Meeting in Valencia - September 2014

- Professor Alan Ebringer
- B.Sc, MD, FRCP. FRACP, FRCPath.
- Professor of Immunology, King's College, London, U.K.

Some autoimmune diseases are caused by bacterial infections.

Meeting in Valencia - September 2014

New possibilities about causes of common autoimmune diseases: rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis.

Anti-bacterial therapy should be used together with anti-TNF and anti-inflammatory biologicals in these autoimmune diseases.

Rheumatic fever as a model

- In patients with severe tonsillitis due to *Streptococcus pyogenes* antibodies will be produced which will attack the heart and cause "rheumatic fever".

- "Molecular mimicry" between the *Streptococcus* and the heart is the cause of this autoimmune disease and can be used as a model for other autoimmune diseases..

Meeting in Valencia - September 2014

- "Molecular mimicry" occurs in rheumatic fever.
- "Molecular mimicry" has been used to show:
- (1) ankylosing spondylitis (KLEBSIELLA), (2) rheumatoid arthritis (PROTEUS) (3) multiple sclerosis (ACINETOBACTER) are autoimmune diseases caused by bacterial infections.

Ankylosing spondylitis

- Disease 1.

Ankylosing spondylitis

In 1973, Terasaki from Los Angeles and D.C.O. James from London showed that 96% of AS patients carry the HLA-B27 antigen, whilst it is only present in 8% of the general population in the UK and USA.

We have used the "molecular mimicry" model of rheumatic fever to explain these results.

Ankylosing spondylitis

- AS is a disease which starts between the ages 15 and 30 years and is characterised by BACKACHE and muscle stiffness.

- In AS, muscle stiffness is relieved by exercise.

Ankylosing spondylitis

- There are approximately one million individuals in the U.K. and 5 million in the US who suffer from some feature of ankylosing spondylitis.

- Approximately 30% of AS patients will also suffer during their lifetime from an episode of uveitis or iritis.

Ankylosing spondylitis

HLA antigens are antigenic markers present on all nucleated cells and are inherited co-dominantly from both parents.

Ankylosing spondylitis

- The explanation for the HIGH FREQUENCY of HLA-B27 in AS patients is the “central problem in AS research”, today.

Ankylosing spondylitis

- Which microbe ?
- HLA-B27 lymphocytes were injected into a rabbit and antiserum obtained.
- Antiserum reacted with *Salmonella*, *Shigella*, *Yersinia* and *Klebsiella*.

Ankylosing spondylitis

- Patients with AS tested for presence of faecal *Salmonella, Shigella, Yersinia* and *Klebsiella*.

- Only *Klebsiella* microbes isolated.

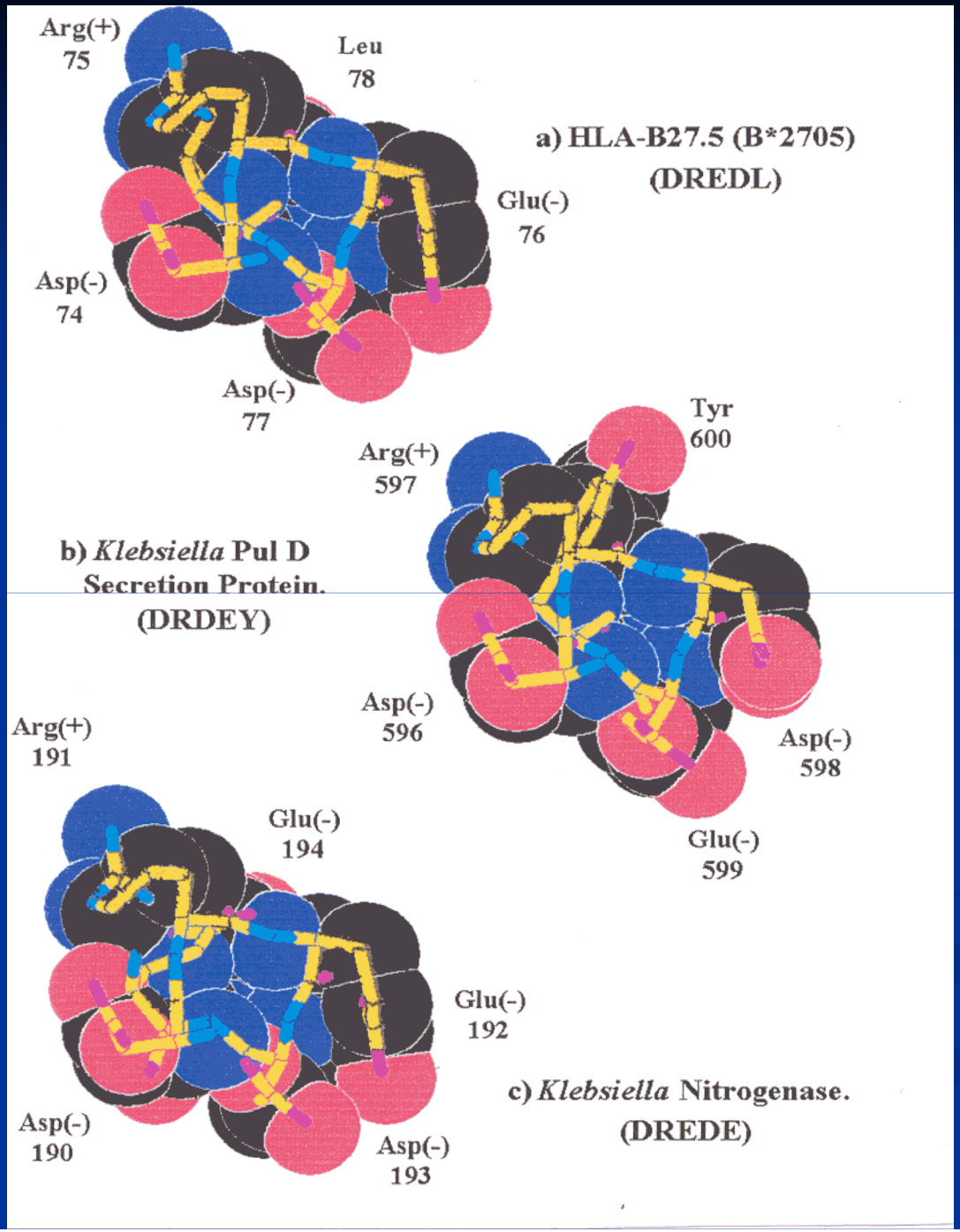
Ankylosing spondylitis

- There are 2 molecules in *Klebsiella* showing MOLECULAR MIMICRY, one against HLA-B27 and the other one against "spinal collagens".

Ankylosing spondylitis

- The "pullulanase-D" (London) and "nitrogenase reductase" (La Jolla-Calif) molecules of *Klebsiella* resemble HLA-B27.

