty's syndrome patients in our study. From these data, it is unclear whether anti-GPI Abs positivity is correlated with the occurrence of extraarticular manifestations in Japanese patients. However, some patients with C1q immune complexes were followed. Two anti-GPI Ab-positive patients among four RA patients (50%) had elevated levels of C1q immune complexes.

Anti-GPI Ab-positive patients had elevated levels of several Abs, including IgG and ANA ($P = 0.0003$ and $P = 0.002$, respectively). In the anti-GPI Ab-positive population, 70% of SLE patients had arthritis. It could be argued that among anti-GPI Ab-positive patients with RA or SLE, anti-GPI Abs are correlated with the occurrence of arthritis. Another possibility is that anti-GPI Abs are expressed as a result of polyclonal activation.

In summary, these findings suggest that anti-GPI Abs are correlated with severe arthritis in Japanese patients with RA, and so might be a useful arthritic marker in some RA patients. The role of this Ab in disease progression remains to be elucidated, and it is possible that there is an autoAb-dependent pathway in the development of human arthritis.[21]

Acknowledgments This work was supported in part by the Japanese Ministry of Science and Culture (I.M., T.S.) and PRESTO, Japan Science and Technology Agency (I.M.). I.M. is also a recipient of a fellowship from the Japan Intractable Diseases Research Foundation, Uehara Memorial Foundation, and Japan Rheumatoid Foundation. We thank Mrs. Chitose Okabe and Miss Hitomi Ikeda for their excellent technical assistance and Dr. Marianne Kimura for her critical reading of the manuscript.

References

1. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356–61.
2. Matsumoto I, Staub A, Benoist C, Mathis D. Arthritis provoked by linked T and B cell recognition of glycolytic enzyme. Science 1999;286:1732–5.
3. Matsumoto I, Maccioni M, Lee DM, Maurice M, Simmons B, Brenner M, et al. How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. Nat Immunol 2002;3:360–5.
4. Maccioni M, Zeder-Lutz G, Huang H, Ebel C, Gerber P, Hergueux J, et al. Arthritogenic monoclonal antibodies from K/BxN mice. J Exp Med 2002;195:1071–7.
5. Schaller M, Burton DR, Ditzel H. Autoantibodies to GPI in rheumatoid arthritis: linkage between an animal model and human disease. Nat Immunol 2001;2:746–53.
6. Matsumoto I, Lee DM, Goldbach-Mansky R, Sumida T, Hitchon CA, Schur PH, et al. Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and a spectrum of other chronic autoimmune disorders. Arthritis Rheum 2003;48:944–54.
7. Schubert D, Schmidt M, Zaiss D, Jungblut PR, Kamradt T. Autoantibodies to GPI and creatine kinase in RA. Nat Immunol 2002;3:411.
8. Kassahn D, Kolb C, Solomon S, Bochtler P, Illges H. Few human autoimmune sera detect GPI. Nat Immunol 2002;3:411–2.
9. Benoist C, Mathis D. A revival of the B cell paradigm for rheumatoid arthritis pathogenesis? Arthritis Res 2000;2:90–4.
10. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
11. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725
12. Steiner G, Smolen J. Autoantibodies in rheumatoid arthritis and their clinical significance. Arthritis Res 2002;4(Suppl 2):S1–5.
13. Jouen F, Vittecoq O, Leguillou F, Tabti-Titon I, Menard JF, Mejjad O, et al. Diagnostic and prognostic values of anti glucose-6-phosphate isomerase antibodies in community-recruited patients with very early arthritis. Clin Exp Immunol 2004;137:606–11.
14. Herve CA, Wait R, Venables PJ. Glucose-6-phosphate isomerase is not a specific autoantigen in rheumatoid arthritis. Rheumatology (Oxford). 2003;42:986–8.
15. Soltys AJ, Axford JS, Sutton BJ. Rheumatoid factors: where are we now? Ann Rheum Dis 1997;56:285–6.
16. Lee DM, Schur PH. Clinical utility of the anti-CCP assay in patients with rheumatic diseases. Ann Rheum Dis 2003;62:870–4.
17. Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831–5.
18. Vencovsky J, Machacek S, Sedova L, Kafkova J, Gatterova J, Pesakova V, et al. Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. Ann Rheum Dis 2003;62:427–30.
19. Meyer O, Labarre C, Dougados M, Goupille P, Cantagrel A, Dubois A, et al. Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five years radiographic damage. Ann Rheum Dis 2003;62:120–6.
20. van Gaalen FA, Toes RE, Ditzel HJ, Schaller M, Breedveld FC, Verweij CL, et al. Association of autoantibodies to glucose-6-phosphate isomerase with extraarticular complications in rheumatoid arthritis. Arthritis Rheum 2004;50:395–9.
21. Matsumoto I, Sumida T. B cells and immunoglobulins dependent mechanism in rheumatoid arthritis. Ther Apher 2002;6:317–9.

