Chapter 17
Rheumatoid Arthritis Sera Are Cytotoxic to Cells Bearing HLA and Collagen Susceptibility Sequences

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Introduction: The Association of the 'Shared Epitope' EQ(R)RAA with the ESRRAL Sequence of *Proteus* Haemolysin and the Link of *Proteus* Urease with Collagen

The HLA-DR alleles, DRB1*0401, *0402, *0405, *0101 and *1402, which share an amino acid sequence EQR(K)RAA in the DRB1 chain, have been linked with a susceptibility to develop rheumatoid arthritis.

It has been suggested that an environmental factor interacting with a genetic predisposition contributes to the pathogenesis of rheumatoid arthritis.

We have reported that rheumatoid arthritis patients with active disease have elevated levels of antibodies to the urinary microbe *Proteus mirabilis* and this has been confirmed by several independent groups, such as one in the UK (Senior et al. 1995) and another one in the USA (Newkirk et al. 2005).

The level of anti-*Proteus* antibodies correlates with urinary isolation rates of *Proteus mirabilis* from patients with active rheumatoid arthritis.

Amino acid homologies and immunological crossreactivity between the susceptibility motif EQR(K)RAA and *Proteus mirabilis* haemolysin ESRRAL as well as type XI collagen LRREI and *Proteus mirabilis* urease IRRET have been demonstrated in studies from the Immunology Unit at King's College in London.

Ankylosing spondylitis is a chronic inflammatory disorder affecting predominantly the lumbar spine and where 95% of patients are HLA-B27-positive. Increased levels of antibodies to the gut microbe *Klebsiella pneumoniae* have been reported, suggesting an aetiological role for this microbe in the pathogenesis of this disease. Sera from patients with this condition have been used as 'disease controls' for these cytotoxicity studies in patients with rheumatoid arthritis.

Amino acid sequence homologies have been identified between HLA-B*2705 (QTDRED) and two enzymes present in *Klebsiella pneumoniae*, nitrogenase reductase (QTDRED) and pullulanase secretion protein pul D (DRDE) (Fielder et al. 1995).

Patients with active ankylosing spondylitis have been shown to have elevated levels of antibodies to all three peptide sequences compared to control groups.

The cytotoxic activity of sera from rheumatoid arthritis patients and ankylosing spondylitis has been investigated to determine whether tissue-damaging properties were present in patients when compared to sera obtained from healthy individuals.

Sera from Rheumatoid Arthritis and Ankylosing Spondylitis Patients

Sera were collected from patients with active rheumatoid arthritis, (erythrocyte sedimentation rate > 15 mm/h) attending the Department of Rheumatology at the Lister Hospital in Stevenage and ankylosing spondylitis patients attending the 'Ankylosing Spondylitis Research Clinic' of the Middlesex Hospital in London.

The diagnosis of rheumatoid arthritis was made according to the American Rheumatism Criteria and the diagnosis of ankylosing spondylitis was made according to the New York criteria.

Antibodies against synthetic peptides 15-/16-mer peptides containing ESRRAL, EQRRAA, LRREI, IRRET, EDERAA,

QTDRED and DRED as well as control sequences were measured in the sera obtained from rheumatoid arthritis and ankylosing spondylitis patients.

There were 51 patients with rheumatoid arthritis, 18 men and 33 women with a mean age of 49 years (range: 28–70 years). Their mean (± standard error) erythrocyte sedimentation rate was 45.4 ± 9.6 mm/h.

There were 34 patients with ankylosing spondylitis, 26 men and 8 women with a mean age of 46 years (range: 23–69 years). Their mean (± standard error) erythrocyte sedimentation rate was 48.2 ± 3.7 mm/h.

Sera were also obtained from 38 healthy blood donors, 18 men and 28 women with a mean age of 40 years (range: 24–57 years).

Synthetic Peptides and ELISA

Peptides were prepared by solid phase synthesis and analysed for purity by high-performance liquid chromatography as previously described. All peptides had at least 90% purity. The molecular mimicry sequences are shown in bold.

