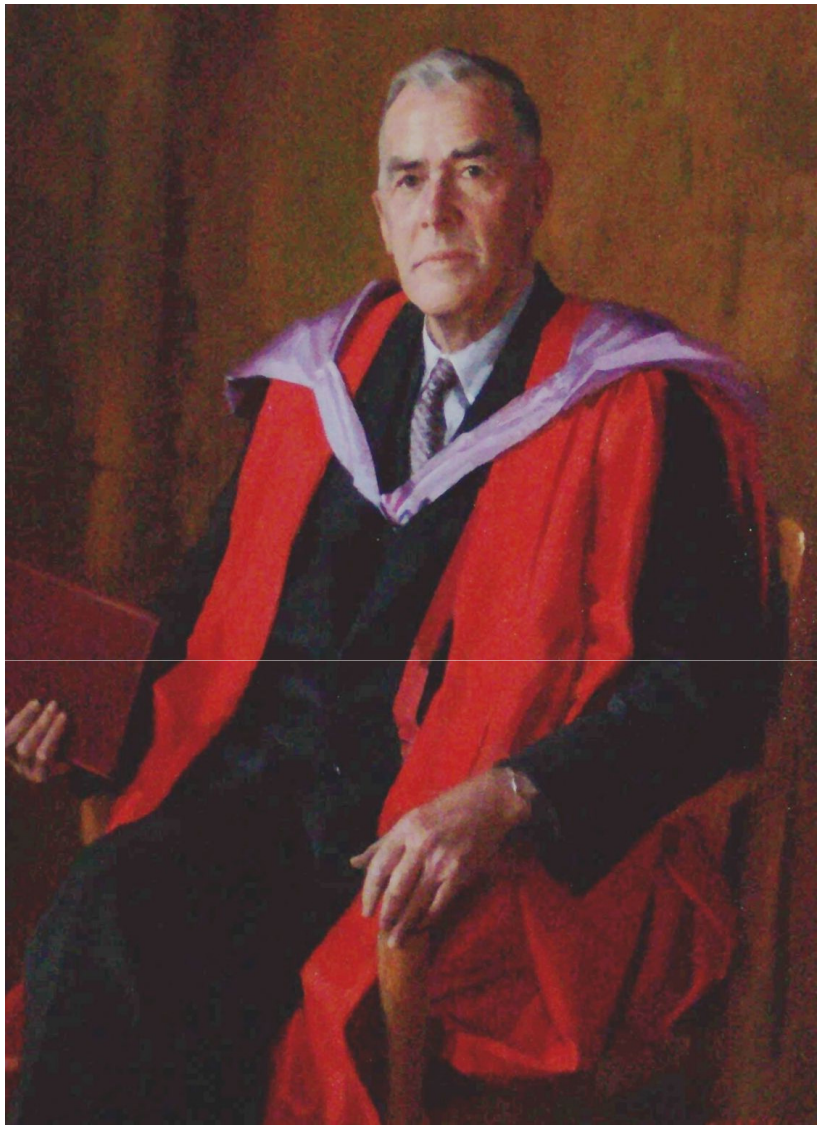
Prophylaxis of Autoimmune Disease

DD Adams[1], JG Knight[2], A Ebringer[3]

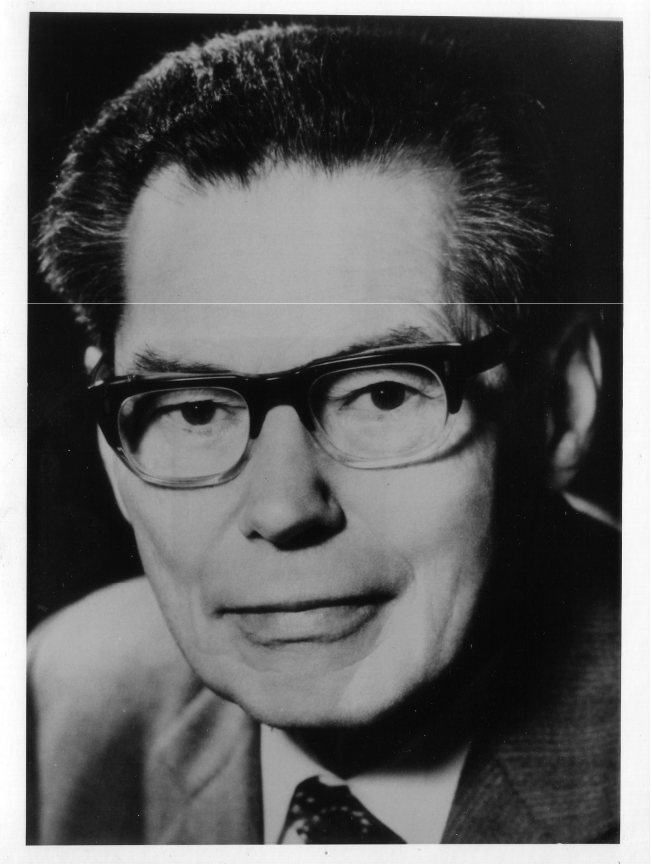
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Sir Charles Hercus

