Chapter 13
The 'Shared Epitope', *Proteus* Haemolysin, Type XI Collagen and Rheumatoid Arthritis

Contents

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

The association between rheumatoid arthritis and some subtypes of HLA-DR4 is well known and a particular region of the DRβ1 chain, from positions 70–74 coding for amino acids Gln-Arg-Arg-Ala-Ala (QRRAA) and found in DRB1*0101 (DR1) and some DR4 subtypes DRB1*0404 (DW14) and DRB1*0405 (Dw15), has been identified as the molecular sequence responsible for the susceptibility to rheumatoid arthritis (Nepom et al. 1989).

This susceptibility sequence has been given the name of the 'shared epitope'.

The sequence closely resembles that found in DRB1*0401 (DR4/Dw4) individuals, there being only one conservative substitution at position 71, from arginine to lysine (QKRAA). These two amino acids are positively charged, and thus the overall shape and charge configuration of these two sequences are similar.

The glutamic acid occupying position 69 is common to all DRβ1 molecules.

Our group has reported an amino acid homology between an outer membrane haemolysin protein of *Proteus mirabilis* and the 'shared epitope' found in patients with rheumatoid arthritis.

Early studies from our laboratory showed that anti-HLA-DR4 tissue typing sera bound significantly to *Proteus mirabilis* compared to non-HLA-DR4 tissue typing sera, but no such interaction was found with *Escherichia coli* bacteria.

The hexamer sequence ESRRAL of *Proteus mirabilis* haemolysin is hydrophilic which suggests that it may be immunogenic and could be involved in antibody interactions.

The present study was undertaken to compare the spatial configurations of EQRRAA/ESRRAL sequences for similarity using molecular modelling, and to determine if antibodies to the

ESRRAL peptide and to other similarity sequences of *Proteus mirabilis* were present in active rheumatoid arthritis patients.

Investigations were also carried out to determine if there was any link between *Proteus* bacteria and collagens.

Molecular Modelling of ESRRAL from *Proteus* with EQRRAA of HLA-DR1/4

Comparison of space filling models of a predicted ESRRAL sequence of *Proteus* haemolysin and EQRRAA sequence within DRB1*0101 (HLA-DR1) from known crystallographic structure was carried out to study spatial configuration. The ESRRAL model was constructed as a helix with torsional angles corresponding to those observed in the known EQRRAA sequence and the two sequences were superimposed (root mean square (RMS) = 0.046).

The ESRRAL and EQRRAA sequences were also compared with the sequence EDERAA present in DRB1*0402 (HLA-DR4/Dw10) which is not associated with rheumatoid arthritis.

Molecular modelling of the two structures ESRRAL and EQRRAA showed a common surface of homologous residues. The positions 32, 34, 35 and 69, 71, 72 form a motif common to HLA-DR and *Proteus mirabilis* occupying the same stereochemical space.

However, the EDERAA sequence of DRB1*0402 (HLA-DR4/Dw10), which is not associated with rheumatoid arthritis, differed from both the ESRRAL and EQRRAA motifs (Fig. 13.1).

Molecular Modelling of IRRET from *Proteus* Urease with LRREI of Type XI Hyaline Cartilage

Rheumatoid arthritis is a systemic disorder and cartilage destruction is a major feature of the disease. Therefore, a search of the protein database was made for any sequence of