The test peptides were:

1. SQKDLL**EQRRAA**VDTY of HLA-DRB1*0404
2. LGSS**ESRRAL**QDSQR of *Proteus mirabilis* haemolysin
3. QSLDS**LRREI**EQMRR of type XI collagen
4. FAESR**IRRET**IAAED of *Proteus mirabilis* urease
5. Control peptide SQKDIL**EDERAA**VDTY of HLA-DRB1*0402
6. CKAKA**QTDRED**LRTLL of HLA-B*2705
7. RPTVIR**DRDE**YRQASS of *Klebsiella pneumoniae* pullulanase
8. ASLEHEEGKILRAQLE of human myosin
9. KKLTEKEAELQAKLE of *Streptococcus pyogenes*
10. LGSISRSELARQDSQR of scrambled haemolysin
11. RPTVRSDIDYRQAESR of scrambled pullulanase

The ELISA was carried out as previously described.

All assays were carried out in duplicate and under code, so that the status of each serum sample under investigation was not known to the tester.

Preparation of Sheep Red Blood Cells for Cytotoxicity Assay

A 20-ml aliquot of sheep blood (Unipath Ltd, Hampshire, UK) was washed twice with 0.85% sodium chloride (saline) solution in a centrifuge (Heraeus, minifuge) at 750 × g (3,000 rpm) for 15 min. Supernatant was discarded, and 600 µl of the packed cells were removed and placed into each of two universal containers. The cells in each container were resuspended in 10 ml saline and 10 ml of a 1/20,000 tannic acid (Aldrich Chemical Co, Dover, UK) in saline added to each container, followed by mixing and incubation for 15 min at 37°C in a water bath. After centrifugation for 5 min, the supernatant was discarded and cells resuspended in 20-ml saline and washed. One container was later used as a negative control.

The cells in the other container were resuspended in 10-ml saline and 10-ml antigen (2 mg/ml) added, followed by mixing and incubation for 30 min at 37°C in a water bath. After washing by centrifugation for 5 min, the supernatant was discarded, the cells were washed 3 times with saline and both coated and uncoated (Control) cells resuspended in 50 ml of saline.

Serum samples were inactivated in a water bath at 56°C for 30 min.

The cytotoxicity assay was carried out as follows: 100 ml of serum diluted 1/8 in saline was added to 96 well microtitre plates (Dynatech) together with 100 µl of peptide-coated sheep red blood cells and the plates incubated at 37°C for 30 min. A 100-µl aliquot of guinea pig complement (Calbiochem Ltd., Nottingham, UK) diluted 1/10 in saline was added followed by mixing, then incubated at 37°C for 30 min and later at 4°C overnight to allow the unlysed cells to settle.

A 100-µl aliquot of test supernatant was removed and placed into wells of a microtitre plate and absorbance measured 570 nm.

Minimum lysis was calculated from peptide-coated cells treated with saline plus complement, and absorbance value obtained from the minimum lysis was subtracted from each test value. One hundred percent lysis was calculated from

100 μl of uncoated cells treated with 200 μl of distilled water and this was labelled as 'maximum lysis'.

Percentage lysis was determined using the following formula:

$$\%\text{Lysis} = \frac{(\text{Test lysis} - \text{minimum lysis})}{(\text{Maximum lysis} - \text{minimum lysis})} \times 100$$

All assays were carried in duplicate and under code, in that the tester did not know which sera came from patients or control subjects.

Antibody Absorption Assay

Serum samples from five rheumatoid arthritis patients with high antibody levels to the individual peptides were absorbed with 250 μl of packed sheep red blood cells coated with ESRRAL, EQRRAA, IRRET and LRREI peptide sequences in a plastic tube overnight at 4°C, with gentle rotation. The absorption process was repeated until the antibody level for each sample was below the mean value of the control subjects, measured by ELISA.

The absorbed sera were then tested for cytotoxic activity against sheep red blood cells coated with EQRRAA and LRREI peptides, as described above.