- "Pullulanase-A" resembles collagens found in I (spine), III (muscles) and IV (uvea). This explains backache, muscle stiffness and uveitis.


Arg(+)
75
Leu
78
Glu(-)
76
Asp(-)
74
Asp(-)
77
a) HLA-B27.5 (B*2705)
(DREDL)
Tyr
600
Arg(+)
597
b) Klebsiella Pul D
Secretion Protein.
(DRDEY)
Asp(-)
596
Asp(-)
598
Glu(-)
599
Arg(+)
191
Glu(-)
194
Glu(-)
192
Asp(-)
190
Asp(-)
193
c) Klebsiella Nitrogenase.
(DREDE)

Ankylosing spondylitis

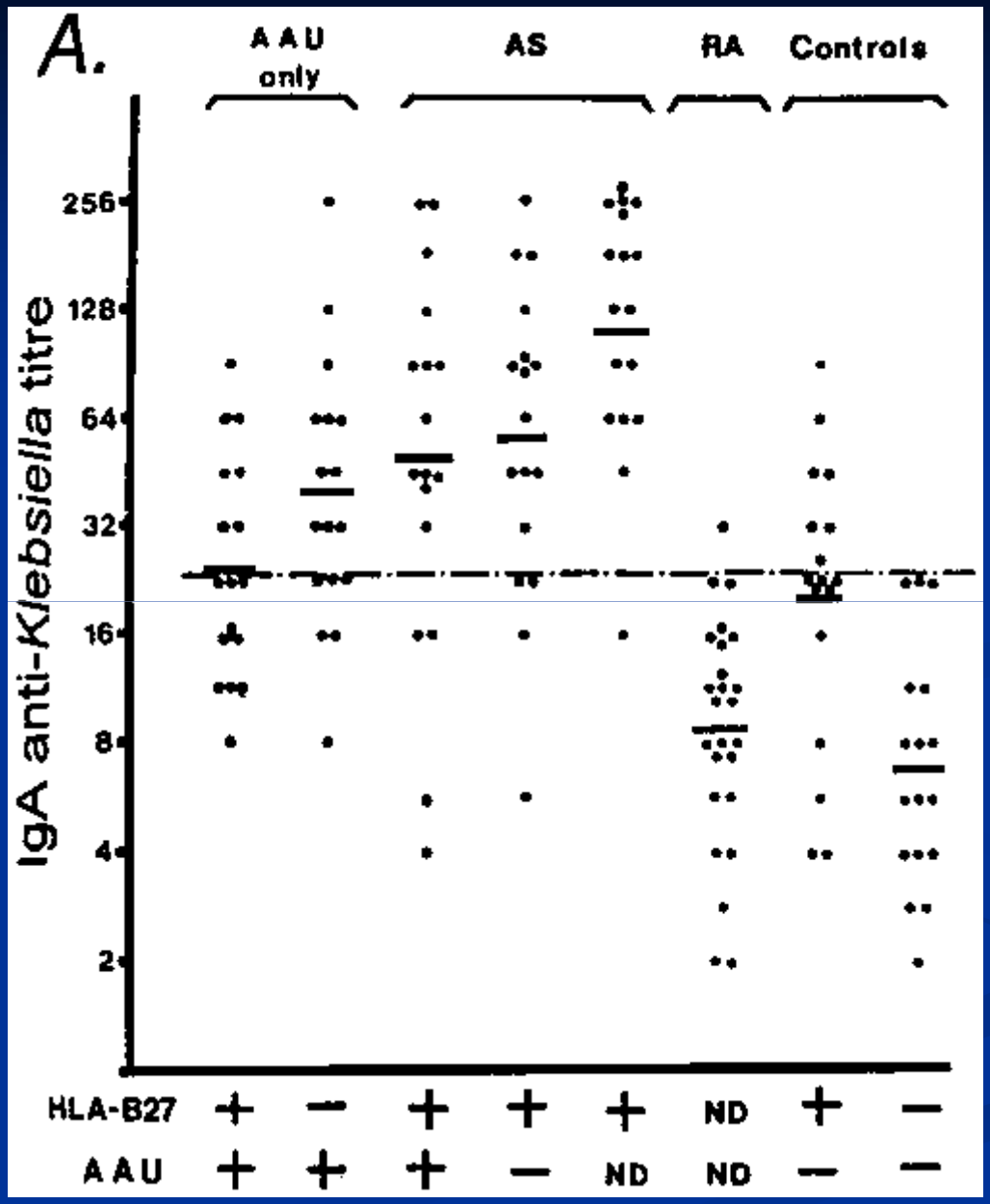
- AS sera are cytotoxic for sheep red cells coated with HLA-B27, haemolysin

  or pullulanase peptides but NOT RA

  or blood donor sera.
- The presence of auto-antibodies to HLA-B27 antigen and to spinal collagens makes AS an autoimmune disease.

