Elevated levels of anti-glucose-6-phosphatase isomerase (GPI) IgG in serum and synovial fluid from patients with inflammatory arthritis

Monica Maria Schaller, William Stohl, Soon-Min Tan, Vivian M Benoit, David M Hilbert, and Henrik J Ditzel

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Extended Report:

Elevated levels of anti-glucose-6-phosphate isomerase (GPI) IgG in serum and synovial fluid from patients with inflammatory arthritis

M Schaller, W Stohl, SM Tan, VM Benoit, DM Hilbert, HJ Ditzel

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ABSTRACT

Objectives: In K/BxN mice, anti-glucose-6-phosphate isomerase (GPI) antibodies (Abs) are arthritogenic, and their adoptive transfer into naïve mice induces arthritis. Anti-GPI Abs develop in many human RA patients and are associated with more severe forms of the disease. To elucidate the anti-GPI IgG profiles among different patient groups, we analyzed a panel of concomitantly-obtained sera and synovial fluids (SF) from patients with a variety of arthritides.

Methods: Blood and SF obtained concomitantly from 91 clinically well-defined arthritis patients were tested for concentrations of total anti-GPI IgG, anti-GPI IgG subclasses, B lymphocyte stimulator (BLyS), and APRIL by ELISA.

Results: Although anti-GPI IgG was detected in sera and SF of patients with many arthritic diseases, it was preferentially associated with inflammatory arthritis in general, and with RA in particular. The anti-GPI IgG subclass usage was skewed and varied among the different arthritic disease groups. Inverse correlations between serum levels of BLyS and anti-GPI IgG and positive correlations between serum levels of APRIL and anti-GPI IgG were observed among immune-based arthritis patients and RA patients but not among non-immune-based patients. No such correlations were appreciated in SF from any group of arthritis patients.

Conclusion: Elevated circulating anti-GPI Ab levels are not unique to RA patients but are present in many patients with inflammatory arthritis. The difference in anti-GPI IgG subclass usage among disease groups may influence effector function and disease outcome. The inverse correlation between serum BLyS and anti-GPI IgG levels suggests that anti-GPI B cells may be regulated differently from other autoantibody-producing B cells. Anti-GPI Abs may serve a pathogenic function in humans by promoting the maintenance of existing disease.

Keywords: rheumatoid arthritis, glucose-6-phosphate isomerase, BLyS, human autoantibodies, IgG subclasses
INTRODUCTION

The pathogenesis of rheumatoid arthritis (RA) is multi-factorial, encompassing genetic influences on susceptibility, environmental factors, immune mechanisms, and amplifying cytokine networks that perpetuate inflammation (1-4). The precise mechanism(s) of disease development, however, remains unclear. The association of RA with certain HLA-DR-shared epitope-bearing alleles points to a central role for CD4+ T cells in the initiation and propagation of joint inflammation (5). A pathogenic role for autoantibodies in RA and other forms of inflammatory arthritis is less certain, but support for this notion comes from studies utilizing a T cell receptor (TCR)-transgenic arthritis mouse model, K/BxN. In these mice, glucose-6-phosphate isomerase (GPI) can serve as an autoantigen for both B and T cells, and adoptive transfer of anti-GPI Abs to naïve mice can induce inflammatory arthritis with features similar (but not identical) to human RA (6). In addition, immunization of genetically-unaltered mice with GPI has been shown to induce peripheral polyarthritis (7). We previously detected anti-GPI IgG Abs in a large proportion of RA patients but rarely in patients with Sjögren’s syndrome, Lyme arthritis, or osteoarthritis (OA) or in healthy age- and gender-matched individuals (8), and elevated levels of anti-GPI Abs have been associated with more severe forms of RA (9). To assure that the observed serum anti-GPI IgG reactivity was due to GPI-specific Abs, anti-GPI IgG mAbs were generated from one of the RA patients and shown to be of high affinity and highly specific for GPI (8). The presence of anti-GPI Abs in RA patient sera has been confirmed by others, albeit in most studies at a frequency lower than what we had observed (10-14).