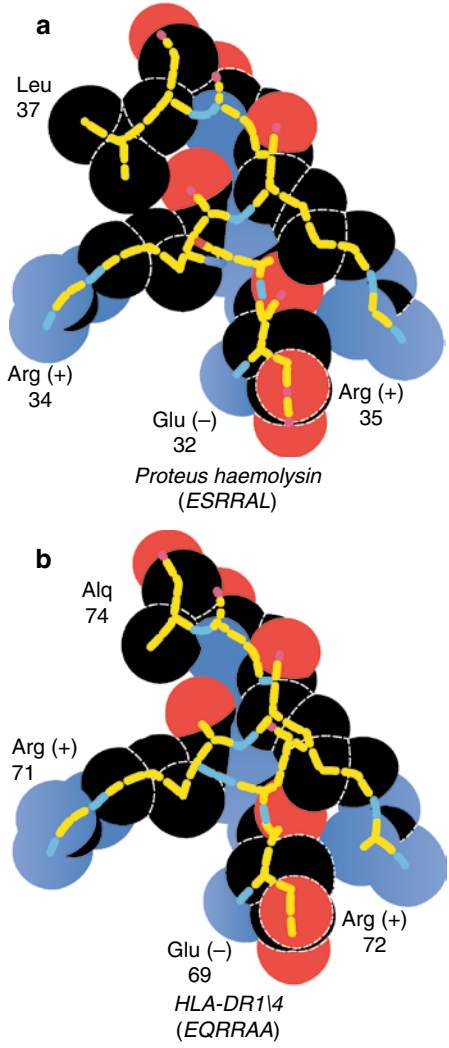

FIGURE 13.1 Comparison of space filling models. (**a**) ESRRAL sequence of *Proteus mirabilis* haemolysin predicted from known crystallographic structure. (**b**) EQRRAA sequence within DRB1*0101 (HLA-DR1), predicted from known crystallographic structure. (**c**) EDERAA sequence of DRB1*0402 (HLA-DR4/Dw10), an HLA group not associated with rheumatoid arthritis (Reprinted with permission from Springer Science+Business Media, Wilson et al. (1995))

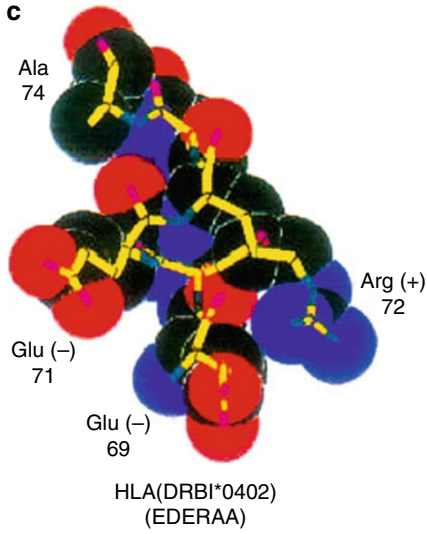

FIGURE 13.1 (continued)

Proteus mirabilis showing some 'molecular mimicry' or structural similarity to any collagens.

One way of distinguishing urine cultures is by determining whether the bacterial colonies obtained are either urease-positive or urease-negative. Urease is not present in *Escherichia coli* but is present in *Proteus* bacteria. Thus, the hypothetical question arose if the *Proteus* urease molecule had any similarity or showed any 'molecular mimicry' to collagens known to be present in joint tissues, hyaline cartilage and tendons.

A computer analysis was carried out to determine if there was any molecular association between *Proteus* urease and collagens.

An amino acid homology was identified between *Proteus mirabilis* urease (IRRET), amino acid residues 421–425 and α2(XI) collagen (LRREI) residues 421–425 (Kimura et al. 1989).

Type XI collagen is a component of hyaline cartilage which is composed of three different polypeptide subunits α1(XI), α2(XI) and α3(XI).

Northern blot analysis shows that the α2(XI) collagen gene is expressed in hyaline cartilage but not in adult liver, skin or tendons.

Comparison of space filling models was made of the predicted IRRET sequence of *Proteus mirabilis* urease and LRREI of α2(XI) collagen. Both sequences were constructed as helices and the two sequences superimposed (RMS = 0.011). Structures were modelled using Alchemy III (Tripos ASSOC Inc, St. Louis, USA).

Molecular modelling of the two sequences IRRET and LRREI also showed a common surface of homologous residues.

The positions 338, 339, 340 and 421, 422, 423 form a motif common to type XI collagen and *Proteus mirabilis* urease occupying the same stereochemical space (Fig. 13.2).

The common surface of homologous residues observed in the sequences ESRRAL/EQRRAA and IRRET/LRREI, which may be involved in immune interactions, consists of two positively charged arginines and one negatively charged glutamic acid.