The exploration of joint-specific immunoreactions on immunoglobulins G of anti-glucose-6-phosphate isomerase antibody-positive patients with rheumatoid arthritis

ISAO MATSUMOTO[1,3], YOSHIFUMI MURAKI[1], TAKANORI YASUKOCHI[1,3], ZHANG HUA[1,3], YUKO KORI[1], TAICHI HAYASHI[1], DAISUKE GOTO[1], SATOSHI ITO[1], AKITO TSUTSUMI[1], KOTARO IKEDA[2], HIROSHI SUMICHIKA[4] and TAKAYUKI SUMIDA[1]

Divisions of [1]Rheumatology and [2]Orthopedic Surgery, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tenodai, Ibaraki 305-8575; [3]PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama; [4]Mitubishi Pharma Corporation, 1000, Kamoshida-cho, Aoba-ku, Yokohama, Japan

Received April 13, 2005; Accepted May 16, 2005

Abstract. The pathogenic role of autoantibodies in rheumatoid arthritis (RA) remains elusive. Anti-glucose-6-phsosphate isomerase (GPI) antibodies (Abs) are candidates for arthritogenic Abs because they directly induce arthritis in mice. High titers of anti-GPI Abs are found in some RA patients with severe forms. The aim of this study was to analyze the role of IgG, including anti-GPI Abs, in the joints of RA patients. Synovial tissue was obtained from 6 patients with RA (3 anti-GPI Abs- positive and 3 anti-GPI Abs-negative) and compared histologically and immunohistochemically for IgG and C3 deposition. IgG fractions were separated from the sera of anti-GPI Abs-positive RA patients and healthy subjects, and injected into the metacarpophalangeal joints of 4 cynomolgus monkeys. On day 16, the joints were harvested and examined histologically and immunohistochemically. The expression of the C5a receptor (C5aR) molecule in the synovium was quantified by real-time PCR using cDNA from the monkeys' joints. The synovia of anti-GPI Abs-positive RA patients showed diffuse infiltration of cells, including mast cells, and strong deposition of IgG and C3. In monkeys, IgG from RA patients, including anti-GPI Abs, resulted in recruitment of granulocytes and mononuclear cells, strong deposition of IgG on the articular surface, and overexpression of C5aR, but no joint swelling. No infiltrated cells or IgG deposition were observed in monkeys injected with IgGs from healthy subjects. Our results suggest that IgG fraction from RA patients, including that of anti-GPI Abs, may play a role in the synovitis of RA, although the pathogenesis of human anti-GPI Abs is still uncertain.

Correspondence to: Dr Isao Matsumoto, Division of Rheumatology, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan
E-mail: ismatsu@md.tsukuba.ac.jp

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

Introduction

Recently, the K/BxN T cell receptor transgenic mouse model was described as a model of inflammatory arthritis, characterized by arthritic manifestations similar to those of rheumatoid arthritis (RA) (1). Matsumoto *et al* (2) reported that arthritis could be almost entirely sustained by autoantibodies to the self-antigen glucose-6-phosphate isomerase (GPI). However, once the pathogenic antibodies (Abs) have been produced by the autoimmune reaction, there is no further requirement for lymphoid cells (3). The effector mechanisms of anti-GPI Abs have been confirmed by the requirement of innate immune system players (e.g. complement cascade, FcγR, neutrophils and mast cells) (4-7). More recently, immunization with human GPI was reported to provoke arthritis in DBA/1 mice, supporting the notion that autoimmunity to GPI directly plays some role in arthritis in genetically unaltered mice (8). These results also indicated that ubiquitous antigens might be the targets of arthritogenic Abs.

