Antibodies to *Klebsiella pneumoniae* nitrogenase reductase in patients with ankylosing spondylitis

The strong link between ankylosing spondylitis (AS) and HLA-B27 has been well established. Any aetiological agent or mechanism implicated in AS must provide an explanation for the link with HLA-B27. An amino acid sequence homology, QTEDRED, found in the variable region of B*2705, (residues 72–77) and the KP2 component of *Klebsiella pneumoniae* nitrogenase enzyme (residues 188–193) has been reported. Furthermore, AS patients were shown to have increased concentrations of antibodies to a homologous sequence of both B*2705 and the KP2 component of *Klebsiella pneumoniae* nitrogenase reductase through some authors have been unable to confirm these results. Antibody affinity is often lower with peptide sequences compared with binding by the native protein and this could be due to conformational changes between peptide and the native protein molecule, thereby accounting for these differences in reactivity.

In the light of these conflicting findings, this study was undertaken to measure antibodies to the native KP2 component of nitrogenase reductase enzyme of *Klebsiella pneumoniae*, to determine if it has a part to play in the development of disease, and whether anti-nitrogenase antibodies are correlated with C reactive protein (CRP) concentrations.

Serum samples were obtained from AS patients attending the AS Research Clinic at the King's College Hospital, London. The diagnosis of AS was according to the New York criteria and that of RA by the American Rheumatism Association.

In the study, IgG, IgA, and IgM immunoglobulin antibodies were measured in 200 subjects against *K pneumoniae* nitrogenase reductase. The groups examined were as follows: 100 patients with AS (71 male, 29 female, median age 47 years); 50 RA patients (15 male, 35 female, median age 57 years), and 50 healthy control subjects (30 male, 20 female, median age 44 years).

<table>
<thead>
<tr>
<th>Antibody class</th>
<th>Healthy controls (n=50)</th>
<th>RA patients (n=50)</th>
<th>AS patients (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>0.22 (0.01)</td>
<td>0.25 (0.01)</td>
<td>0.32 (0.01)</td>
</tr>
<tr>
<td>IgA</td>
<td>0.15 (0.02)</td>
<td>0.17 (0.02)</td>
<td>0.42 (0.02)</td>
</tr>
<tr>
<td>IgM</td>
<td>0.01 (0.01)</td>
<td>0.02 (0.01)</td>
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The mean (SEM) of the absorbance values (optical density 630 nm) are given. ** Indicates statistical significance compared with the healthy control group using Student's t test; p<0.001.

Table 1: IgG, IgA, and IgM antibodies against the KP2 component of Klebsiella pneumoniae nitrogenase reductase in serum samples of patients with ankylosing spondylitis (AS), rheumatoid arthritis (RA), and healthy controls.

The KP2 component of Klebsiella nitrogenase, which contains the QTEDRED sequence, was provided by Dr Martin Buck, of the Nitrogen Fixation Laboratory in Sussex University and purified as previously described. Antibody levels against the KP2 component of *K pneumoniae* nitrogenase reductase were measured by enzyme linked immunosorbent assay (ELISA) as previously described. All assays were carried out under code, so that the status of each serum sample under investigation was not known to the tester. CRP concentrations were determined by the single radial immunodiffusion method of Mancini et al and the results expressed as mg/l of serum. The mean optical density (OD) units of IgG, IgA, and IgM immunoglobulin antibodies against the KP2 component of *K pneumoniae* nitrogenase reductase were calculated to determine the antibody titre, which is often lower with peptide compared with binding by the native protein molecule, thereby accounting for these differences in reactivity.

The authors gratefully acknowledge the support of the Arthritis and Rheumatism Council and the Trustees of the Middlesex Hospital. The late Dr WJSimmons is also thanked for providing the antipeptide antibodies. The late Dr RFGraves is greatly thanked for providing the anti-peptide antibodies. The late Dr RF Graves is greatly thanked for providing the anti-peptide antibodies.

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**Figure 1** Correlation analysis between class specific IgG (1a) and IgA (1b) anti-*K pneumoniae* (KP2) nitrogenase reductase antibodies and CRP concentrations in serum samples of ankylosing spondylitis patients.

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Lack of association between HLA DBR1* alleles and RS3PE syndrome

In 1985, McCarty and colleagues reported a syndrome characterised by symmetrical and acute synovitis, marked pitting oedema, seronegativity resulting from the absence of rheumatoid factors (RF), increased acute-phased reactants, lack of bony erosions on radiography, and benign and short course (RS3PE syndrome: remitting seronegative symmetrical synovitis with pitting oedema). Most patients were older men. A pitting oedema was also observed in other conditions (polymyalgia rheumatica (PMR) and late onset peripheral spondylarthropathy (LOPS)). Whether the RS3PE syndrome represents a distinct clinical entity remains controversial.