BLyS, a recently identified member of the tumor necrosis factor (TNF) ligand superfamily (15-20), if administered in vivo to mice induces B cell expansion and polyclonal hypergammaglobulinemia (15) which is, at least in part, consequent to inhibition of B cell apoptosis and enhanced B cell survival (21-24). Constitutive overexpression of BLyS in blys-transgenic mice leads to elevated serum titers of multiple autoantibodies, including anti-dsDNA and rheumatoid factor (RF) autoantibodies (20, 25-26). Increased levels of BLyS are found in patients with conditions associated with polyclonal hypergammaglobulinemia and elevated circulating autoantibody titers, including systemic lupus erythematosus (SLE), RA, Sjögren’s syndrome, and HIV infection (27-32). Positive correlations between circulating BLyS and autoantibody levels were observed in most of these studies (27-30), suggesting a direct role for BLyS in the development of elevated circulating autoantibodies.

In this report, we analyzed concomitantly-obtained serum and SF samples from patients with different arthritic diseases to evaluate the anti-GPI IgG responses in more detail. Our results show that anti-GPI Abs are not specific for RA but are rather broadly associated with inflammatory arthritis. Moreover, an inverse correlation between serum levels of anti-GPI IgG and BLyS was observed, indicating that the anti-GPI autoimmune response exhibits features distinct from other systemic autoantibody responses.

METHODS AND PATIENTS

Subjects were recruited for this study from the Los Angeles County + University of Southern California Medical Center (LAC + USC MC). Diagnosis was based on established clinical criteria (40). Sera and SF samples were concomitantly obtained from 91 patients diagnosed with RA (n=37), crystal-induced arthritis (Crys, including 12 gout and 2 CPPD (n=14)), seronegative spondylarthropathies (SpA) including 6 Reiter’s syndrome and 5 ankylosing spondylitis (n=11), osteoarthritis (OA) (n=13), traumatic arthritis (Tr) (n=6) and 10 with other forms of arthritis.
including GC arthritis (n=1), culture-negative infectious arthritis (n=1), adenocarcinomatous arthritis (n=1), undifferentiated inflammatory polyarthritis (n=1), polymyalgia rheumatica (n=1), TB arthritis (n=2), and SLE (n=3). Patients with RA, SpA, undifferentiated inflammatory arthritis, polymyalgia rheumatica, and SLE were classified as “immune-based arthritis”, whereas patients with Crys, OA, Tr, and infectious arthritis were classified as “non-immune-based arthritis”. The demographic and medication data of the study patients are listed in table 1.

### Table 1  Subject demographic and medication

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<thead>
<tr>
<th></th>
<th>RA (n=37)</th>
<th>OA (n=13)</th>
<th>Tr (n=6)</th>
<th>Crys (n=14)</th>
<th>SpA (n=11)</th>
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Abbreviations: F, female; M, male; Ar, Arabic; As, Asian; B, Black; H, Hispanic; W, White; Pred, prednisone; MTX, methotrexate; AZA, azathioprine; SSA, sulfasalazine; HCQ, hydroxychloroquine; Lef, leflunomide; Etan, etanercept; Inflix, infliximab.

BLyS and APRIL levels in the sera and SF of the majority of these patients have recently been reported (41). Coded serum and SF samples were shipped from LAC + USC MC to The Scripps Research Institute for blind measurement of total anti-GPI antibody and IgG subclass concentrations and to our collaborators at Human Genome Sciences (HGS, Rockville, MD) for blind measurement of BLyS and APRIL concentrations by ELISA. Protocol and the use of all human samples were approved by the IRBs of The Scripps Research Institute and USC.

**Purification of anti-GPI Abs from serum and SF of RA patients**

IgG from ten to thirty ml of serum from RA patients with a high titer of anti-GPI IgG (as previously determined by ELISA) or control serum from a healthy individual were purified using a protein A-Sepharose 4 fast flow column (Amersham, Piscataway, NJ). Subsequently, the anti-GPI IgG Abs were purified by binding to an affinity column of recombinant human GST-GPI
coupled to CNBr-activated Sepharose 4B (Amersham, Piscataway, NJ). Recombinant human GPI was cloned and purified as described previously (42). Bound IgG was eluted at pH 3.3 using 100 mM citric acid and dialyzed against PBS. These purified anti-GPI preparations were used as standards for the ELISAs.