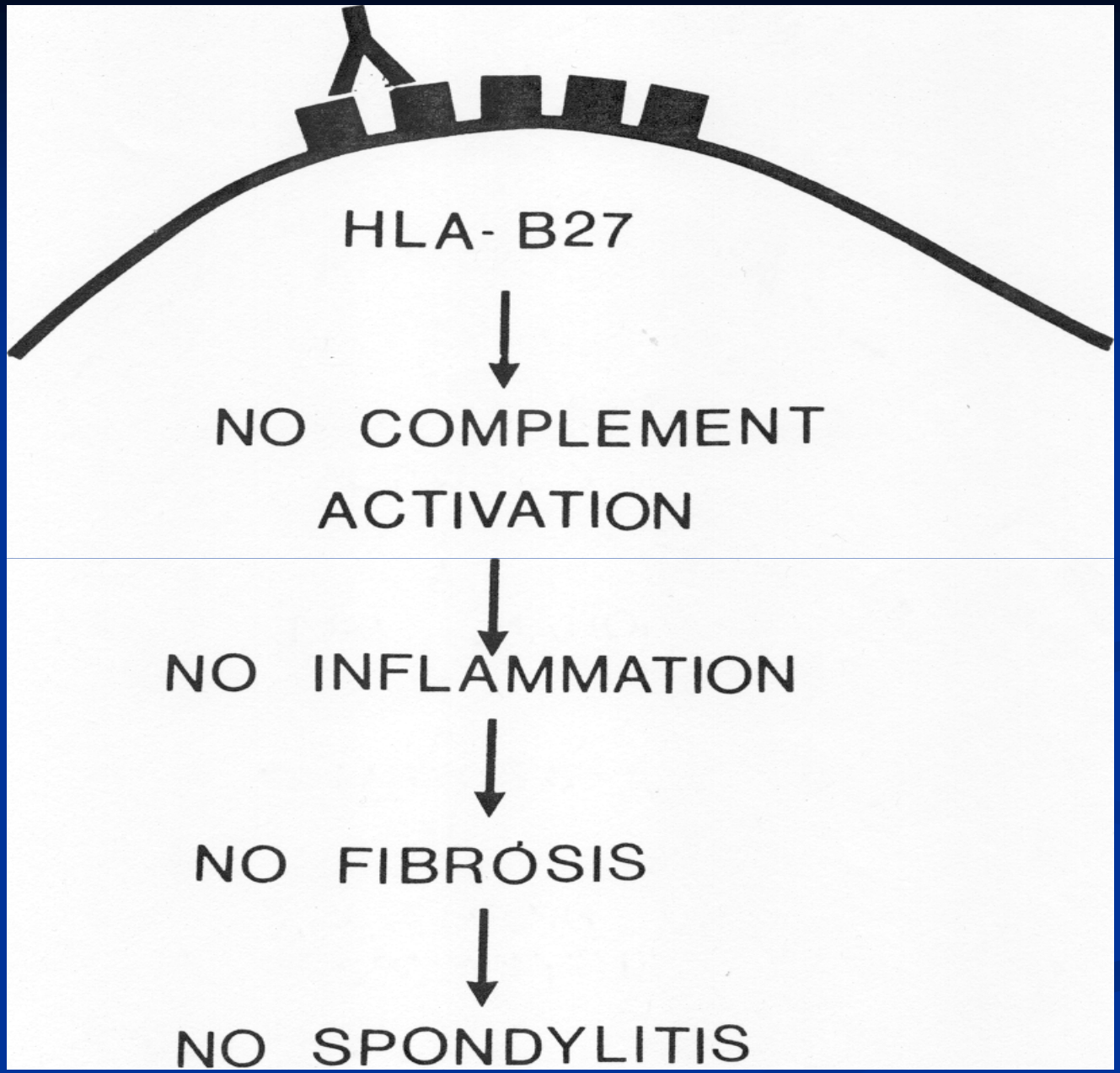
Ankylosing spondylitis

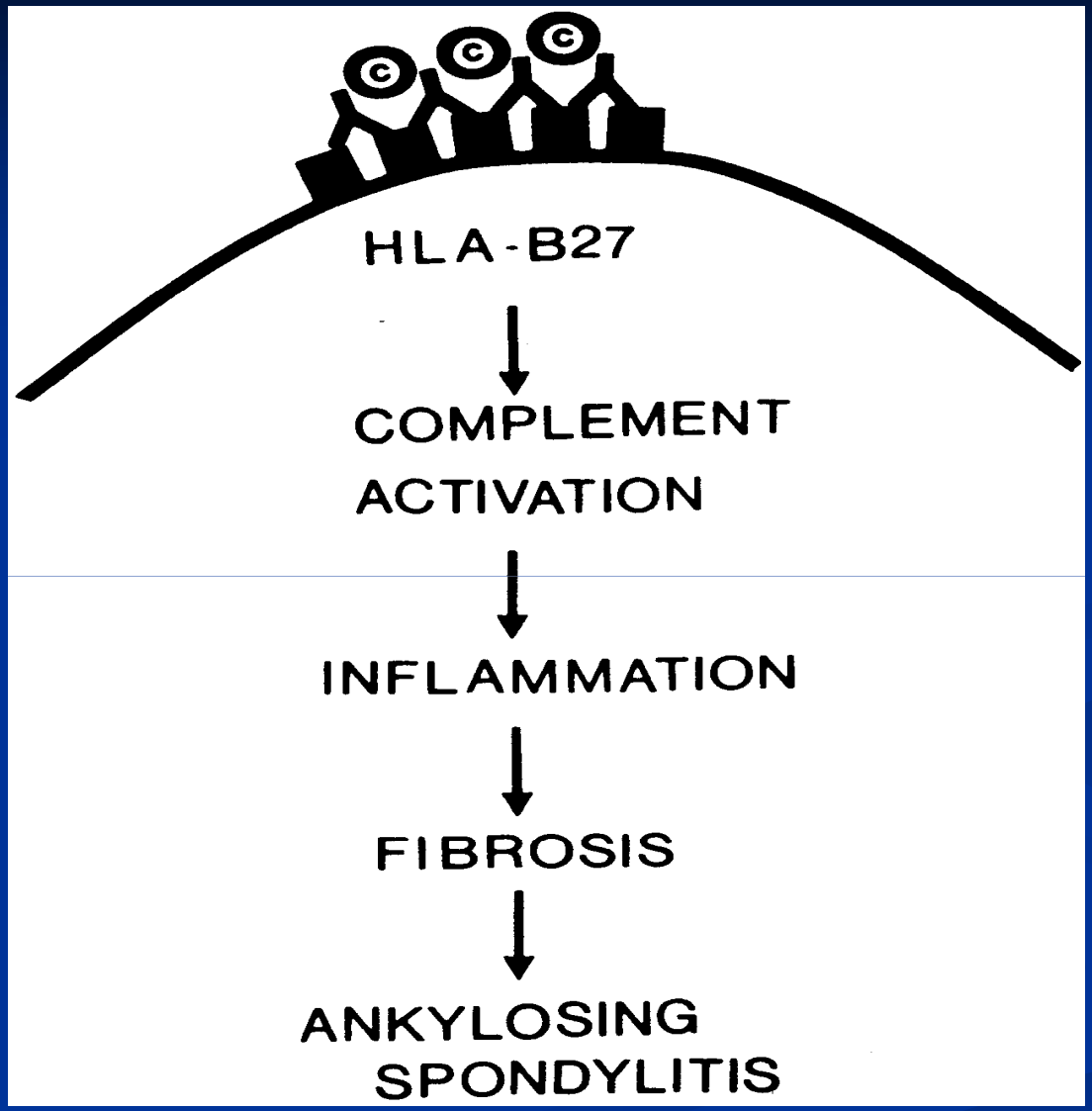
- Dutch ankylosing spondylitis patients have elevated levels of antibodies against *Klebsiella*.


A.
AAU only
AS
RA
Controls
IgA anti-Klebsiella titre
256
128
64
32
16
8
4
2
HLA-B27 + − + + + ND + −
AAU + + + − ND ND − −

Ankylosing spondylitis

- The complement cascade is activated when 2 IgG antibodies are close enough to activate C1q.
- If the antibodies are too far apart, C1q will NOT be activated.
- Therefore CYTOTOXIC activity will occur only when high antibody levels are present.