First Study with Rheumatoid Arthritis Patients, Measuring Antibodies Against ESRRAL and the *Proteus* Haemolysin Protein

Sera were collected from active rheumatoid arthritis patients having an erythrocyte sedimentation rate above 15 mm/h. The rheumatoid arthritis patients were attending the Rheumatology Department at the Lister Hospital in Stevenage, Herts and active ankylosing spondylitis patients, who acted as 'disease controls' were attending the 'Ankylosing Spondylitis Research Clinic' of the Middlesex Hospital.

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

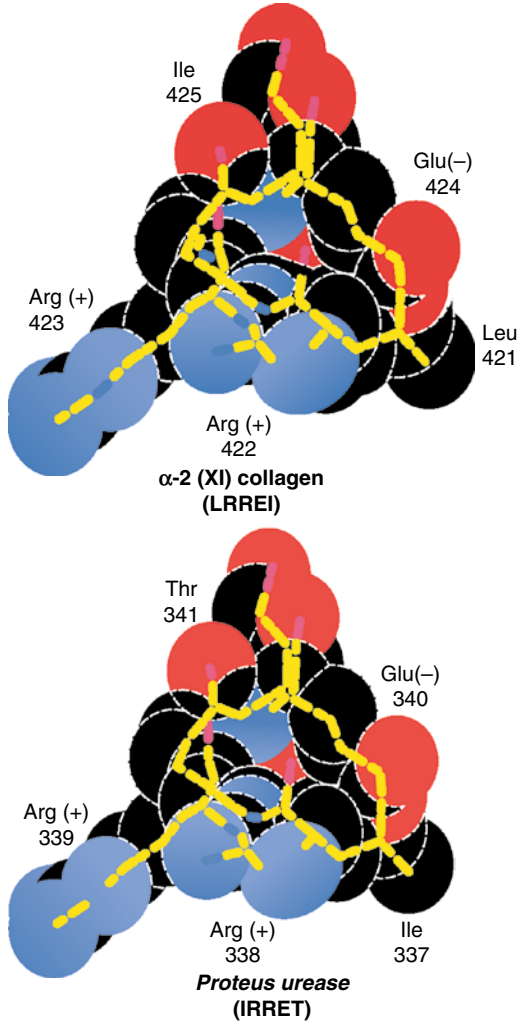

FIGURE 13.2 Space filling model of LRREI sequence of α2(XI) collagen, predicted from known crystallographic structure and IRRET sequence of *Proteus mirabilis* urease predicted from known crystallographic structure (Reprinted with permission from Springer Science+Business Media, Wilson et al. (1995))

In the first study, antibodies were measured against the ESRRAL peptide and the *Proteus* haemolysin protein.

Sera were obtained from 40 rheumatoid arthritis. Their mean age was 59 years (range: 37–77 years), and there were 10 men and 30 women. Their mean erythrocyte sedimentation rate (± standard error) was 46.2 ± 4.0 mm/h. The female to male ratio in the rheumatoid arthritis patients was 3:1.

Sera were also obtained from 30 ankylosing spondylitis patients. Their mean age was 49 years (range: 27–76 years), and there were 23 men and 7 women. The male to female ratio was 3.3:1. Their mean erythrocyte sedimentation rate (± standard error) was 30.1 ± 5.2 mm/h.

There were also 30 healthy control subjects. Their mean age was 23 years (range: 21–27 years). There were 15 male and 15 female healthy control subjects.

ELISA Studies with (a) Synthetic Peptides and (b) *Proteus* Haemolysin Protein

Synthetic Peptides

Peptides were prepared by solid phase synthesis and analysed for purity by high-performance liquid chromatography as previously described.

The test peptide was LGSIS**ESRRAL**QDSQR (16-mer), which represents amino acid residues 27–42 of *Proteus mirabilis* haemolysin (hpm B) and has in its middle the ESRRAL sequence.

The control peptide SQKDIL**EDERAA**VDTY (16-mer) from DRB1*0402 (HLA-DR4/Dw10) was used for comparison. It has in its middle the EDERAA sequence and this HLA group is not associated with rheumatoid arthritis.