Autoantibodies against GPI in serum and SF samples

ELISA was performed as described previously (8). Briefly, human recombinant purified GPI (hGPI) or rabbit GPI (rGPI) from muscle Type XI (EC 5.3.1.9, Sigma, Saint Louis, MO) were coated at 5 µg/ml in PBS overnight at 4°C. As standard, serial dilutions (0.02-5 µg/ml) of a serum with known concentration of total IgG (Accurate Chemical, M1590, Westbury, NY) was coated on the same ELISA plate, allowing calculation of the exact concentration of anti-GPI IgG from this standard curve. After blocking for 2 h at 37°C with 3% BSA, sera and SF samples diluted 1:50-1:800 were incubated for 3 h at 37°C. For the standard curve, the wells coated with standard serum were incubated with PBS in this step. Following washing with PBS-0.05% Tween 20, the bound IgG from the serum or SF samples and the standard serum were detected with alkaline phosphatase (AP)-labeled F(ab')2 fragment of a goat anti-human IgG Fc-specific antibody (Jackson, West Grove, PA, 1:1000 in PBS), visualized with p-nitrophenyl phosphate (NPP) substrate and read at 405 nm. As an internal standard, two RA sera were included, one with high and one with a moderate anti-GPI titer as well as the affinity-purified anti-GPI Ab preparation obtained from RA patients. The exact concentration of anti-GPI IgG (in µg/ml), in the patient sera or SF, was calculated from the standard curve in the linear range. Inter-assay variation was < 15%. For the anti-GPI IgG subclass distribution analysis the following subclass-specific Abs: murine anti-human IgG1 at 1 µg/ml, murine anti-human IgG2 and murine anti-human IgG4 at 5 µg/ml (all BD Pharmingen, San Diego, CA), murine anti-human IgG3 at 5 µg/ml (Zymed, San Francisco, CA) were incubated for 1 hr at 37°C. Bound anti-IgG subclass specific Abs were detected with an AP-labeled goat anti-murine F(ab')2 fragment specific antibody (Jackson, West Grove, PA), and visualized with NPP substrate and read at 405 nm. As standards, serial dilutions (0.02-20.0 µg/ml) of a serum with known concentration of each of the four IgG subclasses as well as a serum for specific quantification of IgG2 (both from Accurate Chemical, M1564 Westbury, NY) were used. The exact concentration of anti-GPI Abs (in µg/ml) in the patient sera and SF were calculated from the linear range of the standard curves, as described above. Inter-assay variation was < 20%. The total anti-GPI IgG concentration as measured by adding the individual anti-GPI subclass concentrations was shown to correlate (r=0.768) with the total anti-GPI IgG concentration measured using a polyclonal anti-Fc Ab, such as represented in fig 1A, 1B and fig 2A indicating that the anti-Fc Ab detected the different subclasses equally well.

BLyS and APRIL levels in serum and SF samples

BLyS and APRIL levels were determined by ELISA as described previously (29, 32, 43).

Statistical analysis

All analyses were performed using either Statview 4.1 (Abacus Concepts Inc., Cary, NC) or SigmaStat (SPSS, Chicago, IL) software. Since neither sera nor SF samples were distributed normally, nonparametric testing using Mann-Whitney rank sum test between 2 groups, or Kruskal Wallis one-way ANOVA on ranks among 3 or more groups were performed. Correlations were determined by Spearman rank order correlation.
RESULTS
Detection of anti-GPI Abs in serum and SF

Several previous studies of circulating anti-GPI Ab levels have reported results in terms of arbitrary units (10-14). To quantify anti-GPI Ab concentrations as µg/ml of IgG, a human IgG anti-GPI Ab standard was prepared by affinity purification of sera from RA patients with high-titer anti-GPI Ab. Analysis of the purified human anti-GPI IgG showed that the antibody preparation bound specifically to hGPI (but minimally to a panel of control antigens, fig 1A) and had a functional affinity comparable to that of serum from 60 day-old arthritic K/BxN mice (fig 1B). In contrast, little GPI binding was observed in total IgG from sera of healthy donors purified with the same procedure used for the human RA sera and the flowthrough from the GPI column of the RA IgG fraction.

