on timely referral should also include variables as discussed above. This is also suggested in gender studies in other health domains [6]. Multivariate analyses in large prospective studies will allow firm conclusions to be reached.

Evaluation of the PPP was supported by a grant from the Belgian Rheumatology Research Fund.

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Accepted 20 November 2005

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Characterisation of Th1/Th2 type, glucose-6-phosphate isomerase reactive T cells in the generation of rheumatoid arthritis

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Ann Rheum Dis 2006;**65**:968–969. doi: 10.1136/ard.2005.045286

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterised by an unknown inflammatory process in multiple joints. The K/BxN T cell receptor transgenic mouse model is a striking model of inflammatory arthritis characterised by arthritic manifestations similar to those of RA.[1] Matsumoto *et al* reported that arthritis could be provoked by linked T and B cell recognition of a ubiquitously expressed self antigen glucose-6-phosphate isomerase (GPI).[2] Recently, immunisation with recombinant human GPI was reported to induce T cell dependent arthritis in DBA/1 mice,[3] supporting the notion that GPI reactive T cells have a crucial role in the induction of arthritis.

In our previous study we reported the presence of high titres of anti-GPI antibodies (Abs) in some patients with RA, although a few control subjects were also positive.[4] To examine the role of GPI-specific T cells in patients with RA, we investigated the spontaneous Th1/Th2 response to GPI in patients with RA, systemic lupus erythematosus (SLE), and in healthy subjects with anti-GPI Abs.

To select anti-GPI Ab positive patients, an enzyme linked immunosorbent assay (ELISA) was performed using two different sources of GPI: a recombinant human GPI (huGPI), and a rabbit muscle GPI (raGPI; Sigma Chemical Co, St Louis, MO, USA), which have been described in detail previously.[4] Fifteen anti-GPI Ab positive patients with RA (from 185 with RA), four patients with SLE (from 135 with SLE), and four healthy subjects (from 145 controls) were studied (table 1). To analyse the possible relationship between HLA-DRB1 and anti-GPI Ab positivity, HLA-DRB1 alleles were screened. As shown in Table 1, 10 (67%) patients with RA and anti-GPI Abs shared the HLA-DRB1*0405 allele, which is one of the genes for susceptibility to RA in Japanese people, and five (33%) patients were DRB1*0901. In a recent report, the DRB1*0405 and *0901 alleles showed the most significant associations with RA in Korean people.[5] However, none of the four patients with SLE or four control subjects positive for anti-GPI Abs retained these alleles, suggesting a strong linkage between anti-GPI positive patients with RA with anti-GPI Abs and HLA DRB1*0405 and *0901 alleles (table 1).

To investigate the pathogenic relevance of GPI reactive T cells in subjects with anti-GPI Abs, a magnetic activated cell sorting cytokine secretion assay was performed using peripheral blood mononuclear cells plus GPI (in the presence of 10 μg purified human GPI protein digested by thrombin or 13.5 ng thrombin as a control). As a positive control, we used staphylococcal enterotoxin B (1 μg/ml). Cells (2×10^6) were harvested Ab-Ab directed against CD45 and either interferon (IFN) γ or interleukin (IL) 4 conjugates, and stained with phycoerythrin (PE)-conjugated anti-IFNγ or anti-IL4. Cells were magnetically labelled by anti-PE Ab microbeads, and were analysed on a FACSCalibur flow cytometer (Becton Dickinson). IFNγ secreting T cells were detected in seven (47%) patients with RA (RA3, 6, 7, 9, 10, 11, 15). IFNγ may be produced by GPI reactive T cells (table 1). IL4 secreting T cells were detected in four (27%) patients (RA1, 3, 7, 10), although they were less frequent than IFNγ+ T cells. Three patients (RA3, 7, 10) had both IFNγ and IL4 secreting T cells. In contrast, only one healthy subject (control 2) showed weak response to GPI (IFNγ and IL4). Interestingly, all seven patients with RA bearing GPI reactive IFNγ+ T cells shared either DRB1*0405 or *0901 (table 1). Our results demonstrated that GPI-specific Th1 and Th2-type cells (especially

Table 1 Anti-GPI Abs and DRB1 genotype in patients with RA, SLE, and in healthy subjects

Subject	huGPI	raGPI	DRB1 genotype	IFNγ+ T cells	IL4+ T cells
RAI	1.32	2087	0405 0901	4	**9**
RA2	2.73	3.02	0409 0803	0	0
RA3	1.33	1.15	0405 1501	**190**	**12**
RA4	2.43	2.55	0101 0803	0	0
RA5	1.79	3.14	0405 0401	6	3
RA6	1.65	2.67	0802 0901	**15**	3
RA7	1.88	1.15	1402 0901	**55**	**10**
RA8	2.60	3.47	0405 0901	0	0
RA9	2.46	1.70	1502 0405	**8**	1
RA10	1.93	2.65	0405 0803	**16**	**24**
RA11	1.72	0.95	0405 0803	**49**	3
RA12	1.40	1.61	0405 1502	7	0
RA13	1.49	0.94	0405 0803	0	0
RA14	1.39	0.99	0405 1502	0	0
RA15	2.48	3.32	0901 0901	**20**	2
SLE1	3.68	1.86	0803 1404	0	0
SLE2	1.32	1.38	0427 0427	0	0
SLE3	1.91	2.41	0101 0428	2	0
SLE4	2.89	2.97	0803 0803	0	0
Control1	3.75	3.54	1501 1403	2	0
Control2	3.05	3.19	1302 0803	**9**	**4**
Control3	2.19	3.28	1501 0803	3	0
Control4	2.51	2.59	1329 1406	1	1

The cut off optical density was calculated from an ELISA of 145 healthy subjects, the mean value + two standard deviation was 1.32 to human recombinant GPI, and 0.94 to rabbit native GPI. Double positive populations were considered anti-GPI Ab positive. For MACS cytokine secretion assay, positive cell numbers were determined after subtracting control cells, either reacted on thrombin or spontaneously secreting cytokines, from GPI reactive IFNγ+ and IL4+ T cells. The cut off cell numbers were calculated from the reaction of four patients with SLE and four control subjects who were all anti-GPI Ab positive. The mean value + two standard deviation was 7.6 to IFNγ, and 3.6 to IL4. Bold numbers indicate a positive reaction to GPI.
Abs, antibodies; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; IFN, interferon; IL, interleukin.

Th1-type cells) were frequently detected in patients with RA with anti-GPI Abs, suggesting that these cytokines may be associated with the production of arthritogenic Abs, especially when associated with HLA-DRB1*0405 or *0901.

In conclusion, our findings suggest that GPI reactive IFNγ+/IL4+ T cells may have a crucial role in the generation of arthritis in HLA-DRB1*0405 or *0901 positive patients with RA and anti-GPI Abs.

We thank Mrs Titose Okabe and Miss Yuri Ogamino for their excellent technical assistance.
This work was supported in part by a grant from the Japanese Ministry of Science and Culture (IM, TS). IM was a recipient of a fellowship from the Japan Intractable Diseases Research Foundation, Uehara Memorial Foundation, and Japan Rheumatoid Foundation.

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Accepted 26 October 2005

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