Two other control peptides, YASGASGAS (9-mer) and DAHKSEVAHRFLDLGEENFKALVL (24-mer), were used to exclude nonspecific binding.

Sera were tested against the *Proteus mirabilis* haemolysin peptide and control peptides 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.

Antibodies to the *Proteus mirabilis* haemolysin peptide LGSIS**ESRRAL**QDSQR, of the IgG class, containing in the middle the ESRRAL sequence, 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 0.263 ± 0.011 OD units and this was significantly higher than the mean in ankylosing spondylitis patients which was 0.162 ± 0.012 OD units ($t = 5.98, p < 0.001$) or the mean in healthy control subjects which was 0.158 ± 0.014 OD units ($t = 5.90, p < 0.001$) (Fig. 13.3).

There was no significant reactivity by the rheumatoid arthritis patients or ankylosing spondylitis patients against the DRB1*0402 (HLA-DR4/Dw10) SQKDIL**EDERAA**VDTY (16-mer) peptide, an HLA group not associated with rheumatoid arthritis when compared to controls.

Proteus Haemolysin

Purification of the 63-kDa outer membrane haemolysin protein was carried as originally described for *Escherichia coli* (Schnaitman 1974).

Sera obtained from the same subjects as above were tested against *Proteus mirabilis* haemolysin (63 kDa) protein and against two control haemolysin proteins, obtained from *Streptococcus pyogenes* and *Vibrio parahaemolyticus* (Sigma Chemical Company Ltd) by ELISA.

Briefly, polystyrene microtitre plates (Dynatech) were coated with the haemolysin protein (2.0 μg/well) and incubated overnight at 4°C. Serum samples were diluted 1/200 in PBS-Tween and the plates saturated with 0.1% BSA-PBS-Tween. The remainder of the assay procedure was as previously described for the synthetic peptides.

The difference between the titres in ankylosing spondylitis patients and healthy controls, when tested against the *Proteus mirabilis* haemolysin, was not significant.

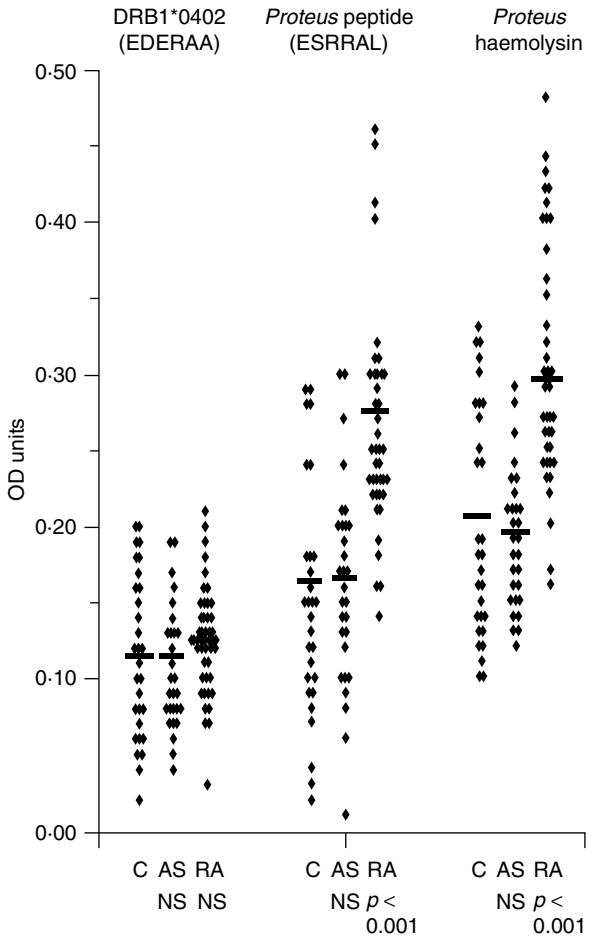

FIGURE 13.3 Antibody titres for IgG immunoglobulin in controls (*C*) and patients with ankylosing spondylitis (*AS*) or rheumatoid arthritis (*RA*) when tested by ELISA against the synthetic peptides EDERAA, ESRRAL and the haemolysin protein of *Proteus mirabilis*. *OD* optical density. Bars indicate means. *NS* not significant when compared to controls (Reprinted with permission from Springer Science+Business Media, Wilson et al. (1995))

There was no elevation in IgA and IgM antibodies against the *Proteus* haemolysin peptide.