HLA- B27
NO COMPLEMENT ACTIVATION
NO INFLAMMATION
NO FIBRÓSIS
NO SPONDYLITIS


HLA-B27
COMPLEMENT
ACTIVATION
INFLAMMATION
FIBROSIS
ANKYLOSING
SPONDYLITIS

Rheumatoid arthritis

- Disease 2.

Rheumatoid arthritis

- In 1978, STASTNY from Dallas showed that 80% of rheumatoid arthritis (RA) patients carery the HLA-DR4 antigen, whilst it is present in only 35% of the general population. Similar results in RA patients were reported from Europe.

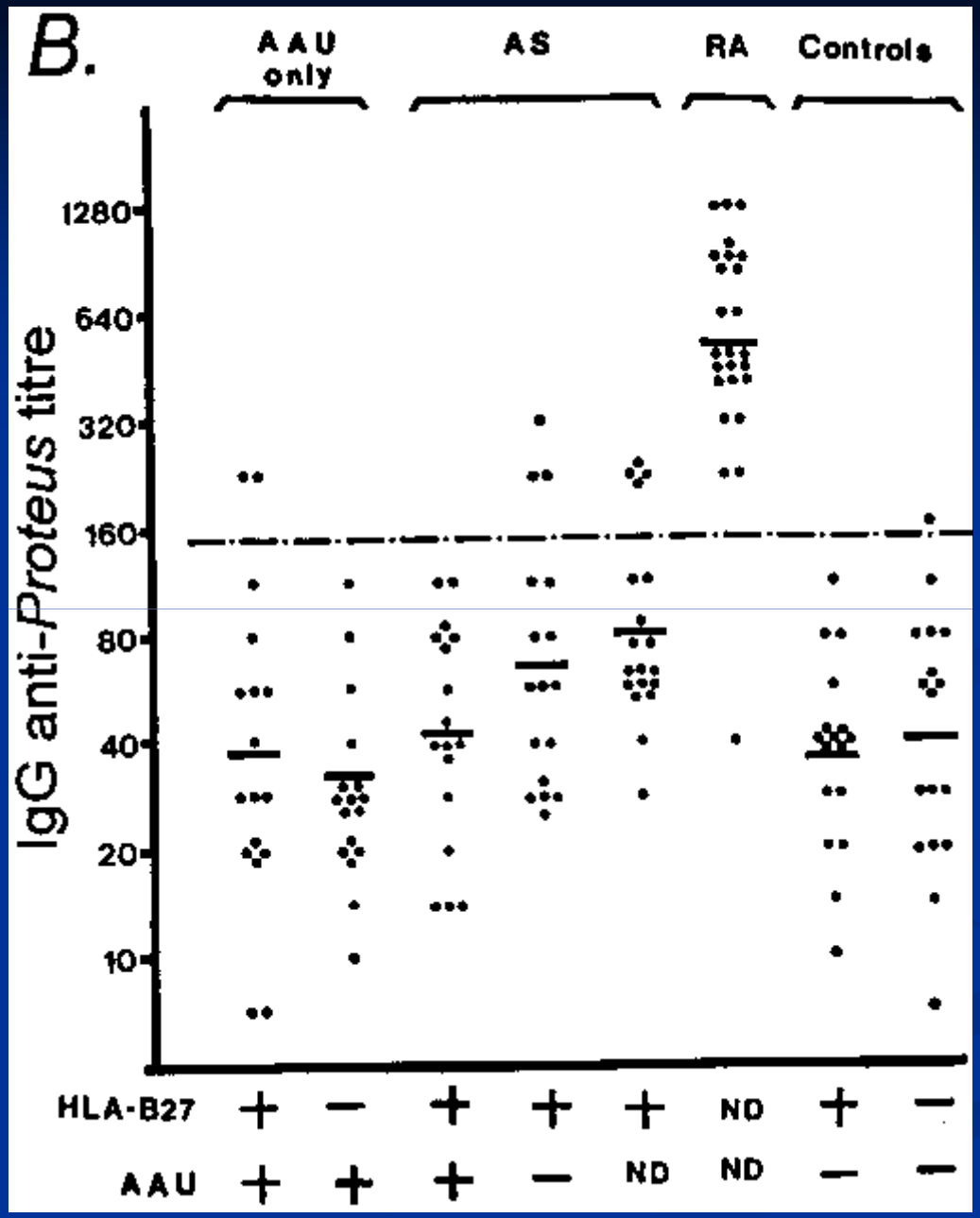
Rheumatoid arthritis

- We injected HLA-DR4 lymphocytes into rabbits and they made antibodies against PROTEUS bacteria.

This was the EUREKA moment, since the second commonest cause of urinary tract infections is caused by PROTEUS bacteria.

Antibodies in Dutch sera

- Antibodies to *Klebsiella* have been measured in Dutch patients with AS, uveitis and RA, and compared to tissue typed HLA-B27 positive and HLA-B27 negative blood donors.