The first report on anti-GPI Abs in humans showed a high frequency of Abs in the sera of RA patients (9), though their frequency is still debated (10-14). Our anti-GPI Abs assay (10) seemed to be highly specific because it employs two different GPIs; a recombinant human GPI and a rabbit native GPI. Our results showed that only 15% of RA patients had high titers of anti-GPI Abs, although the severity of arthritis correlated with serum levels of anti-GPI Abs (10). Other authors have shown that extra-articular complications in RA are associated with serum levels of anti-GPI Abs (15). However, the arthritogenic role of these Abs remains unclear. To explore the role of human IgG including anti-GPI Abs in the joints, we compared the synovia of anti-GPI Abs-positive or -negative RA patients by histology and immunohistochemistry. We demonstrated the diffuse infiltration of inflammatory cells, including mast cells, in the synovium, lack of germinal-center

Table I. Clinical data and anti-GPI titer in six patients with RA.

	Age/Sex	Disease duration (years)	RF	CRP (mg/dl)	Rheumatoid nodule	Hu-GPI Abs OD405	Ra-GPI Abs OD405	Anti GPI-Abs
RA1	68/F	23	+	4.01	-	2.43	2.55	+
RA2	66/F	20	+	4.47	+	1.78	3.13	+
RA3	71/F	25	+	0.92	+	2.6	3.47	+
RA4	72/F	24	+	1.1	-	0.62	0.05	-
RA5	67/F	20	-	3.78	-	0.59	0.34	-
RA6	60/F	15	+	1.61	-	1.07	0.02	-

Cutoff values; Hu-GPI, 1.32 OD, Ra-GPI, 0.94 OD. RF, rheumatoid factor; +, positive; -, negative.

like structures, and the formation of immuno-complexes with C3 on the synovial surface.

There are significant differences between mice and humans with regard to the immune system, especially the innate immune system, including complement and immuno-complex-Fc receptor cascades (16). To investigate whether human anti-GPI Abs present in the serum have arthritogenic properties, we injected serum anti-GPI Abs, obtained from patients with RA, into several strains of mice. However, this did not produce swelling of the joints in mice (unpublished data). The results of these preliminary experiments suggested that human GPI Abs did not solely induce arthritis, although we could not confirm the differences in the innate immune system between humans and mice. In such experiments, it is better to use monkeys because they are genetically closer to humans than mice. Moreover, recent studies reported that orally-administered C5aR antagonist is only effective in humans and cynomolgus monkeys (17). Based on these properties, we used cynomolgus monkeys in this study. Intra-articular injection of human serum IgGs containing anti-GPI Abs in cynomolgus monkeys resulted in immunocomplex formation on the articular surface and intra-articular accumulation of granulocytes and mononuclear cells. These findings suggest that RA IgG including anti-GPI Abs, present in serum, preferentially form on the articular surface of immunocomplexes that might induce complement activation via C5aR-bearing cells, resulting in minor synovitis. However, the direct pathogenic role of IgG from human RA is still uncertain.

Patients and methods

Patients and tissue samples. Patients with RA were recruited from the Autoimmune Disease Clinic at Tsukuba University Hospital. Samples of synovial tissue were obtained from six RA patients including three anti-GPI Abs-positive, and three anti-GPI Abs-negative, who underwent knee replacement surgery. The study was approved by the local ethics com-mittee, and written informed consent was obtained from all participants. The diagnosis of RA was based on the criteria of the American College of Rheumatology (18). Table I summarizes the clinical data of participating patients.

Enzyme-linked immunosorbent assay for GPI. To select anti-GPI-positive patients, we used recombinant human GPI (huGPI), or rabbit muscle GPI (raGPI) (Sigma, St Louis, MO), which has been described in detail previously (10). Briefly, both antigens were used at 5 μg/ml (diluted in phosphate-buffered saline, PBS) to coat microtiter plates (12 h at 4°C). After washing, Block Ace (diluted 1/4 in 1xPBS, Dainippon Pharmaceuticals, Osaka, Japan) was used for saturation (30 min at 37°C). After washing, sera (diluted 1/50) were added and the plates were incubated for 12 h at 4°C. After washing again, alkaline phosphatase (AP)-conjugated anti-human IgG (Fc-fragment specific, Jackson Immuno Research, West Grove, PA) was added to the plate (dilution: 1/1000, 1 h, room temperature). After three washes, color was developed with AP reaction solution (containing 9.6% diethanol amine, 0.25 mM $MgCl_2$, pH 9.8) with AP substrate tablets (Sigma; one AP tablet per 5 ml of AP reaction solution). Plates were incubated for 1 h at room temperature and the OD was measured by plate spectrophotometry at 405 nm. Determinations were performed in triplicate, and standardized by reference to a highly positive human anti-GPI serum. The primary reading was processed by subtracting OD readings of control wells (coated with GST and Block Ace for huGPI-GST and raGPI, respectively). The cutoff OD was calculated from the ELISA reaction of 137 healthy control 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 Abs-positive.