Using both the affinity-purified anti-GPI Ab preparation and a standard serum with known total IgG and IgG subclass concentrations as reference standards, we tested sera from 37 patients with RA, 13 with OA, 6 with Tr, 14 with Crys, 11 with SpA, and 10 with other diagnoses. Measurable anti-GPI IgG Abs were routinely detected in all but the OA patient groups (fig 2A), although the anti-GPI IgG concentrations and frequencies of anti-GPI IgG positive sera varied significantly among the patient groups (P < 0.001). Positivity for serum anti-GPI IgG was defined as a concentration greater than the 95th percentile of the anti-GPI concentration of 21 sera from normal healthy controls (> 35.4 µg/ml). Eighteen of 37 RA sera (49%), 3 of 6 Tr sera (50%), 5 of 14 Crys sera (36%), 4 of 11 SpA sera (35%), 3 of 10 Other sera (30%), 1 SLE, 1 TB arthritis, and 1 undifferentiated inflammatory polyarthritis sera) and 0 of 13 OA were positive for anti-GPI IgG Ab. The median concentration of anti-GPI IgG Ab (32.8 µg/ml, range: 0.8-72 µg/ml) in serum from RA patients was significantly higher than that in OA patients (0.5 µg/ml, P< 0.0001) or in normal healthy control sera (7.6 µg/ml, P=0.028). However, no statistically significant differences in the median concentration of anti-GPI IgG Ab was found between the RA and the Crys (29.7 µg/ml), Tr (31.0 µg/ml), SpA (27.7 µg/ml) or Other (32.0 µg/ml) groups.

We also measured anti-GPI IgG Ab in SF samples using the cut-off value described for the serum samples (> 35.4 µg/ml) to determine positivity (fig 2B). Twenty of 37 RA SF (54%), 5 of 13 OA SF (38%), 5 of 14 Crys SF (36%), 2 of 6 Tr (33%), 5 of 11 SpA SF (45%) and 6 of 10 Other (60%) were positive. The median concentration of anti-GPI Abs in the SF samples was significantly higher than that of the serum samples (P < 0.001). The median SF anti-GPI IgG concentration in the RA (42.1 µg/ml, range: 1-266 µg/ml, P=0.041), SpA (86.9 µg/ml, P=0.03) and the Other groups (87.0 µg/ml, P=0.0257) were significantly higher than the OA group (13.5 µg/ml). Grouping the SF samples according to patients with immune- versus non-immune-based arthritis revealed a significantly higher concentration of anti-GPI IgG in the immune-based arthritis group (P = 0.025; fig 2B).

IgG subclass distribution of anti-GPI Ab in serum differs among arthritis disease groups

In the K/BxN mouse arthritis model, IgG1 subclass Abs have been found to constitute the bulk of the anti-GPI Ab response (45). To examine whether a specific subclass is preferentially used in the human anti-GPI Ab response, the serum concentration of the different subclasses (IgG1-4) was determined in patients with RA (n=37), Crys (n=12), OA (n=12) and Reiter’s syndrome (n=6). The anti-GPI IgG response in the different patient groups was composed of distinctly different IgG subclasses (fig 3). Positivity was defined as a serum anti-GPI IgG concentration...
greater than the 95th percentile of the mean anti-GPI concentration of 21 sera from healthy controls (IgG1> 29.0 µg/ml, for IgG2> 12.5 µg/ml, for IgG3> 6.1 µg/ml and for IgG4> 2.0 µg/ml). Only one RA sera, and none from the other arthritis groups, exhibited an IgG1 subclass anti-GPI response, the subclass generally constituting the major part of the Ab response to proteins (table 2).