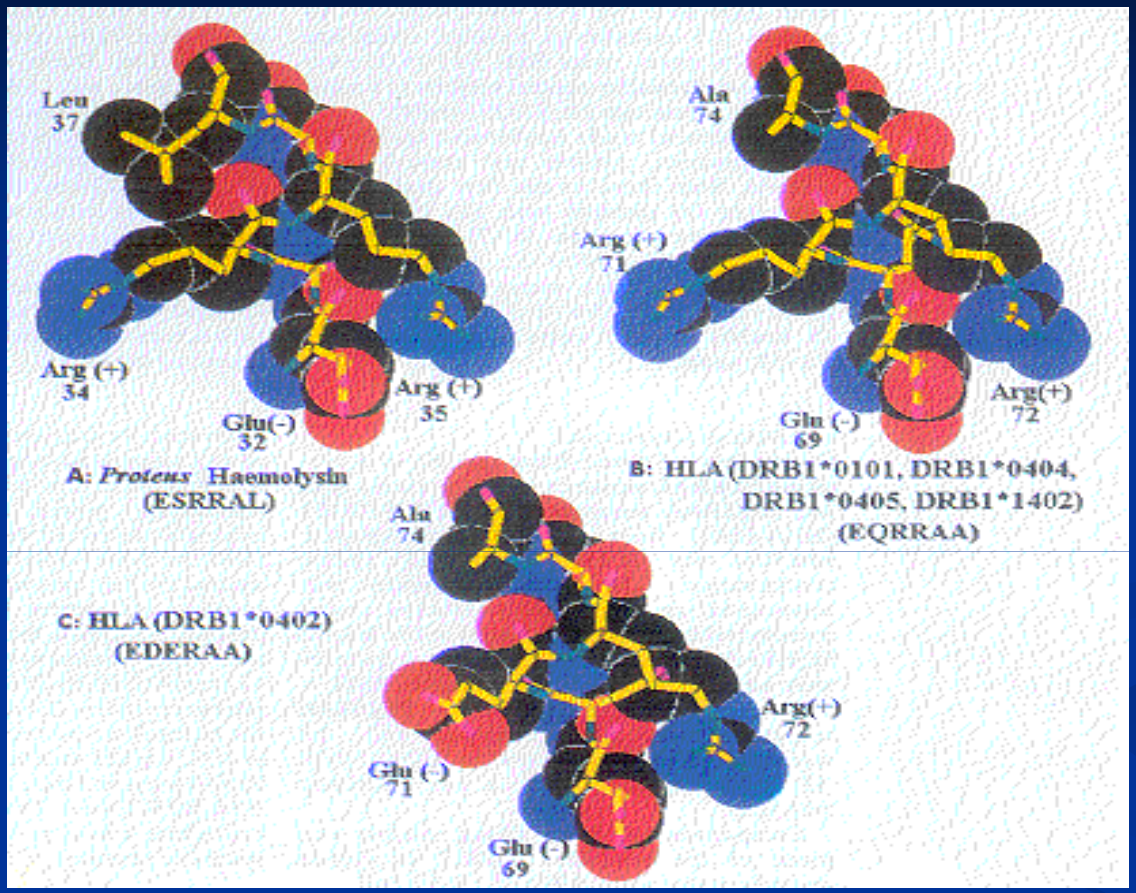

B.
AAU only
AS
RA
Controls
IgG anti-Proteus titre
1280
640
320
160
80
40
20
10
HLA-B27 + − + + + ND + −
AAU + + + − ND ND − −

Rheumatoid arthritis

- The microbe *Proteus mirabilis* contains sequences which resemble HLA-DR1/4 molecules.

- *Proteus* haemolysin contains the ESRRAL resembles stereochemically the EQRRAA sequence found in HLA-DR1/4 molcules.

Comparison of space filling models



A: ESRRAL sequence of Proteus mirabilis haemolysin
*B: EQRRAA sequence within DRB1*0101 (HLA-DR1)*
*C: EDERAA sequence of DRB1*0402 (HLA-DR4/Dw10)*
(predicted from known crystallographic structure)

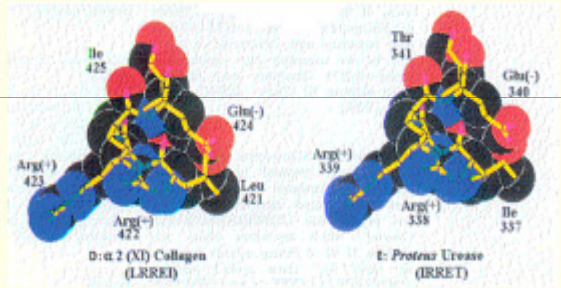
Rheumatoid arthritis

Proteus urease antibodies

attack synovial tissues because of molecular mimicry

Rheumatoid arthritis

Comparison of Space filling Models



D: predicted LRREI sequence of α 2(XI) collagen

E: predicted IRRET sequence of *Proteus mirabilis* urease

Multiple sclerosis

- Disease 3.

Multiple sclerosis

- It was only in 1930's, some 40 years after PASTEUR, that it was discovered if you inject "brain homogenates" into experimental animals, you produce a disease, called "experimental allergic encephalomyelitis" which resembles human "multiple sclerosis" (MS).

Multiple sclerosis

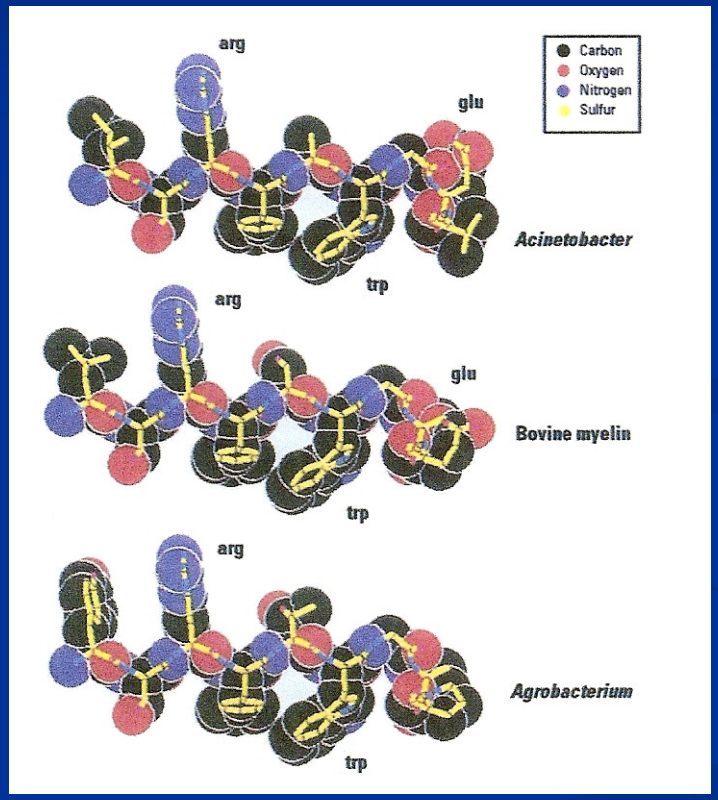
- Eylar et al., from San Diego, published a paper in "Science 1970;168:1220-23" that a 9-amino acid bovine encephalitogenic peptide when injected into into guinea pigs produces classical "hind quarters paralysis" of EAE.

Multiple sclerosis

- We took Eylar's encephalitogenic peptide "FSWGAEGQK" and asked the computer which bacteria or viruses have this sequence ?

  The answer was 3 bacteria; *Acinetobacter, Agrobacterium* and *E.coli.*

Multiple sclerosis



Multiple sclerosis

- ***Acinetobacter*** is found in soil, in faeces, muddy water, sewage, on human and animal skin and in the maxillary nasal sinuses and it also crossreacts with myelin.

Agrobacterium is in soil and ***E.coli*** is found everywhere.

Multiple sclerosis

- A first study was carried out on 29 MS patients

  obtained from the Institute of Neurology, National Hospital Queen Square, London (9 males, 17 females) (Age 29-55 years) and compared to

  20 CVA (cerebro-vascular accident – stroke) (Mean Age 80.5 years) as well as

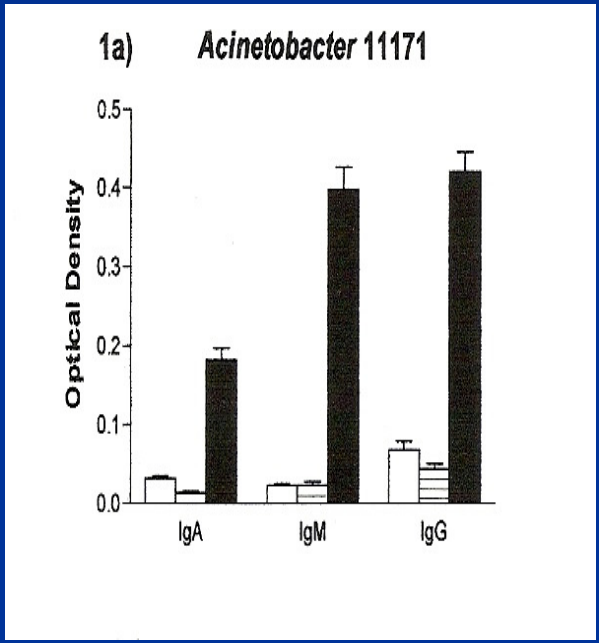
  25 healthy blood bank controls.