Histopathological and immunohistochemical examinations. After knee replacement surgery (written informed consent was also obtained), the synovium from patients with RA were embedded in optimal cutting temperature (OCT) compound, frozen in dry ice isopentane, and 5-μm thick sections were mounted at -25°C. Slides were stored at -80°C until use, then acetone fixed for 30 sec and dried for 30 min. Tissue sections were lightly counterstained with hematoxylin-eosin (H&E) or standard toluidine blue. Stained sections were examined at 1000 diameters and at least 100 fields were evaluated in each specimen. Total mast cells / randomized 10fields were counted in three anti-GPI Abs-positive and -negative RA. The deposition of C3 and IgG was detected by 1/100 diluted fluorescein iso-thiocyanate (FITC)-conjugated sheep anti-

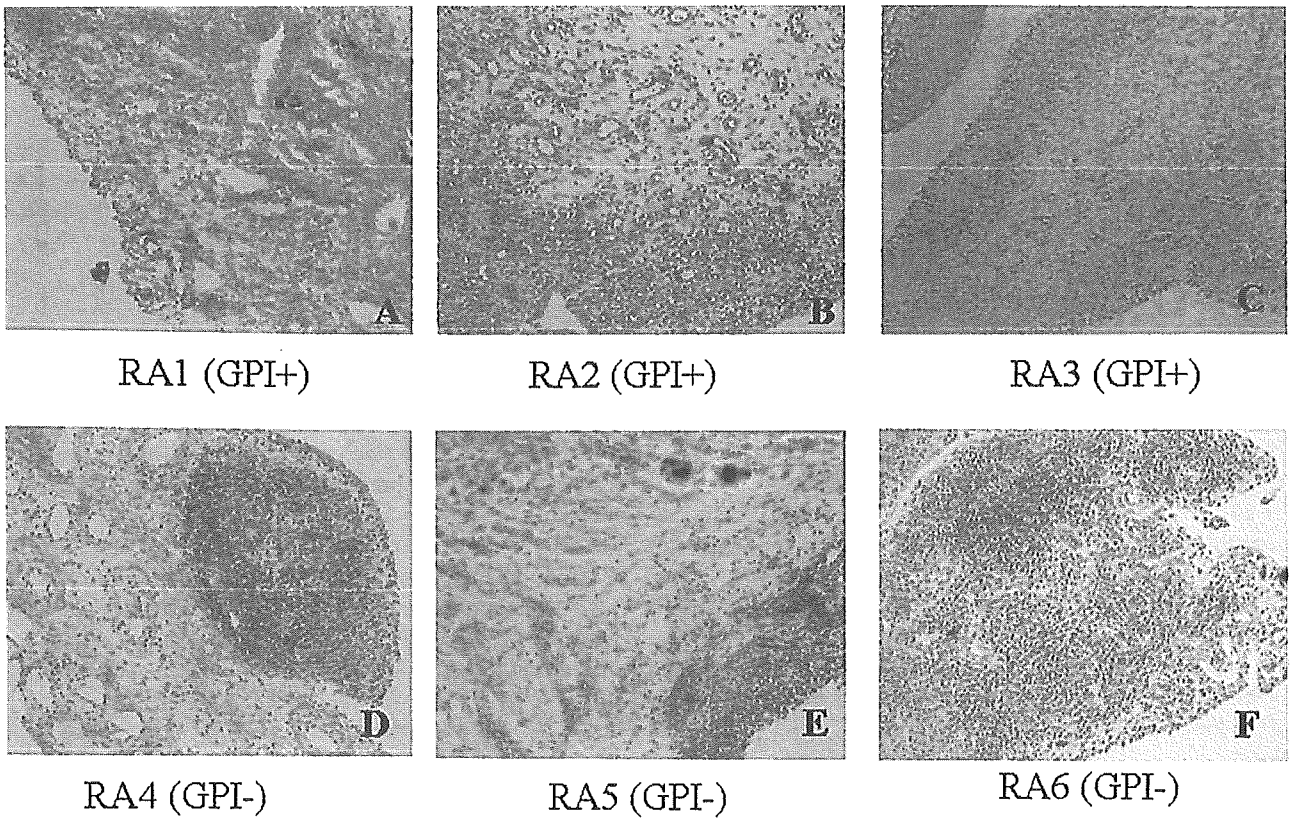