Dr HD Purves, MSc, MB ChB, FRSNZ
Physicist, chemist, mathematician, clinician

- With CE Hercus abolished NZ's goitre endemic.
- With TH Kennedy discovered antithyroid drugs.
- With WE Griesbach solved pituitary cytology.
- With DD Adams discovered thyroid-stimulating antibodies



Graves' Disease

1838 Robert Graves in Dublin identified the syndrome of tachycardia, goitre, weight loss and exophthalmos.

1933 Charles Harington isolated thyroxine, the thyroid hormone, and realised that its excessive production causes the thyrotoxicosis of Graves' disease.

1956 Adams and Purves in Dunedin, using ^{131}I for a bioassay of thyroid-stimulating hormone in baby guinea pigs, discovered **long-acting thyroid stimulator (LATS)** which proved to be an autoantibody that causes thyrotoxicosis by accidentally reacting with the thyroid gland's receptor for thyroid-stimulating hormone from the pituitaty gland, the conductor of the endocrine orchestra.

Thyroid-stimulating autoantibodies (TSab)

Thyroxine contains 4 atoms of **iodine**, enabling bioassay of thyroid-stimulating substances in ^{131}I injected mice by measurement of their blood radioactivity before and after injection of test materials.
An in vitro neutralization step, using the human thyroid receptor, revealed **LATS protector**, thyroid stimulating autoantibodies reactive with the human thyroid's receptor for thyroid-stimulating hormone (TSH), in contrast to the slightly different mouse TSH receptor. This step enabled demonstration that TSab are present in all definite cases of Graves' disease, in amounts that correlate with the patient's thyroid activity as noted clinically and by patients' thyroid uptake of ^{131}I.
Adams, Kennedy, Stewart. *BMJ 1974;2: 199-201.*
Also Allison Knight, Cague, Adams *in* Manual of Clinical Immunology. *Eds.* Rose and Friedman, 2nd Edition, American Society for Microbiology, Washington, pp. 391-402, 1980.



Dr Allison Knight, BSc(Hons) PhD

- With DDA showed that thyroid-stimulating autoantibodies show a fine variation between patients.
- In family studies, with Barbosa, showed that type1diabetes is not caused by autoantibodies.

  Diabetes Research 1988; 9:1.
- An opposite result in similar study of schizophrenic families would confirm its autoimmune basis.



Dr John G Knight, BSc(Hons) PhD

- Disproved loss of suppressor T cells as the cause of autoimmunity in NZB mice.
- Proved forbidden clone theory for Graves' disease with measurement of κ and λ light chains.
- With DDA, solved inheritance of autoimmune disease with the H gene theory.
- Author of the Knight model (autoimmune) of schizophrenia.



The Knight model of schizophrenia.

(Knight JG, *Lancet* 1982; ii: 1073-1076.)
Forbidden clones of B lymphocytes develop by somatic mutation and make autoantibodies which act on neuronal receptors to affect the function of the brain's limbic system.
Familial aggregation is due to H and V genes.
The wait for V gene mutation explains discordance of MZ twins and the juvenile grace gap (relative absence in children).
Also explained are:
1 Kety's adoptive studies (*Arch Gen Psych* 1974; 30: 121)
2 Remission and relapse.
3 The rheumatoid arthritis/schizophrenia discordance (Mellsop et al *Aust NZ J Med* 1974; 4: 247-252)
Confirmation from predisposition by MHC and B cell light chain V genes (Harrison & Owen, *Lancet* 2003; 361: 417-419)