Multiple sclerosis

- All ELISA tests were carried out blind, in that the person doing the assay did not know which was a sample from an MS patient, CVA patient or from a healthy blood donor subject.

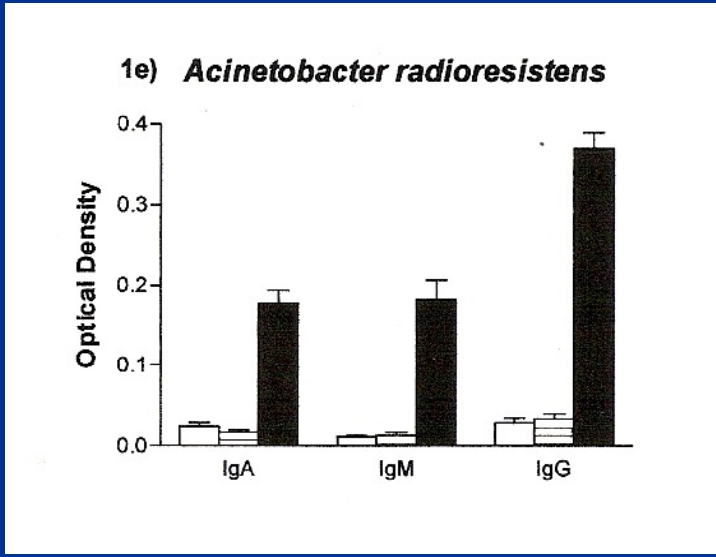
Multiple sclerosis

- Clear= controls, hatched =CVA, dark= MS



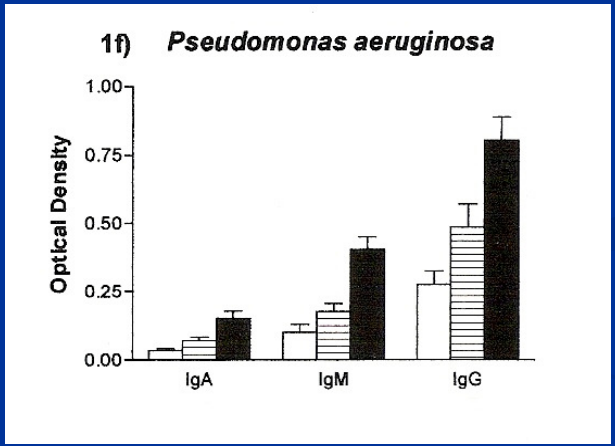
Multiple sclerosis

- Clear = controls, hatched = CVA, dark =MS



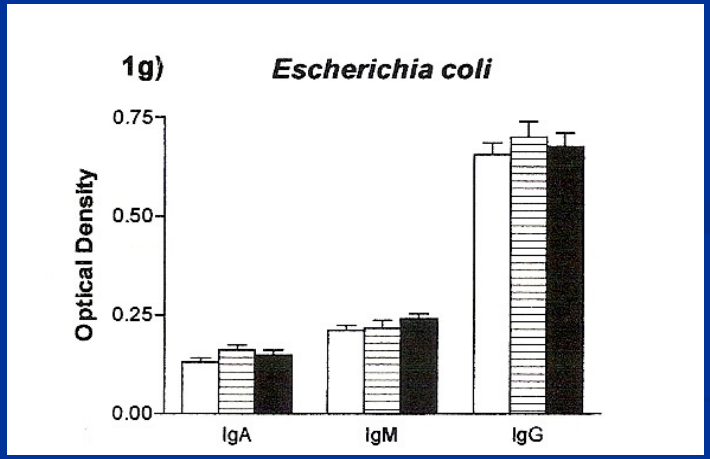
Multiple sclerosis

- Clear = controls, hatched = CVA, dark = MS.



Multiple sclerosis

- Clear = controls, hatched =CVA, dark = MS



Multiple sclerosis.

- The results of the FIRST study showed that MULTIPLE SCLEROSIS patients have elevated antibodies to the nasal microbe *Acinetobacter*.
- They also have slightly elevated levels to *Pseudomonas* bacteria.

However they do not have elevated levels against the ubiquitous microbe *Escherichia coli*.

These results suggest that *Acinetobacter* bacteria may be involved in the aetiology of MS.