The syndrome was initially reported to be associated with HLA-B (B7) rather than HLA-DR (DR4) antigens. Recently, an association with HLA-A2 was reported. The association between RA and HLA-DRB1* alleles was confirmed using the shared epitope (SE) (p=0.29). RA, rheumatoid arthritis. SE, subtypes encompassing the shared epitope. X, all alleles except DRB1*0101, *0401, *0404, *0405, *0408, *0101, *1002, *1001, *1402) is well established. Older age onset RA and seronegative RA are known to be poorly or slightly related to DR4.

In 88 patients, an association was reported between the HLA-DR antigens that contain the SE and PMR.

We studied the HLA-DR polymorphism in RS3PE syndrome patients: HLA-DRB1* type (PCR-SSO), people who typically as HLA-DR1 or DR4 had a subtyping for HLA-DRB1*0101 to *0104 and HLA DRB1*0401 to *0422 was performed in 12 white patients (eight male and four female; mean age: 72, range: 62–84) admitted to our department during the past 10 years. They all had typical RS3PE syndrome with acute onset symmetrical polyarthritis involving the wrists, metacarpophalangeal and proximal interphalangeal joints, tenosynovitis of the flexor of the hands, and a marked pitting oedema. Three patients had a carpal tunnel syndrome and one had a PMR five years before this polyarthritis with pitting oedema. Three patients had a carpal tunnel syndrome and one had a PMR five years before this polyarthritis. The erythrocyte sedimentation rate (ESR) was increased in RS3PE patients (41.6%) as well established.

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Table 1 HLA DRB1* frequencies in RS3PE patients. Comparative study with seronegative RA and controls

<table>
<thead>
<tr>
<th>HLA DRB1*</th>
<th>RS3PE (n=12)</th>
<th>Seronegative RA (n=15)</th>
<th>Healthy controls (n=104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*0101</td>
<td>1</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>*0102</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>*0401</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>*0405</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>*0101</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>*0102</td>
<td>5</td>
<td>*8</td>
<td>*8</td>
</tr>
<tr>
<td>*0401</td>
<td>4</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>*0402</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>*0405</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>*1001</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*7 test between RS3PE and controls (p=0.08); * test between seronegative RA and controls (p=0.29). RA, rheumatoid arthritis. SE, subtypes encompassing the shared epitope. X, all alleles except DRB1*0101, *0401, *0404, *0405, *0408, *0101.

We data suggest that there is no association between HLA-DRB1* alleles and RS3PE syndrome. A slight increase in DRB1* alleles encompassing the SE was observed in this series, but without significance. The overrepresentation of the SE in the RA group argues against a poor expression of this motif in our RA patients. It has been previously reported, HLA-DRB1*01 and *04 were slightly increased in our seronegative RA patients. In the RS3PE patients, there was no influence of the SE on the clinical presentation. In general, all of our patients had a favourable outcome and none had developed bony erosions on radiographs or typical RA. Thus, it may be suggested that this good outcome could be related to the poor expression of the shared motif in these patients. It may also be advanced that HLA-DRB1* typing in patients with RS3PE syndrome provides no useful information. As a link with HLA B7 and/or A2 has been reported, analysis of HLA A and B locus may only be performed in RS3PE syndrome. The long term follow up of patients with RS3PE syndrome has provided the diagnosis of more well defined conditions such as RA, PMR, spondylarthropathies, and malignancies. As it has been previously suggested, RS3PE could be considered as a heterogenous syndrome and this may explain the lack of association with a specific HLA-DR antigen.

Rupture of a non-aneurysmatic aortic trunk in a patient with giant cell arteritis

Giant cell arteritis is a chronic, granulomatous inflammation of the temporal arteries that affects persons over the age of 50. Temporal arteritis is sometimes the first evidence of a more disseminated disease and involvement of all the larger arteries including the aorta has been observed. Many studies describe appearance of murmurs and the formation of aortic aneurysms in patients suffering from temporal arteritis. Weak extremity pulses, arterial occlusion and changes of Raynaud’s phenomenon may occur, symptoms resulting from the narrowing or occlusion of branches of the thoracic aorta. Death from rupture of aneurysms in giant cell arteritis is well described. It has also been suggested that the extra-articular disease polymyalgia rheumatica is an expression of the generalised form of giant cell arteritis and the name polymyalgia arteritis may be more appropriate.
A 74 year old woman was admitted to the university hospital in Lund because of tenderness over the right masseter muscle. It propagated towards the temporal region and spread similarly on the left side and over the back of the head. On admission the erythrocyte sedimentation rate (ESR) was 42 mm 1st h and serum electrophoretic analysis indicated slight inflammatory activity. The patient presented with a minor exophthalmus resulting from an earlier episode of Grave’s disease, but was otherwise previously healthy and in good physical condition. The subcutaneous temporal vessels were swollen and tender. On the diagnosis of probable temporal arteritis, 30 mg/day of prednisolone were prescribed. A biopsy, showing inflammatory cell infiltrates in the arterial wall, verified the diagnosis. The ESR decreased and the symptoms declined and the prednisolone was reduced to 5 mg/day. On several check ups over five years, there were no signs or symptoms of active disease and the ESR was at all times below 15 mm 1st h. At the age of 79, the patient collapsed during a walk and was declared dead upon arrival of the ambulance.