**Table 2 Percentage of positive patient sera for anti-GPI IgG subclasses**

<table>
<thead>
<tr>
<th></th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
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<tr>
<td>RA (n=37)</td>
<td>2.7</td>
<td>2.7</td>
<td>21.6</td>
<td>35.1</td>
</tr>
<tr>
<td>Crys (n=12)</td>
<td>-</td>
<td>58.3</td>
<td>8.3</td>
<td>-</td>
</tr>
<tr>
<td>Reiter’s syndrome (n=6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>66.6</td>
</tr>
<tr>
<td>OA (n=12)</td>
<td>-</td>
<td>33.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NS (n=21)</td>
<td>-</td>
<td>4.8</td>
<td>4.8</td>
<td>4.8</td>
</tr>
</tbody>
</table>

RA=rheumatoid arthritis, Crys= crystal-induced arthritis, OA= osteoarthritis, NS=normal sera

The RA sera predominantly contained IgG3 and IgG4 anti-GPI Abs; OA and Crys sera predominantly contained IgG2 anti-GPI Abs; and Reiter’s syndrome sera predominantly contained IgG4 anti-GPI Abs (fig 3, table 2). The median concentration of anti-GPI IgG3 in RA sera was significantly higher than in Crys (P=0.003), Reiter’s syndrome (P=0.016) and OA sera (P=0.003). The sum of the anti-IgG1-4 concentrations correlated well (r² = 0.768) with the directly measured total IgG anti-GPI concentrations, indicating that the secondary antibody used in the ELISA was able to detect anti-GPI IgG of different subclasses.

**Correlations between anti-GPI IgG concentration and BLyS or APRIL protein levels in serum and SF samples of arthritic patients**

Previous studies have demonstrated positive correlations between serum BLyS and specific autoantibody levels (27-31). To assess whether similar correlations exist between BLyS or APRIL and anti-GPI IgG levels, we measured anti-GPI levels in paired sets of sera and SF in which BLyS and APRIL levels had already been determined (41). Surprisingly, serum anti-GPI concentrations correlated inversely, rather than positively, with serum BLyS levels among all arthritis patients regardless of their diagnoses (fig 4A, p=0.008), but did correlate positively with serum APRIL levels (fig 4B, p=0.028). These correlation patterns were mimicked when only patients with immune-based arthritis (fig 4 E-F, p=0.010, p=0.089) or only RA patients (fig 4 I-J, p=0.052, p=0.014) were evaluated but not when patients with non-immune-based arthritis were evaluated (data not shown). In contrast to the serum findings, no significant correlations between the concentrations of anti-GPI IgG and BLyS or APRIL in SF were appreciated in any of these patient cohorts (fig 4 C-D, G-H, K-L).

The inverse correlation among RA patients between serum levels of BLyS and anti-GPI IgG was especially surprising, since serum levels of BLyS and RF have previously been shown to positively correlate with each other (29). When we focused on the 18 RA patients for whom we had concurrent serum BLyS and RF levels, we again found a positive correlation between the levels of serum RF and BLyS (fig 5A) with no correlation appreciated between serum RF levels and serum APRIL levels (fig 5B). Among this subset of RA patients, we again appreciated an inverse correlation between the levels of serum anti-GPI IgG and serum RF levels (fig 5C).
DISCUSSION

To assess the potential human relevance of anti-GPI-mediated arthritis in K/BxN mice, we measured anti-GPI levels in sera and SF from human patients with a wide variety of arthritic conditions. We detected anti-GPI Abs in a large proportion of RA patients (fig 2), although at a frequency somewhat lower than that previously reported (49% vs. 64%) (8) and as well as in sera and SF from patients with other forms of arthritis. Serum anti-GPI IgG Abs levels were significantly higher in patients with immune- versus non-immune based arthritis, demonstrating that anti-GPI Abs are not disease-specific for RA but, rather, are prevalent among patients with a wide variety of immune-based inflammatory arthritis. Neither the lack of specificity for RA nor their likely secondary, rather than primary, role in the various disease states negates the importance of these Abs in perpetuating arthritis or the possibility that blocking the anti-GPI Ab response may improve disease outcome.

In K/BxN mice, the arthritogenic effect of anti-GPI response is mediated by Abs of a distinct subclass, IgG1, which activates the alternative complement pathway (50). We found that the human serum anti-GPI IgG subclass composition among the various arthritis disease groups was skewed (table 2). The IgG4 subclass represented a major part of the anti-GPI IgG response in Reiter’s syndrome and RA, but was not present in the other disease groups. Interestingly, IgG3 and IgG4 in humans, similar to IgG1 in mice, are supported by Th2 cytokines, while the antibody subclass, which generally dominates the immune response in both species (IgG1 in humans and IgG2a in mice, respectively), is supported by Th1 cytokines. A shift in the balance from Th1 to Th2 cytokines could substantially improve the arthritis conditions in a collagen induced arthritis mice model (51). Further studies to determine whether the differences in IgG subclass distribution of the anti-GPI Abs, found in different arthritis diseases, are indeed pathogenically important are therefore needed.