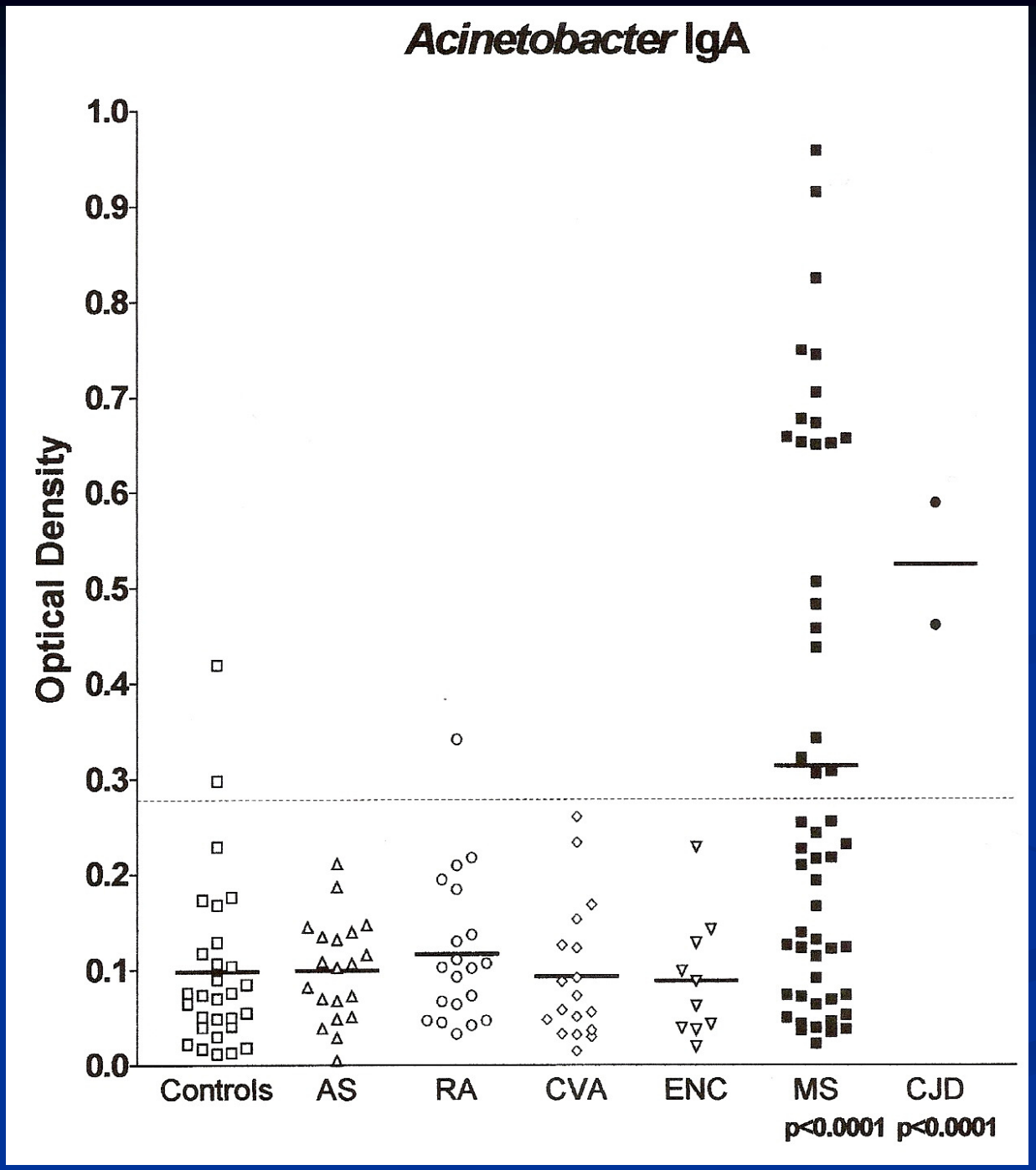
Multiple sclerosis

- These results have been published:

"Antibody responses to *Acinetobacter* and *Pseudomonas* in multiple sclerosis".
Hughes et al., Clin & Diagn Lab Immunology 2001; 8: 1181-8.

Multiple sclerosis 2nd study

- 53 multiple sclerosis (MS)
- 2 sporadic Creutzfeldt-Jakob disease (sCJD)
- 18 stroke (Cerebro-vascular accident) (CVA)
- 10 encephalitis
- 20 rheumatoid arthritis (RA)
- 20 ankylosing spondylitis (AS)
- 29 healthy controls


Acinetobacter IgA
Optical Density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Controls
AS
RA
CVA
ENC
MS
CJD
p<0.0001
p<0.0001

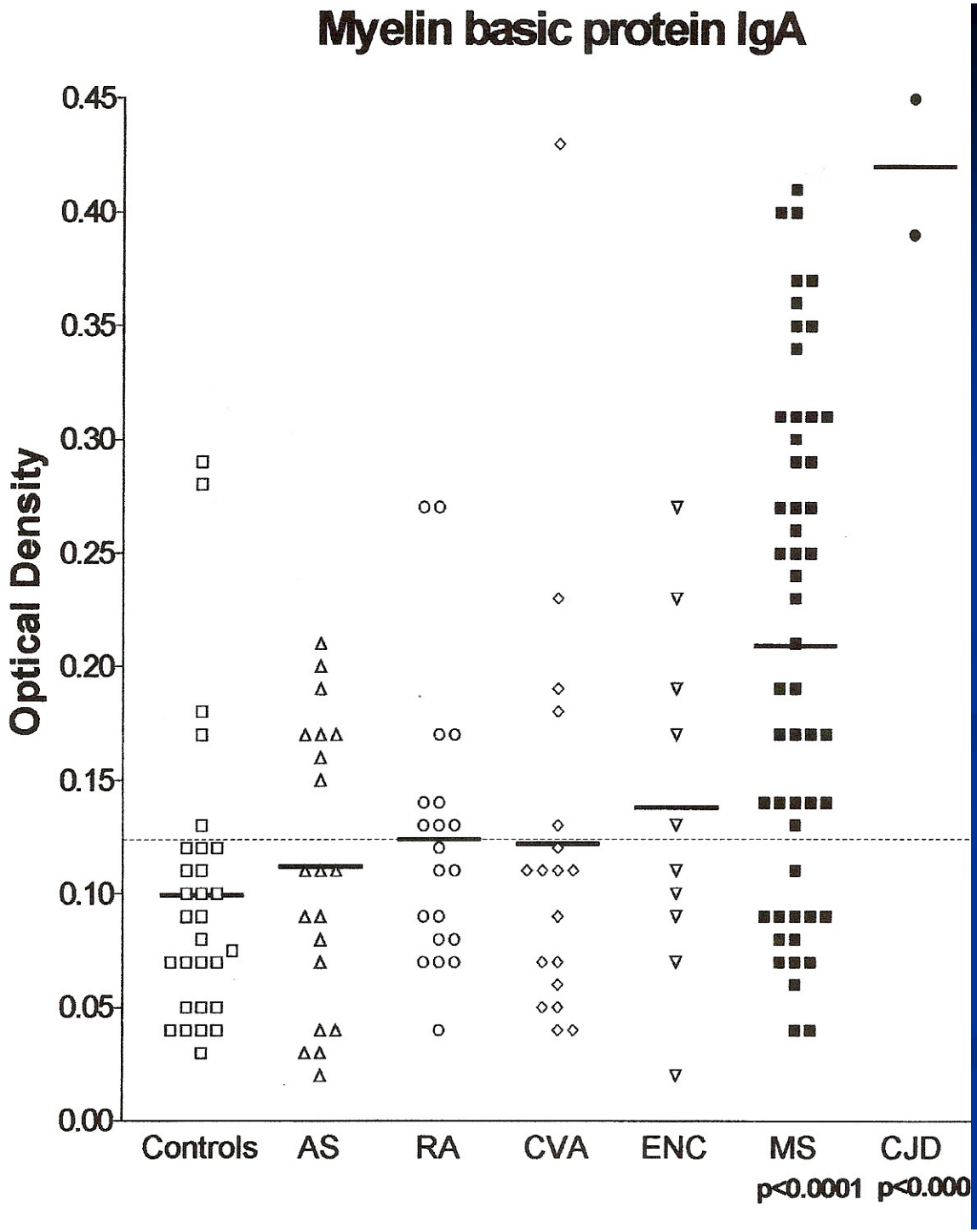
Sporadic-Creutzfeldt-Jakob disease

- 2 patients with sporadic-Creutzfeldt-Jakob disease also had elevated levels of antibodies to ACINETOBACTER bacteria.