Antibodies to Peptide Antigens in Rheumatoid Arthritis Patients

*HLA-DRB1*0404 Peptide*

The levels of IgG antibodies to the HLA-DRB1*0404 peptide SQKDLL**EQRRAA**VDTY of the IgG class were significantly elevated in rheumatoid arthritis patients when compared to ankylosing spondylitis patients or healthy controls. The mean

(± standard error) level in rheumatoid arthritis patients was 0.537 ± 0.013 OD units and this was significantly higher than the level of 0.134 ± 0.006 OD units found in ankylosing spondylitis patients ($t = 23.65, p < 0.0001$) or the level of 0.152 ± 0.010 OD units found in healthy controls ($t = 21.62, p < 0.0001$).

There was no significant difference between ankylosing spondylitis patients and healthy controls when tested against the DRB1*0404 peptide.

Proteus mirabilis Haemolysin Peptide

The levels of IgG antibodies to the *Proteus mirabilis* haemolysin peptide LGSIS**ESRRAL**QDSQR were significantly elevated in rheumatoid arthritis patients when compared to ankylosing spondylitis patients or healthy controls. The mean (± standard error) level in rheumatoid arthritis patients was 0.590 ± 0.015 OD units and this was significantly higher than the level of 0.155 ± 0.008 OD units found in ankylosing spondylitis patients ($t = 22.17, p < 0.0001$) or the level of 0.170 ± 0.010 found in healthy controls ($t = 21.38, p < 0.0001$). There was no significant difference between ankylosing spondylitis patients and healthy controls when tested against the *Proteus mirabilis* haemolysin peptide.

Type XI Collagen Peptide

The levels of IgG antibodies to the type XI collagen peptide GSLD**LRREI**EQMRR were significantly elevated in rheumatoid arthritis patients when compared to the ankylosing spondylitis or healthy controls. The mean (± standard error) level in rheumatoid arthritis patients was 0.490 ± 0.009 OD units, and this was significantly higher than the level of 0.133 ± 0.007 OD units found in ankylosing spondylitis patients ($t = 30.08$, $p < 0.0001$) or the level of 0.136 ± 0.007 OD units found in healthy controls ($t = 26.17$, $p < 0.0001$).

There was no significant difference between the ankylosing spondylitis patients and healthy controls when tested against the type XI collagen peptide.

Proteus mirabilis Urease Peptide

The levels of IgG antibodies to the *Proteus mirabilis* urease peptide FAESR**IRRET**IAAED were significantly elevated in rheumatoid arthritis patients when compared to ankylosing spondylitis patients or healthy controls. The mean (± standard error) in rheumatoid arthritis patients was 0.514 ± 0.010 OD units and this was significantly higher than the level of 0.146 ± 0.007 OD units found in ankylosing spondylitis patients ($t = 26.13$, $p < 0.0001$) or the level of 0.171 ± 0.012 OD units found in healthy controls ($t = 22.0$, $p < 0.0001$).

There was no significant difference between the ankylosing spondylitis patients and healthy controls when tested against the *Proteus mirabilis* urease peptide.

*DRB1*0402 Peptide*

There was no significant reactivity (mean ± standard error) to the DRB1*0402 peptide SQDIL**EDERAA**VDTY in the two groups of patients, rheumatoid arthritis (0.132 ± 0.007) or ankylosing spondylitis (0.113 ± 0.008), when compared to healthy blood donor subjects (0.131 ± 0.009).

Cytotoxicity Studies in Rheumatoid Arthritis Patients

Sera from rheumatoid arthritis and ankylosing spondylitis patients as well as sera from healthy blood donors were tested for reactivity in a complement-mediated cytotoxicity assay with sheep red blood cells coated with HLA.DRB1*0402