Necropsy revealed in the aortic trunk, 3 cm above the valvular plane, a rupture within a small area of pronounced wall thinning (< 0.5 mm) and haemopericardium. There were no aortic aneurysm formation or dissection and only minor arteriosclerotic lesions were seen and focal precipitation of metachromatic material, representing myxoid degeneration. The remaining temporal artery was not examined, as active arteritis had not been suspected at the time of necropsy.

Pathogenetically, we suggest that the intense aortic inflammation dissolved the elastic fibres, and attenuated the wall. It also obliterated some of the afferent arterioles (vasa vasorum), leading to multifocal ischaemic wall necrosis, followed by rupture of the vessel wall, without prior formation of an aneurysm. Despite focally intense inflammatory infiltrates, it is impossible to determine whether this was an acute exacerbation of the disease or part of a chronic, active inflammation. Clinically, however, there was no sign of disease activation. It is well known that giant cell arteritis can lead to death by rupture of aortic aneurysms. In this patient, there was inflammatory and ischaemic aortic wall necrosis without an aneurysm. This has, to our knowledge, not been reported before.

We are indebted to Professor Emeritus Nils Jonsson, for his critical examination of this report.

**Authors’ reply**

Schlesinger and colleagues confirm our findings on the frequency of a normal serum uric acid in acute gout. However, they have misread our statement concerning the comparative serum uric acid values during and between episodes. On the basis that in 70% of patients the serum uric acid was lower during the acute episode we found that serum uric acid concentrations can either be higher or lower with acute episodes than during the intercritical period. Patients receiving long term allopurinol treatment tended to be more likely to have lower serum uric acid during the acute episode (p<0.01). Patients not receiving allopurinol tended to have higher serum uric acid concentrations during the acute gouty episodes than during the interim (p<0.005). More study is needed with more sequential observations examining the relation between timing of episodes and changes in serum uric acid.

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**Figure 1** Serum uric acid values before, during, and after an episode of acute gout in four patients.

**Figure 2** Low power microscopical section of the aortic wall close to the rupture (and fig 1). Note the patchy necroses and disruption of the elastic fibres. Van Gieson and elastica staining. Bar represents 1 mm.
more sequential studies of this kind are needed before deciding on the correct qualifying adverb.

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Correspondence to: Dr P E McGill.


Clinical guidelines

Suarez-Almazor and Russell raise several important points in their excellent review about the plethora of clinical guidelines issued by various official, unofficial, learned or merely pretentious bodies.¹

The potential consumer (usually a full time clinician) is advised to evaluate the soundness of the guidelines on the basis of the strength of evidence on which the guidelines are based (that is, double blind randomised trials versus consensus of opinion) and how clearly the supporting evidence is described.

In reality most guidelines are like an old staircase, with some solidly anchored steps, interspersed with others supported by nothing more than the rickety whim of current opinion. In the end therefore, before deciding that it is safe to climb the stairs, the consumer places his trust more in the pedigree of the authors than in anything else.

More fundamental than “safety” however is the question of why it was built in the first place. This is very seldom stated, though it is generally assumed to be a worthy reason, which all would share.

Even worthy reasons can have competing effects however, and unless the primary goal was clearly identified at inception and its priority maintained against the sometimes competing interests of other worthy goals throughout the development of the guidelines, it can be very difficult to discern the distortions that might have been introduced when you only see the final product.

Guidelines written for the primary purpose of reducing practice variability for cost management, or as a perceived protection against litigation, may also achieve a benefit in reducing morbidity but are likely to be different from guidelines written for the primary purpose of morbidity or mortality reduction, with cost containment the incidental benefit.

For example, guidelines with the title of “postoperative care after knee replacement” are going to differ in important detail if written for the primary purpose of reducing length of hospital stay, from ones written with the primary aim of maximising functional outcome.

It should be incumbent on the authors of guidelines to state what their primary purpose was in writing them, which in a manner analogous to a well structured study with an a priori defined primary outcome measure, can then be subjected to assessment.

In the absence of such a statement, caveat emptor, you may be climbing the wrong staircase.

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