- Further studies are required with CJD patients.

Multiple sclerosis

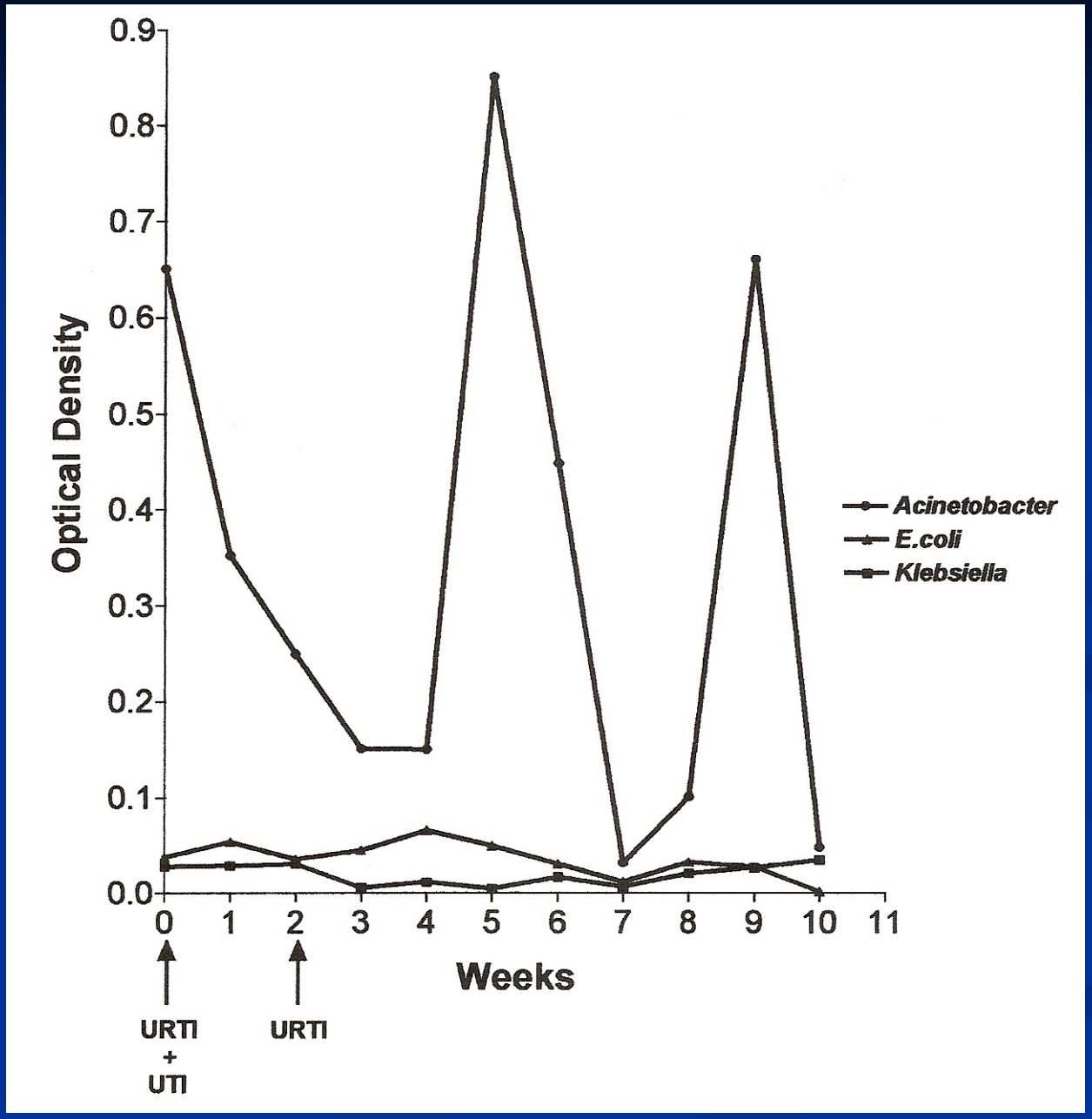
- We have also measured the IgA antibody titre to “myelin basic protein” in these patients.


Myelin basic protein IgA
Optical Density
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Controls
AS
RA
CVA
ENC
MS
CJD
p<0.0001
p<0.000

Multiple sclerosis

- One 38 year-old, female multiple sclerosis (MS) patient was followed for 10 weeks with regular measurements of antibodies to *Acinetobacter, Klebsiella* and *E.coli*.

During this period she developed 2 relapsing neurological episodes.


Optical Density
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11
Weeks
URTI + UTI
URTI
Acinetobacter
E.coli
Klebsiella

Acinetobacter and maxillary sinus

- Casiano et al. from Miami showed that *Acinetobacter* bacteria were the most frequently isolated microbes from the maxillary sinuses, when measured by "endoscopically directed nasal culture".

Casiano et al. Laryngoscope 2001; 111;1333-7.

Multiple sclerosis and sinusitis

- Dr. Dick's group showed in a study of 92 MS patients sinusitis was present in 70% of MS patients from London, compared to a frequency of 17% in controls.

Gay et al. "Multiple sclerosis associated with sinusitis". Lancet 1986; i :815-9.

Pathogenesis of MS

- Pathogenesis:

IgG1 and IgG3 antibodies
which activate complement cascade
readily cross the blood-brain barrier

Pathogenesis of MS

- *Acinetobacter* sinusitis ► Anti-*Acinetobacter* antibodies ► IgG1 and IgG3 complement dependent autoantibodies causing myelin tissue damage

Conclusions

- (1) Multiple sclerosis is probably caused by exposure to *Acinetobacter* bacteria in nasal sinuses.

  (2) *Acinetobacter* shows molecular mimicry or similarity to myelin.

Conclusions

- (3) MULTIPLE SCLEROSIS patients should be treated with ***anti-Acinetobacter*** **therapy.**

(4) Multiple centre studies are required to resolve these questions in both multiple sclerosis and sporadic Creutzfeldt-Jakob disease.

Conclusions

- (5) Ankylosing spondylitis is caused by exposure to Klebsiella bacteria in the colon, predominantly in HLA-B27 individuals who have a high starch diet.

(6) A "low starch diet" might be beneficial in AS patients.

Conclusions

- (7) Rheumatoid arthritis is caused by an upper urinary tract infection by Proteus bacteria.

(8) This explains why rheumatoid arthritis is 3-4 times more frequently in women.

General conclusion

- Several autoimmune diseases are caused by bacterial infections:

Ankylosing spondylitis by *Klebsiella*
Rheumatoid arthritis by *Proteus*
Multiple sclerosis by *Acinetobacter*