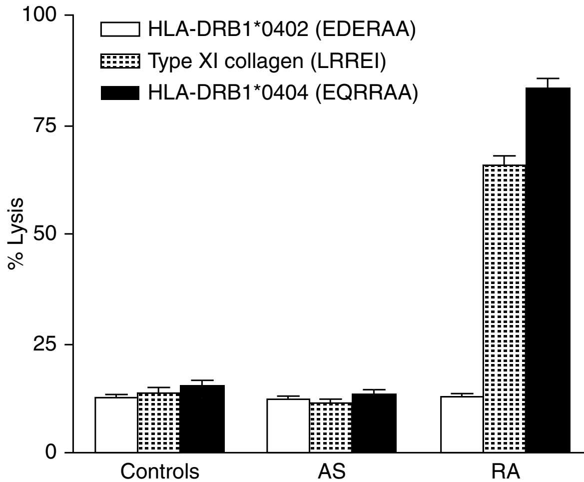

FIGURE 17.1 Percentage lysis of sheep *red* blood cells coated with HLA-DRB1*0404 (*EQRRAA*), DRB1*0402 (*EDERAA*) and type XI collagen (*LRREI*) peptides, via complement-mediated cytotoxicity by control sera and sera from ankylosing spondylitis (*AS*) rheumatoid arthritis (*RA*) patients (With kind permission from Wilson et al. (2003))

(EDERAA) peptide, type XI collagen (LRREI) peptide and HLA-DRB1*0404 (EQRRAA) peptide (Fig. 17.1).

EQRRAA Peptide

The sera from rheumatoid arthritis patients showed significantly higher levels of cytotoxic activity against sheep red blood cells coated with SQKDLL**EQRRAA**VDTY from HLA-DRB1*0404 when compared to ankylosing spondylitis patients or healthy controls. The mean (± standard error) percentage lysis in rheumatoid arthritis patients was 83.3±2.2% and this was significantly higher than the level 13.7±0.8% found in ankylosing spondylitis patients (t=25.63, p<0.0001) or the level of 15.5±1.2% (t=25.06, p<0.0001) found in healthy controls.

There was no significant difference in the cytotoxic activity of the ankylosing spondylitis sera or the ones obtained from healthy controls, when tested against sheep red blood cells coated with the HLA-DRB1*0404 (EQRRAA) peptide.

LRREI Peptide

The sera from rheumatoid arthritis patients showed significantly higher levels of cytotoxic activity against sheep red blood cells coated with GSLDS**LRREI**EQMRR from type XI collagen when compared to ankylosing spondylitis patients or healthy controls.

The mean (± standard error) percentage lysis in rheumatoid arthritis patients was 65.7±2.2%, and this was significantly higher than the level of 11.5±0.9% found in ankylosing spondylitis patients ($t = 19.16$, $p < 0.0001$) or the level of 13.7±1.3% found in healthy controls ($t = 18.43$, $p < 0.0001$).

There was no significant difference in the cytotoxic activity of the ankylosing spondylitis sera or the ones obtained from healthy controls when tested against sheep red blood cells coated with the type XI collagen peptide.

EDERAA Peptide

There was no significant cytotoxic reactivity against sheep red blood cells coated with SQKDIL**EDERAA**VDTY of HLA-DRB1*0402 in the rheumatoid arthritis or ankylosing spondylitis sera when compared to sera from healthy blood donors.

Correlation Between ESRRAL and IRRET Antibodies

There was a significant correlation between anti-*Proteus mirabilis* haemolysin peptide (ESRRAL) antibodies and percentage lysis of sheep red blood cells coated with SQKDLL**EQRRAA**VDTY of HLA-DR1*0404 ($r = 0.970$, $p < 0.0001$) (Fig. 17.2a).