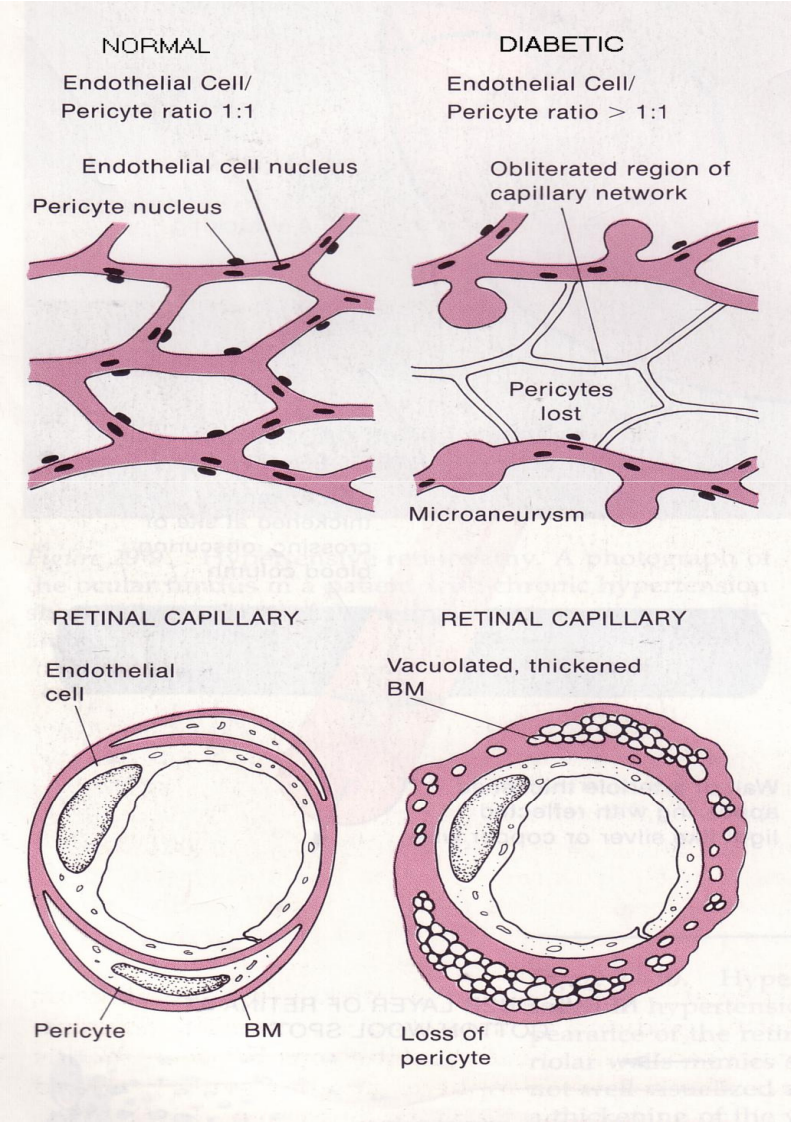
Discovery of dopamine receptor autoantibodies by Dr Fabienne Brilot and colleagues.

F Brilot et al Brain 2012 Nov; 135(Pt 11): 3453-68.
Antibodies to surface dopamine-2 receptor in autoimmune movement and psychiatric disorders.

These autoantibodies, found in children with encephalitis, are precisely the ones that Dr John Knight postulated to cause schizophrenia. Dr Fabienne Brilot has been invited to test blood from acute schizophenic patients for the presence of these autoantibodies. If she finds them, she will have establised the autoimmune basis of schizophrenia, confirming the Knight Model of Schizophrenia, published in the Lancet 1982; ii: 1073-6, with more deatail on page 39 in “Autoimmune Disease” by DD Adams and CD Adams, Springer Briefs in Public Health, 2013.

Association of pericyte loss with collapse of the retinal vasculature.

- From Gordon Klintworth, in Pathology, edited by Rubin and Farber, 1988



The cause of diabetic retinopathy; cross-tissue T cell auto-reactivity

2008 Adams has postulated:

1 That destruction of the retinal pericytes causes the vascular collapse of diabetic retinopathy.

2 That the pericytes are destroyed by forbidden clones of cytotoxic T cells, antigenically related to those that destroy the pancreatic islet β cells that make insulin.

(Adams DD. Autoimmune destruction of pericytes as the cause of diabetic retinopathy.
Clinical Ophthalmology 2008; 2: 295-298)

Revolutions in Medicine

I. Achieved: **The Germ Theory of Disease.** 1870

Led by Louis Pasteur and Robert Koch. Bitterly resisted.

Solved of the aetiology of a score of major diseases,
including tuberculosis, plague, cholera, typhoid, tetanus,
diphtheria, pneumonia, erysipelas, syphilis, meningitis.

Discovered the immunity system and rational immunization.

Founded aseptic surgery, led by Joseph Lister.

II. Pending: **Conquest of the Autoimmune Diseases.** 2010s

Theoretical basis:

The **Forbidden Clone Theory** of the pathogenesis.

The **H Gene Theory** of the genetics.

Specific **Microbial Triggers** are the cause.

Major technology to develop:

Finding and negating **microbial triggers**.

Selective destruction of **forbidden clones**.

The three types of adverse immune responses.