However, antibodies of the IgG class to *Proteus mirabilis* haemolysin protein (63 kDa) were significantly elevated in rheumatoid arthritis patients when compared to ankylosing spondylitis patients and healthy controls.

The mean (± standard error) in rheumatoid arthritis patients was 0.304 ± 0.013 OD units and this was significantly higher than the mean in ankylosing spondylitis patients which was 0.190 ± 0.075 OD units ($t = 7.11$, $p < 0.001$) or the mean in healthy control subjects which was 0.201 ± 0.014 OD units ($t = 5.52$, $p < 0.001$) (Fig. 13.3).

In contrast, there was no elevation in IgA or IgM antibodies against the *Proteus* haemolysin protein and no significant reactivity by the rheumatoid arthritis patients against the two control *Streptococcus pyogenes* and *Vibrio parahaemolyticus* haemolysin proteins.

Second Study with Rheumatoid Arthritis Patients, Measuring Antibodies Against Urease Proteins

In the second study, antibodies were measured against two different preparations of bacterial urease, one from *Bacillus pasteurii* urease and the other one from *Proteus mirabilis* urease.

Sera were obtained from 20 rheumatoid arthritis patients. Their mean age was 56 years (range: 20–75 years), and there were 5 men and 15 women. The female to male ratio in the rheumatoid arthritis patients was 3:1. Their mean (± standard error) erythrocyte sedimentation rate was 59.8 ± 7.7 mm/h.

Sera were also obtained from 40 ankylosing spondylitis patients. Their mean age was 45 years (range: 21–76 years). There were 29 men and 11 women. The male to female ratio in the ankylosing spondylitis patients was 2.6:1. Their mean (± standard error) erythrocyte sedimentation rate was 25.9 ± 3.6 mm/h.

There were also 15 healthy control subjects and their mean age was 29 years (range: 21–52 years).

Purification of the 280-kDa *Proteus mirabilis* urease protein was carried out by the method of Jones and Mobley (Jones and Mobley 1989).

Sera were obtained from different subjects than the ones investigated in the first study and were tested against *Proteus mirabilis* urease protein and *Bacillus pasteurii* urease (Sigma Chemical Company) by ELISA. Polystyrene microtitre plates (Dynatech) were coated with the urease protein (2 μg/well) and assays carried out as before.

Antibody titres against *Proteus mirabilis* urease protein of the IgG class 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 0.68 ± 0.07 OD units and this was significantly higher than the mean in ankylosing spondylitis patients which was 0.39 ± 0.02 OD units ($t = 5.09$, $p < 0.001$) or the mean in healthy control subjects which was 0.32 ± 0.04 OD units ($t = 4.06$, $p < .001$), but there was no significant difference in the titres between ankylosing spondylitis patients and healthy controls (Fig. 13.4).

Furthermore, there was no significant reactivity by rheumatoid arthritis patients against *Bacillus pasteurii* urease (Fig. 13.4).

Discussion and Conclusions

In this study, active patients with rheumatoid arthritis have been shown to have an increased titre of antibodies against a synthetic peptide derived from *Proteus mirabilis* membrane haemolysin (hpm B), containing the hexamer sequence ESRRAL which is homologous with the rheumatoid arthritis susceptibility sequence EQ(K)RRAA of the DRβ1 chain of class II major histocompatibility complex molecules.