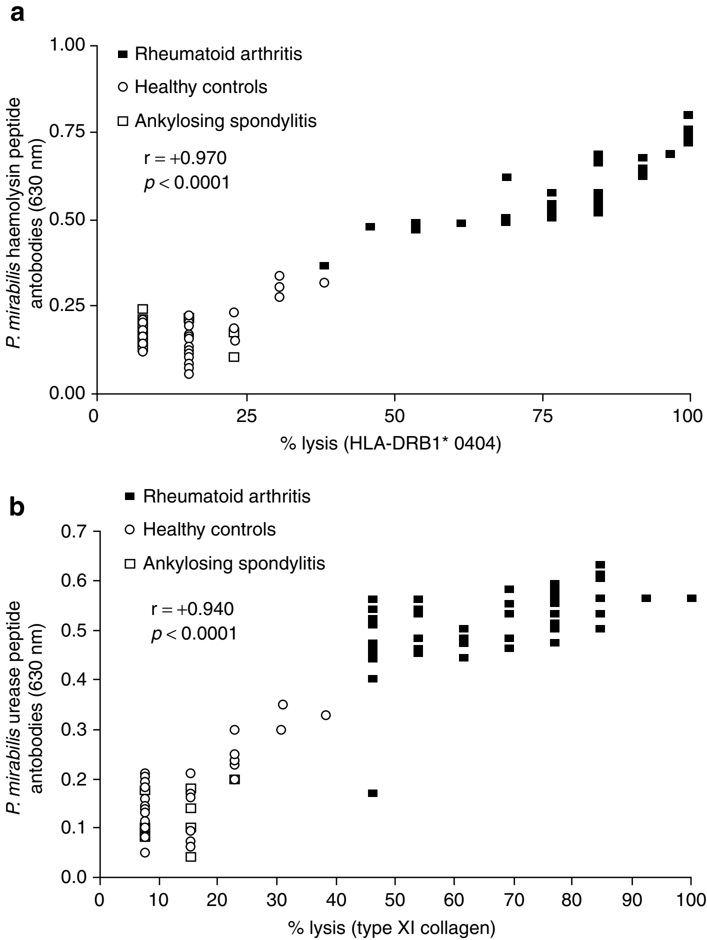

FIGURE 17.2 Correlation of anti-*Proteus mirabilis*haemolysin (**a**) and anti-*Proteus mirabilis*urease (**b**) IgG antibody levels for rheumatoid arthritis and ankylosing spondylitis patients and healthy controls and percentage lysis of sheep *red*blood cells coated with HLA-DRB1*0404 and type XI collagen peptides respectively (With kind permission from Wilson et al. (2003))

There was also a significant correlation between anti-*Proteus mirabilis* urease (IRRET) and percentage lysis of sheep red blood cells coated with GSLDS**LRREI**EQMRR of type XI collagen ($r = 0.940$, $p < 0.0001$) (Fig. 17.2b).

Furthermore, there was also a significant correlation between anti-EQRRAA antibodies and percentage lysis of sheep red blood cells coated with EQRRAA peptide ($r = 0.954$, $p < 0.0001$).

There also was a significant correlation between anti-IRRET antibodies and sheep red blood cells coated with IRRET peptide ($r = 0.948$, $p < 0.0001$).

Results of Absorption Studies

Absorbed sera from 5 rheumatoid arthritis patients were tested for reactivity in complement-mediated cytotoxicity with sheep red blood cells coated with EQRRAA and LRREI peptides.

The mean percentage lysis of sheep red blood cells coated with EQRRAA peptide was 100%.

The mean percentage lysis of sheep red blood coated with LRREI peptides was 82%.

However, after absorption with sheep red blood cells coated with EQRRAA or ESRRAL, the mean percentage lysis was 13.7%.

Furthermore, after absorption with sheep red blood cells coated with LRREI or IRRET peptides, it was 12.2% (Table 17.1).

Absorption analyses with EQRRAA, ESRRAL, LRREI and IRRET thus resulted in the reduction of percentage lysis.

Antibodies to Peptide Antigens in Ankylosing Spondylitis Patients

Levels of IgG antibodies to the HLA-B27 peptide CKAKAQ**QTDRED**LRTLL in ankylosing spondylitis patients were significantly elevated compared to rheumatoid arthritis patients and healthy blood donor controls.

TABLE 17.1 Antibody absorption assay on 5 rheumatoid arthritis sera

Absorbing test peptides	**ELISA (OD units)**		**Percentage LYSIS (%)**	
	Pre-absorption	Post-absorption	Pre-absorption	Post-absorption
HLA-DRB1 *0404	0.63 ± 0.005	0.13 ± 0.009	100 ± 0.0	13.7 ± 1.4
P. mirabilis haemolysin	0.72 ± 0.004	0.11 ± 0.010	ND	ND
Type XI collagen	0.56 ± 0.009	0.12 ± 0.007	82.4 ± 4.2	12.2 ± 1.7
P. mirabilis urease	0.59 ± 0.010	0.13 ± 0.010	ND	ND

The mean ± standard error of the absorbance values (630 nm) and the percentage lysis are given
ND not done

The mean (± standard error) in ankylosing spondylitis patients was 0.491 ± 0.011 OD units and this was significantly greater than the level of 0.181 ± 0.012 OD units found in rheumatoid arthritis patients ($t = 18.52, p < 0.0001$) or the level of 0.223 ± 0.015 OD units ($t = 14.15$, $p < 0.0001$) found in healthy controls.

