Figure 1 Median serum IL1RN levels for patients and controls, with and without joint pain, by genotype. Only the a and b alleles were present in sufficient numbers for comparison. jp, joint pain; njp, no joint pain.

rather than early osteoarthritis were included in the group with no joint pain.

In agreement with previous studies,¹⁰ we found a significant difference (Mann-Whitney test) in serum ferritin levels between men and women in both the patient ($p \leq 0.0019$) and control ($p \leq 0.0031$) groups, where men had higher serum ferritin levels. However, there was no significant difference in median serum ferritin levels in men with or without joint pain, or women with or without joint pain, in either the patient or control groups (data not shown).

A highly significant difference in serum IL1RN level was found between patients with and without joint pain ($p \leq 0.0007$). Although there was no significant difference in serum IL1RN levels between controls with or without joint pain, a similar trend to that found in patients was observed. There was no difference in serum IL1RN level between patients and controls with joint pain, or between patients and controls without joint pain. We did not detect any difference in the IL1RN allele frequency between patients with or without joint pain, or controls with or without joint pain. We did, however, observe a significant trend (sign test $p \leq 0.031$) of increased serum IL1RN levels with genotype (fig 1), where serum IL1RN levels for genotypes were in the order $bb > ab > aa$.

Some studies have shown a significant increase in the frequency of the IL1RN*b allele in autoimmune, particularly inflammatory, disease. At the same time, these diseases are

commonly associated with markedly higher serum levels of IL1RN.⁸ We show an association between genotype and IL1RN levels in patients with haemochromatosis and joint pain. The mechanism by which IL1RN impacts on joint pain in haemochromatosis is unclear, though it is independent of iron levels.

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Human parvovirus B19 viraemia is associated with neither IgM positivity nor anaemia in patients with rheumatic diseases

Y Munakata, T Saito

Human parvovirus B19 (B19) is an erythrovirus and causes anaemia. B19 infection results in not only anaemia but also a variety of symptoms resembling

autoimmune diseases.^{1–3} As B19 is detectable in non-erythroid cells in vivo,^{4,5} B19 infection is of interest for an understanding of the pathogenesis of autoimmune diseases.

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Table 1 B19 in bone marrow, and anaemia in patients with B19 viraemia

Cases	Age	Original disease*	Anti-B19 Abs		Copy number of B19 (copies/mg DNA)	RBC ($\times 10^4$ /ml)	Haemoglobin (g/l)
			IgM	IgG			
1	27	RA	+	+	2081	460	141
2	41	RA	+	+	6929508	260	77
3	40	RA	+	+	881938788	282	86
4	75	RA	—	+	1863	291	92
5	63	SLE	—	+	2287	372	100
6	43	RA	—	+	2472	453	137
7	37	RA	—	+	5867	368	116
8	35	RA	—	+	1084	455	110
9	28	RA	—	+	330	546	132

*All study patients with RA and SLE were diagnosed according to the American College of Rheumatology classification criteria.

We investigated the mode of B19 infection in the bone marrow of patients with rheumatic diseases who had B19 viraemia.

A polymerase chain reaction (PCR) was carried out on peripheral blood samples obtained from 92 febrile adult patients with rheumatic disease in our clinic during 1999–2001 to detect B19 DNA (B19 viraemia); an enzyme linked immunosorbent assay (ELISA) was carried out on serum samples from the patients to detect anti-B19 IgM. Informed consent was obtained from the patients to allow testing of the samples. Bone marrow samples were obtained from nine patients with B19 viraemia. B19 DNA was amplified in a two step PCR: firstly, with outer primers for the NS1 gene (forward primer, 5'-ATTGCATACAGACTTTGAGC-3'; reverse primer, 5'-TCAGAGCTTTCACACCAC-3') and then with inner primers (forward primer, 5'-CAGACTTTGAGCAGGTTATG-3'; reverse primer, 5'-AGTGTAGTTTATGCCAG-3') nested within the first primers. Quantitative PCR for bone marrow samples was carried out as we described previously.⁶ Anti-B19 IgM or anti-B19 IgG in serum samples were measured by a commercial indirect ELISA (Parvo IgM-EIA "SEIKEN" kit or a Parvo IgG-EIA "SEIKEN" kit; Denka Seiken Inc, Tokyo, Japan).

Eighteen patients positive for B19 DNA were regarded as B19 viraemia. We classified patients with B19 viraemia into two groups according to their serum IgM status: (a) four patients with rheumatoid arthritis (RA) and one patient with systemic lupus erythematosus (SLE) (28%) positive for anti-B19 IgM and categorised as having acute primary infection of B19; (b) 13 (72%) patients negative for anti-B19 IgM, including eight, one, and four patients positive for RA, SLE, and haemophagocytic syndrome, respectively. B19 viraemia in patients without anti-B19 IgM may be due to persistent infection of B19 caused by inappropriate virus clearance despite the presence of anti-B19 IgG.

To evaluate the pathological role of B19 infection, we explored the relationship between severity of anaemia and B19 in bone marrow by measuring B19 DNA in bone marrow cells from nine patients with B19 viraemia (eight patients with RA and one patient with SLE) (table 1). Three patients positive for anti-B19 IgM were regarded as having acute primary B19 infection. Among them, markedly high copies of B19 in the bone marrow were detected in two patients (cases 2 and 3) who showed apparent anaemia. Six patients negative for anti-B19 IgM showed no serious anaemia despite the presence of B19 in bone marrow. The average copy number of B19 in bone marrow was relatively small in patients negative for anti-B19 IgM. Four patients (cases 1, 6, 7, and 9) showed no anaemia despite the presence of B19 in bone marrow.

Most studies have found a very low prevalence of anti-B19 IgM in rheumatic diseases, and concluded that B19 infection

is not related to rheumatic diseases.⁷ However, this does not agree with the observation that B19 may cause symptoms resembling rheumatic diseases. Anti-B19 IgM may be associated with anaemia and a higher B19 DNA copy number. However, B19 DNA in bone marrow does not indicate anaemia in B19 viraemic patients with rheumatic diseases, which may suggest that target cells of B19 infection in these patients may not be erythroid lineage cells and that impaired repopulating capacity of infected cells may induce abnormal immunity, such as in the case of the parvovirus minute virus of mice.⁸ On the other hand, it is known that there are three genotypes of B19,⁹ and possibly, a specific genotype of B19 predisposes to persistent infection in patients with rheumatic diseases. We need further investigation to clarify this issue.

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