Figure 1. H&E staining of the synovium of RA patients with and without anti-GPI Abs. H&E staining of RA synovium. A, RA1 (anti-GPI Abs-positive RA) x100; B, RA2 (anti-GPI Abs-positive RA) x100; C, RA3 (anti-GPI Abs-positive RA) x100; D, RA4 (anti-GPI Abs-negative RA) x100; E, RA5 (anti-GPI Abs-negative RA) x100; F, RA6 (anti-GPI Abs-negative RA) x100. Note the diffuse distribution of infiltrated cells throughout the tissue samples in anti-GPI-positive patients (A, B, C). In contrast, note the germinal center-like structure (D, F) or cell aggregation (E) in three anti-GPI negative samples.

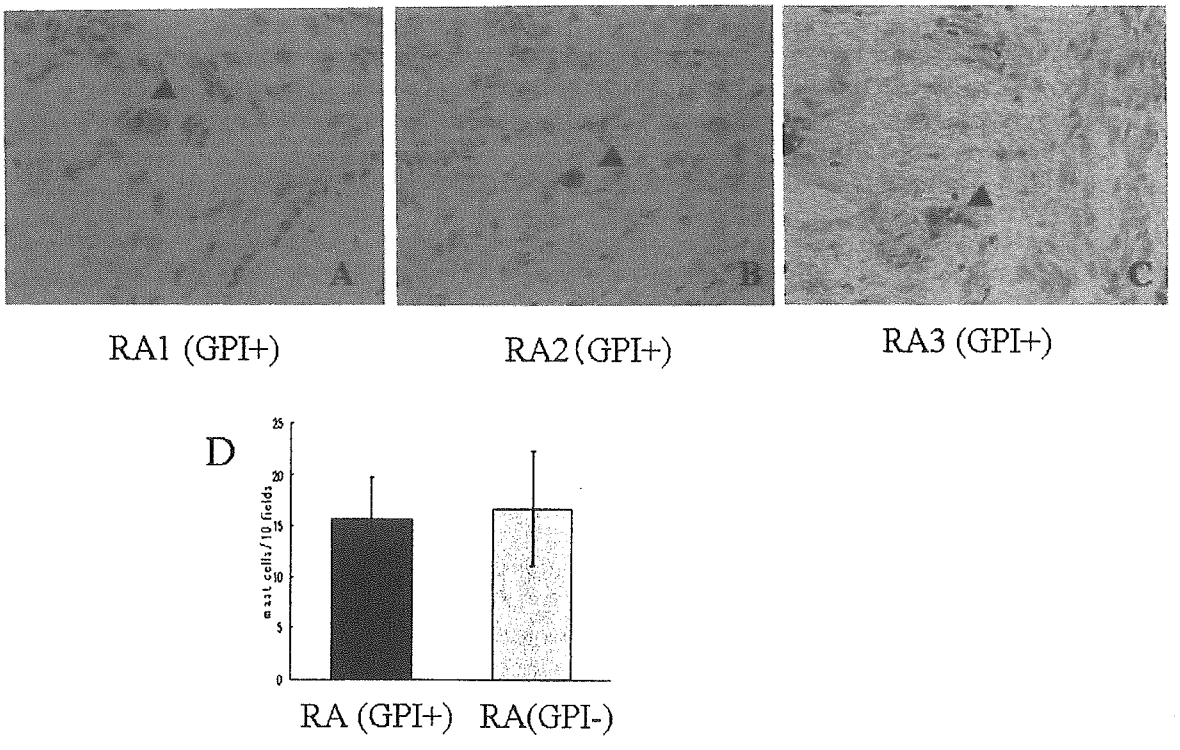

Figure 2. Mast cell involvement in the synovial tissue of RA patients with anti-GPI Abs. Toluidine blue staining (A, B, C) was performed. RA1 (anti-GPI Abs-positive RA) (A, D), RA2 (anti-GPI Abs-positive RA), RA3 (anti-GPI Abs-positive RA). Arrowheads indicate mast cells (A, B, C). Anti-GPI Abs-negative RA patients also had comparable mast cells in the synovium (D). Magnification, x200 A-C.