There was no significant difference in the level of antibodies to the HLA-B27 peptide between rheumatoid arthritis patients and healthy blood donors.

Levels of IgG antibodies to the *Klebsiella pneumoniae* nitrogenase peptide CNSR**QTDRED**ELIIA were significantly elevated in ankylosing spondylitis patients compared to rheumatoid arthritis patients and healthy blood donors.

The mean (± standard error) in ankylosing spondylitis patients was 0.529 ± 0.011 OD units and this was significantly greater than the level of 0.197 ± 0.012 OD units found in rheumatoid arthritis patients ($t = 18.89$, $p < 0.0001$) or the level of 0.233 ± 0.014 OD units ($t = 15.96$, $p < 0.0001$) found in healthy controls.

There was no significant difference in the level of antibodies to the *Klebsiella pneumoniae* nitrogenase peptide between rheumatoid arthritis patients and healthy blood donors.

Levels of IgG antibodies to the *Klebsiella pneumoniae* pullulanase peptide RPTVIR**DRDE**YRQASS were significantly elevated in ankylosing spondylitis patients compared to rheumatoid arthritis and healthy blood donors.

The mean (± standard error) in ankylosing spondylitis patients was 0.530 ± 0.010 OD units and this was significantly greater than the level of 0.183 ±0.012OD units found in rheumatoid arthritis patients ($t = 20.15$, $p < 0.0001$) or the level of 0.218 ± 0.016 OD units ($t = 16.32$, $p < 0.0001$) found in healthy controls.

There was no significant difference in the level of antibodies to the *Klebsiella pneumoniae* pullulanase peptide between rheumatoid arthritis patients and healthy blood donors.

There was no significant reactivity of rheumatoid arthritis and ankylosing spondylitis sera when compared to the sera from healthy blood donors to the following peptides from: human myosin, *Streptococcus pyogenes* and the scrambled peptides from haemolysin or pullulanase.

Cytotoxicity Studies in Ankylosing Spondylitis Patients

Sera from ankylosing spondylitis and rheumatoid arthritis patients, as well as sera from healthy controls were tested for complement-mediated cytotoxicity against sheep red cells coated with HLA-B27*2705 synthetic peptide.

The sera from ankylosing spondylitis patients showed significantly higher percentage lysis for sheep red blood cells coated with CKAKA**QTDRED**LRTLL of HLA.B*2705 peptide when compared to rheumatoid arthritis patients or healthy controls (Fig. 17.3).

The mean (± standard error) percentage lysis in ankylosing spondylitis patients was 69.2 ± 3.1% and this was significantly higher than the level found in rheumatoid arthritis patients which was 14.8 ± 1.1% ($t = 19.28$, $p < 0.0001$) or the level of 16.3 ± 1.4% ($t = 16.13$, $p < 0.0001$) found in healthy blood donors.

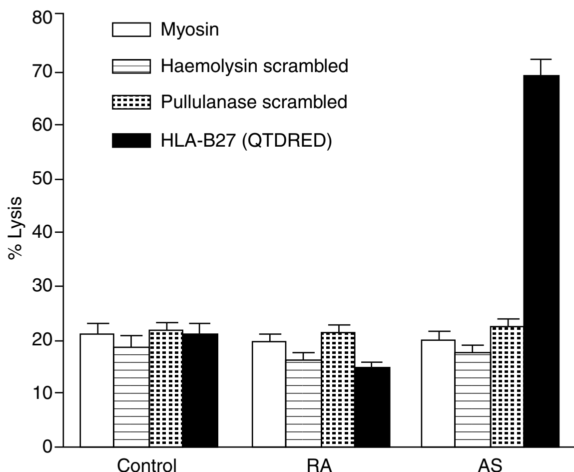

FIGURE 17.3 Percentage lysis of sheep *red* blood cells coated with HLA-B27 peptide (*QTDRED*) and other peptides via complement-mediated cytotoxicity by control sera and sera from patients with rheumatoid arthritis (*RA*) and ankylosing spondylitis (*AS*) (With kind permission from Wilson et al. (2003))

