AB serum. IFN γ -producing T cells were identified using MACS secretion assay as described in fig 1A.

To determine the altered peptide ligands of M3R in patients with SS, we synthesised nine 15mer peptides (VPPGECFI→E/K/LQFLSEPT, VPPGECFIQ→A/V/MFLSEPT, VPPGECFIQFL→M/E/KSEPT, M3R216–230), in which the anchored motif binding to the HLA-DR B1*0901 molecule is conserved, although one amino acid to TCR was different. Altered peptide ligands were selected based on inhibition of IFN γ production by M3R reactive T cells. Figure 1B shows that M3R 223I→K and M3R 224Q→A significantly suppressed the number of IFN γ -producing T cells, suggesting that they are candidates for selection as altered peptide ligands. The inhibition of IFN γ by other cytokines may not be likely, because interleukin 4 producing T cells were not increased (data not shown).

In conclusion, we have provided evidence for the presence of M3R reactive T cells in the serum of patients with SS and shown that VPPGECFKQFLSEPT (M3R 223I→K) and VPPGECFIAFLSEPT (M3R 224Q→A) are candidate altered peptide ligands of the second extracellular domain of M3R. Our findings may provide the basis of a potentially useful antigen-specific treatment for SS using altered peptide ligands of autoantigens recognised by autoreactive T cells.

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IL1RN genotype as a risk factor for joint pain in hereditary haemochromatosis?

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Hereditary haemochromatosis is a genetically determined disease of disrupted iron metabolism caused predominantly by the C282Y mutation in the HFE gene on chromosome 6.¹ If detected early, the symptoms of haemochromatosis can usually be prevented by venesection to remove excess iron and maintain normal iron stores.

One of the most common symptoms of haemochromatosis is joint pain (arthralgia) with or without associated joint disease (arthropathy),^{2–3} and it has been generally assumed that arthritis in haemochromatosis is caused by iron deposition in the joints. Nevertheless, studies have failed to demonstrate any correlation between the level of iron overload and the presence or absence of arthritis or arthralgia,^{4–6} nor does venesection ameliorate joint symptoms.⁶ The interleukin cluster on chromosome 2 includes the interleukin (IL) 1 α , IL1 β , and IL1RN genes, and an association has been demonstrated between an IL1RN polymorphism and several inflammatory disorders, including osteoarthritis⁷ but not rheumatoid arthritis.⁸ This 86 bp polymorphism with five known alleles (a, b, c, d, and e) in intron 2 of the IL1RN gene has also been shown to be associated with expression levels.⁹

As far as we know, this study is the first to investigate genetic associations underlying joint pain in haemochromatosis. All participants gave informed written consent and the study was conducted with institutional ethics approval. In preliminary studies, we found that the frequency of the IL1RN polymorphism was significantly different ($p \leq 0.01$) between patients with haemochromatosis ($n = 313$) and controls ($n = 349$), and the IL1RN*a allele was more common in patients than in controls (data not shown).

We assessed 66 HFE C282Y homozygote patients (29 female, 37 male) and 52 healthy volunteers (25 women, 27 men) who were all wild type for the HFE mutation. All were available for clinical assessment of joint pain. Blood was collected for extraction of DNA and determination of serum ferritin (Immulate, 2000) and serum IL1RN levels (enzyme linked immunosorbent assay (ELISA), R&D, Minneapolis, USA). Clinical data, including the presence of joint pain, were collected through a questionnaire and by subsequent consultation with a physician (JR), who was unaware of their genetic status. Patient and control data were then divided according to whether they experienced joint pain or not. People who experienced joint pain as a result of injury

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

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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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