Rheumatoid arthritis patients were also found to have an elevated titre of antibodies against the native *Proteus*

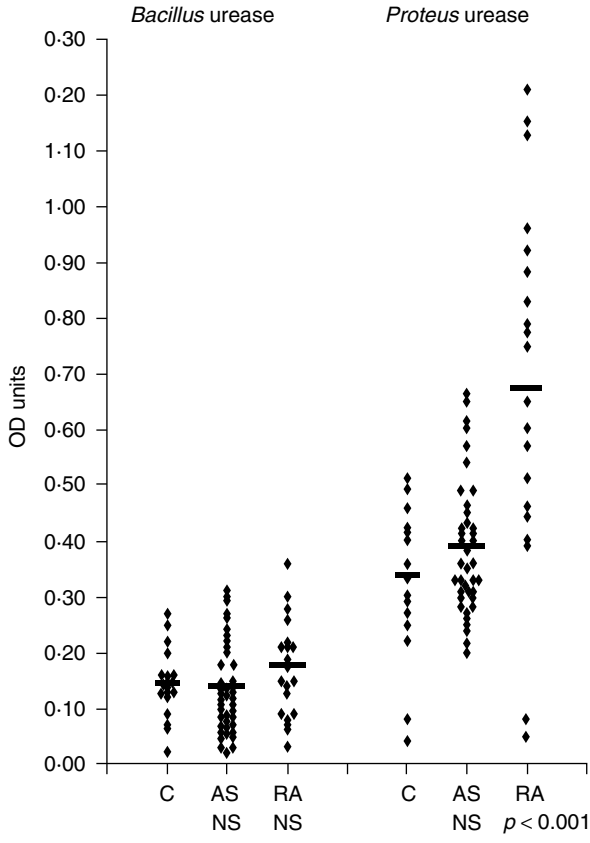

FIGURE 13.4 Antibody titres for IgG immunoglobulin in controls (*C*) and patients with ankylosing spondylitis (*AS*) or rheumatoid arthritis (*RA*) when tested by ELISA against the urease proteins of *Proteus mirabilis* and *Bacillus pasteurii*. *OD* optical density. Bars indicate means. *NS* not significant when compared to controls (Reprinted with permission from Springer Science+Business Media, Wilson et al. (1995))

haemolysin protein but not against the haemolysin proteins found in *Streptococcus pyogenes* or *Vibrio parahaemolyticus*.

The ESRRAL motif was found in three of 67,000 sequences: *Proteus mirabilis*, *Serratia marcescens* and *Vibrio cholerae*.

This demonstration of a significant increase in IgG but not IgA antibodies to *Proteus mirabilis* membrane haemolysin sequence and to the native protein suggests that the site of infection in rheumatoid arthritis patients is not a mucosal surface involving IgA but in some interstitial tissue leading to IgG production.

In a related study, autoantibodies against a 16-mer synthetic peptide of DRB1*0405 (HLA-DR4/Dw15) β1 chain, containing the EQRRAA sequence, were reported to be increased in Japanese patients with rheumatoid arthritis, when tested by ELISA (Takeuchi et al. 1990).

This study also shows that rheumatoid arthritis patients have an increased titre of antibodies to the urease protein derived from *Proteus mirabilis*, containing a sequence IRRET which is homologous with sequence LRREI present in type XI collagen. The IRRET sequence was found in six biological proteins: *Bacillus sphaericus*, murine leukaemia virus, *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Proteus mirabilis* out of 30,000 sequences examined in the protein database. The high level of *Proteus mirabilis* antibodies was not attributable to nonspecific effects of inflammation because the ankylosing spondylitis patients also had increased erythrocyte sedimentation rates, yet their levels of *Proteus mirabilis* antibodies were similar to those found in healthy control subjects.

The ESRRAL and IRRET sequences are both hydrophilic and in helical configurations where they may be involved in immune interactions.

The results of this study suggest that rheumatoid arthritis patients have a significant increase in antibodies to a *Proteus* membrane haemolysin sequence containing a hexamer which is homologous with the rheumatoid arthritis susceptibility sequence described by the 'shared epitope'.

Moreover, the rheumatoid arthritis patients in this study also had a significant elevation in antibodies to *Proteus mirabilis* urease containing a pentamer which is homologous with a sequence in type XI collagen (Wilson et al. 1995).

Further investigations are required to determine if antibodies to ESRRAL/IRRET and haemolysin/urease react with HLA alleles and collagen to produce tissue damage, thereby contributing to the pathogenesis of this disease.

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