There was no significant difference in the cytotoxic activity of rheumatoid arthritis sera or the ones obtained from healthy donors when tested against sheep red blood cells coated with the HLA-B*2705 peptide.

No significant differences were found between ankylosing spondylitis and rheumatoid arthritis patients or healthy controls when testing sheep red blood cells coated with either human myosin, haemolysin scrambled or pullulanase scrambled peptides.

Correlation Between Cytotoxicity and Antibodies

There was a significant correlation between anti-nitrogenase antibodies and percentage lysis of sheep red blood cells coated with HLA-B*2705 (QTDRED) peptides for the

ankylosing spondylitis and rheumatoid arthritis as well as the healthy control subjects ($r = +0.826$, $p < 0.0001$) (Fig. 17.4a).

There was also a significant correlation between anti-pullulanase (DRDE) antibodies and percentage lysis of sheep red blood cells coated with HLA-B*2705 (QTDRED) peptide for the ankylosing spondylitis and rheumatoid arthritis patients as well as the healthy control subjects ($r = +0.823$, $p < 0.0001$) (Fig. 17.4b).

There was also a significant correlation between anti-HLA-B*2705 antibodies and percentage lysis of sheep red blood cells coated with HLA-B*2705 (QTDRED) peptide for the ankylosing spondylitis and rheumatoid arthritis patients as well as the healthy control subjects ($r = +0.817$, $p < 0.0001$).

Discussion and Conclusions

Rheumatoid arthritis patients have been shown to have increased levels of antibodies against synthetic peptides derived from *Proteus mirabilis* haemolysin (ESRRAL), HLA-DRB*0404 (EQRRAA), type XI collagen (LRREI) and *Proteus mirabilis* urease (IRRET).

However, we were unable to find any significant increase in levels of antibodies to HLA-DRB1*0402 (EDERAA) peptide, a histocompatibility group not linked to rheumatoid arthritis.

These findings confirm our previous reports and those of others that rheumatoid arthritis patients have increased levels of antibodies against synthetic peptides containing ESRRAL (Dybwad et al. 1996) and EQRRAA (Takeuchi et al. 1990) sequences.

Further, rheumatoid arthritis sera are cytotoxic for sheep red blood cells coated with HLA-DRB1*0404 and type XI collagen peptides. These two observations have important pathological significance because they link directly an HLA susceptibility type to joint damage through the presence of anti-*Proteus mirabilis* antibodies causing damage to chondrocytes carrying HLA markers and to collagen XI present in hyaline cartilage.