The particular IgG subclass profile observed for each type of arthritides seem to be specific for the anti-GPI response and not a general pattern of autoantibody responses to common autoantigens. In contrast to the subclass profiles we observed for the anti-GPI response, Cook et al. (52) found that the type II collagen response in RA patients were predominantly IgG1 and IgG3, and in OA and SLE patients predominantly IgG4. Further, Cambridge et al. (53) found that the anti-myeloperoxidase response in RA patients were predominantly of the IgG1 and IgG3 subclasses, while Todome et al. (54) found that the anti-streptococcal cell wall peptidoglycan response in RA patients were predominantly IgG2.

Recently, increased circulating levels of BLyS have been detected in several B cell-dependent autoimmune diseases, and a positive correlation between the circulating BLyS levels and the magnitude of systemic autoimmune antibody responses such as anti-dsDNA, anti-Sm, anti-SSA/SSB, anti-phospholipid, and RF has been observed (27-29, 55). These correlations and results from animal models suggest that BLyS may preferentially affect autoactive B cells leading to autoantibody production. It was, thus, highly surprising that instead of observing a positive correlation between serum BLyS and anti-GPI IgG levels, we routinely observed among our arthritis patients positive correlations between serum APRIL and anti-GPI IgG levels but inverse correlations between serum BLyS and anti-GPI IgG (fig 4). The inverse correlations between serum BLyS and anti-GPI IgG levels were appreciated despite a positive correlation between serum BLyS and RF levels in RA patients (fig 5).

A possible explanation for this surprising result is based on the highly complex relation between BLyS and its receptors. The three known receptors for BLyS (BAFF-R, TACI, and
BCMA) are all present on B cells to various degrees (56). The latter two receptors can also bind APRIL (16). Whereas BlyS binding to BAFF-R leads to maturation of B cells at several differentiation stages, BlyS binding to TACI inhibits B cell activation (57), thereby maintaining B cell homeostasis. Promotion of autoantibody production by BlyS may be mediated through BAFF-R, which has been shown to be the major BlyS receptor on human primary B cells (56). However, TACI is up-regulated in activated B cells, and incubation with agonistic anti-TACI antibody inhibits the ability of BlyS to co-stimulate B cell activation (56). It is possible that anti-GPI-producing B cells express high surface levels of TACI, which, upon engagement with BlyS, down-modulate activation of these B cells. Thus, high circulating levels of BlyS could be associated with low levels of circulating anti-GPI Ab.

An important role for APRIL may emerge from the recent findings (38) that plasmablasts (CD38+) up-regulate surface expression of BCMA, rendering them sensitive to survival signals delivered by both BlyS and APRIL. Findings that APRIL circulates at higher concentrations in RA patients (41), and that APRIL levels are positively correlated with anti-GPI Abs concentrations (fig 4B, F, J) may indicate that plasmablasts could be the anti-GPI-producing cells, and are especially sensitive to APRIL. Our findings raise the intriguing question of whether BlyS or APRIL antagonistic therapy would actually lower autoantibody levels of all specificities. Ongoing clinical trials in SLE patients with a neutralizing anti-BlyS Ab may help address this question for SLE-associated autoantibodies.

In conclusion, our results indicate that anti-GPI Abs are frequently found not just in RA patients but in patients with other forms of inflammatory arthritis as well. Assaying for anti-GPI IgG is not straightforward and may account for some of the conflicting results reported. Our data suggest that anti-GPI IgG is not the primary disease-causing event, but may perpetuate disease, in agreement with results from van Gaalen et al. (9), who found high-titers of anti-GPI Abs particularly associated in patients with severe forms of RA, i.e. Felty’s syndrome, vasculitis or rheumatoid nodules. Future studies will address whether neutralization of anti-GPI IgG will alleviate the clinical and/or pathological manifestation of inflammatory arthritis.