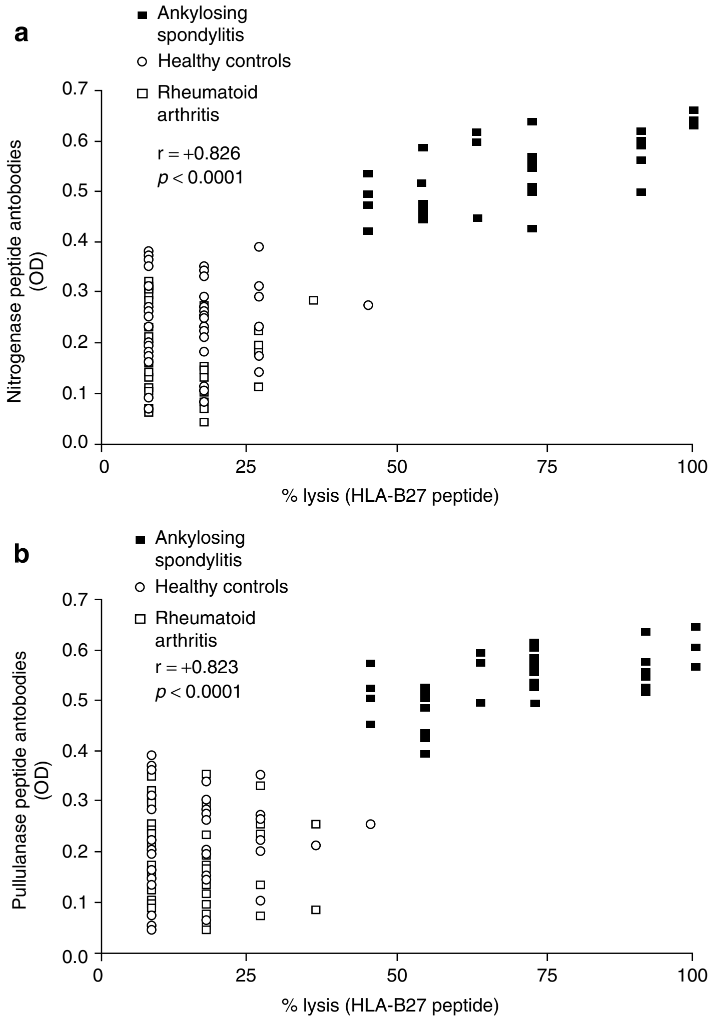

FIGURE 17.4 Correlation of anti-*Klebsiella* nitrogenase (**a**) and anti-*Klebsiella* pullulanase (**b**) IgG antibody levels for ankylosing spondylitis and rheumatoid arthritis patients and controls with percentage lysis of sheep *red* blood cells coated with HLA-B27 peptide (With kind permission from Wilson et al. (2003))

The pathological sequence could be summarised as follows:

> 'shared epitope' ► *Proteus* infection ► anti-*Proteus* antibodies ► joint damage

Similarly, ankylosing spondylitis patients have been shown to have increased levels of antibodies against synthetic peptides derived from *Klebsiella pneumoniae* and HLA-B*2705, and there was a significant correlation between anti-HLA-B*2705 antibodies and percentage lysis of sheep red cells coated with HLA-B*2705 peptide.

A similar situation is known to occur in rheumatic fever, where anti-*streptococcal* antibodies have been found to be cytotoxic for heart and fibroblast cell lines, because of molecular similarity between the *streptococcal* M protein and human cardiac myosin. (Cunningham et al. 1992).

The rheumatoid arthritis and ankylosing spondylitis patients used in our studies were deemed to be active in that their erythrocyte sedimentation rates were elevated. The high levels of *Proteus mirabilis* haemolysin, urease, HLA-DRB1*0404 and type XI collagen peptide antibodies were not due to nonspecific effects of inflammation because although ankylosing spondylitis patients were active, their levels of antibodies were similar to those found in healthy control subjects.

Antibodies against type XI collagen have been described in rheumatoid arthritis patients (Morgan et al. 1987). Furthermore, antibodies to type XI collagen have been found to be arthritogenic in DBA/1 mice (Boissier et al. 1990).

The α2 subunit of type XI collagen is a component of hyaline cartilage and is also present in non-cartilaginous tissue such as vitreous humour, which could be of relevance in episcleritis and scleromalacia perforans, conditions occurring in severe rheumatoid arthritis.

Proteus mirabilis haemolysin is a virulence factor contributing to the pathogenesis of the microorganism (Peerbooms et al. 1984).

The urease molecule of *Proteus mirabilis* facilitates infection of the kidneys and the urinary tract (Musher et al. 1975).

The haemolysin molecule from *Proteus mirabilis* has been shown to be a potent cytotoxic agent against renal proximal tubular epithelial cells, and some of the systemic and vascular manifestations of rheumatoid arthritis could occur as the result of the biological properties of this molecule (Mobley et al. 1991).

In conclusion, it would appear that cytotoxic properties of anti-*Proteus mirabilis* antibodies could be relevant in rheumatoid arthritis (Wilson et al. 2003).

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