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FIGURE LEGENDS

Figure 1  Anti-GPI specific IgG purified from serum of a RA patient with high anti-GPI titer. (A) Samples from different purification steps including the RA serum before purification, the purified total IgG fraction and the purified anti-GPI IgG fraction were tested for their binding to rabbit GPI, hGPI, BSA and GST as control antigens in an ELISA. Mouse K/BxN serum and normal serum from a healthy individual (NS), were included as positive and negative controls. The fraction containing the purified anti-GPI IgG preparation was highly specific for hGPI and did not react with the other control antigens. (B) Titration of the human anti-GPI IgG fraction (●) showed positive GPI reactivity up to 0.6 µg/ml of hGPI, comparable to the mouse K/BxN serum (Δ), whereas no reactivity was found in the remaining (□) RA IgG fraction after affinity purification.
**Figure 2** Anti-GPI IgG concentration in sera and SF of patients with various arthritic diseases. Sera (A) and SF (B) obtained concomitantly from 37 patients with RA, 13 with OA, 6 with Tr, 14 with crystal-induced arthritis (Crys), 11 with seronegative spondyloarthropathies (SpA), 10 with other forms of inflammatory arthritis (Other) and as well as 21 normal healthy control sera (NS) were tested for binding to recombinant hGPI by ELISA. Bound IgG was detected with an alkaline phosphatase-conjugated F(ab)₂ goat anti-human IgG-Fc and the concentration calculated according to a standard serum with known concentration of IgG. The cutoff points for positivity were calculated as the 95% percentile of the concentrations in healthy control subjects (> 35.4 µg/ml). Immune-based arthritis (Imm) includes patients with RA, SpA, SLE, undifferentiated inflammatory polyarthritis, and polymyalgia rheumatica. Non-immune-based arthritis (Non) includes patients with OA, Tr, Crys, infectious arthritis, and adenocarcinomatous arthritis. The lines inside the boxes indicate the medians, the outer boxes indicate 25th and 75th percentiles; the bars extending from the boxes indicate 10th and 90 percentiles.

**Figure 3** Distinct anti-GPI IgG subclass composition observed for patients with different arthritic diseases. Patient sera from 37 RA, 12 OA, 12 Crys, 6 Reiter’s syndrome, and 21 healthy individuals (NS) were tested for binding to hGPI in an ELISA. Bound anti-GPI IgG was detected with subclass specific (IgG1-4) Ab, and the subclass concentration determined and expressed as the median concentration in µg/ml of the different anti-GPI IgG subclasses.

**Figure 4** Correlation between BLyS or APRIL levels and anti-GPI IgG concentration in serum and SF samples of arthritic patients. Correlations representing all 91 arthritis patients (A-D), only immune-based arthritis patients (E-H), or only RA patients (I-L).

**Figure 5** Correlation between rheumatoid factor (RF) and BLyS, APRIL or anti-GPI IgG levels in serum of RA patients. Serum RF values plotted against serum BLyS (A), serum APRIL (B), and serum anti-GPI IgG (C) for the RA patients for whom concurrent serum BLyS and RF levels were available.

**REFERENCES**


Fig. 1
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Fig. 2
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Fig. 3
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A $r = -0.278$  
$p = 0.008$

B $r = 0.240$  
$p = 0.028$

C $r = 0.157$  
$p = 0.139$

D $r = 0.053$  
$p = 0.622$

E $r = -0.353$  
$p = 0.010$

F $r = 0.245$  
$p = 0.089$

G $r = 0.122$  
$p = 0.381$

H $r = -0.205$  
$p = 0.141$

I $r = -0.322$  
$p = 0.052$

J $r = 0.417$  
$p = 0.014$

K $r = 0.296$  
$p = 0.075$

L $r = -0.043$  
$p = 0.797$

Fig. 4

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Serum BLyS (ng/ml)

- A: $r = 0.480$, $p = 0.058$
- B: $r = -0.240$, $p = 0.407$
- C: $r = -0.483$, $p = 0.057$

Serum RF (IU/ml)

Fig. 5
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Elevated levels of anti-glucose-6-phosphatase isomerase (GPI) IgG in serum and synovial fluid from patients with inflammatory arthritis

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