1. Allergy and anaphylaxis.
2. Serum sickness and immune complex disease.
3. Autoimmunity.

Adverse Immune Responses

Type 1. Allergy and anaphylaxis.
Gut worm defense mechanism reacting to non-worm antigens.

Fault: a B lymphocyte IgE clone reactive with an allergen.
e.g., hay fever, anaphylaxis, gut allergy, skin allergy.

Adverse Immune Responses

Type II. Serum sickness and immune complex disease.

Fault: excessive quantity of antigen.
This swamps complement-neutralising mechanisms, causing complement-mediated damage.
Microbes offer picogram quantities of antigen,
not milligrams of horse serum protein,
nor micrograms of released intra-cellular proteins, as from nuclei.
e.g., post-passive immunization serum sickness,
systemic lupus erythaematosus, lupus nephritis.

Adverse Immune Responses

Type III. Autoimmunity.
Fault: forbidden clones, which are anti-microbial lymphocyte clones with accidental host-antigen specificity, arising from unlucky somatic mutations in their V genes.

Type III B. Diseases caused by **B cell forbidden clones**
e.g., Graves' disease, myasthenia gravis, rheumatoid arthritis.

Type III T. Diseases caused by **T cell forbidden clones**
e.g., Diabetes type 1, diabetic retinopathy and
presumably autoimmune hepatitis, Addison's disease
and other autoimmune diseases with specific parenchymal
cell destruction.

The Forbidden Clone Theory

Burnet, a Bacteriologist, accustomed to counting mutation rates in multiplying bacterial cells on blood agar plates, realised that multiplying lymphocyte cells will mutate similarly.
Therefore, he proposed that the Forbidden Clones that cause autoimmune diseases arise by unlucky somatic gene mutations in multiplying lymphocytes.

F. M. Burnet. Autoimmune Disease. *BMJ 1959;2:645-650 and 720-725.*

Confirmation of the Forbidden clone Theory in Graves' disease.

In thyrotoxic patients, affinity chromatography shows that the thyroid-stimulating autoantibodies (TSab) contain only one of two possible light chain types. Therefore, these autoantibodies **originate from single B lymphocytes**, in which a V gene has mutated.

Furthermore, the variation from patient to patient of reactivity with the slightly-differing TSH receptors of various non-human animals shows **the random element** in the somatic mutations forming the TSabs.

Adams, Knight, Knight, Laing *in* Pinchera et al eds *Thyroid Autoimmunity*, Plenum, New York, pp 1-10, 1987

Suppressor T cells (TS) and autoimmunity

1971 Allison et al postulated that lack of suppressor T cells causes the autoimmune diseases. (*Lancet; ii: 135*)
1975 Nachtigal et al proposed that on meeting antigen
mature T cells become Helpers.
immature T cells become Suppressors.
This limits specific immune responses, preventing both leukemia and autoimmunity. (*Transplant Rev*; *26: 87*)
1978 Knight & Adams disproved claims that lack of TS causes autoimmune disease in the New Zealand mice. (*JCLI; 1:151*)
1999 Shevack (in Paul's *Fundamental Immunology*) reported that
the autoimmune diseases are not caused by lack of TS.
2010 Adams, Knight, Ebringer recommend research switch to selective destruction of pathogenic forbidden clones, with methods available. (*Autoimmun Rev* 2010; 9: 525-530)

The H gene theory of inheritance of autoimmune disease.

(Adams & Knight *Lancet* 1982; i: 936-938.)

Histocompatibility antigens, major, minor and the male sex antigen, H-Y, protect from autoimmune disease, with imperfect success, by deleting nascent lymphocyte clones with complementary receptors for antigen.

Hence the effect of :

MHC (HLA) status,

minor histocompatibilty antigen status,

and sex,

on the risks of developing the various autoimmune diseases.

Methods for destruction of forbidden clones

1. Generalised Immune ablation (chemical or radiological) followed by reconstitution with autologous bone marrow cells taken previously from the patient's hip.
This procedure was used to save the lives of three patients with systemic scleroderma of lethal severity (Englert et al, *Int Med J 2005; 35: 436*). Because forbidden clones arise by unlucky somatic mutations in the V genes of multiplying lymphocytes, they are unlikely to recur in the reconstituted immune repertoire.

2. Manufacture and therapeutic use of cytotoxic autoantigen complexes. The autoantigen for Graves' disease was isolated by Vassart & Dumont (Costagliola et al *J Clin Endocrinol Metab 1999; 84: 90*). Similar isolation of the autoantigens of other autoimmune diseases would enable their attachment to a cytotoxic agent, such as bungarotoxin or ^{131}I, to make a "magic bullet" (therapeutic complex) for curing the disease by selectively destroying its pathogenic forbidden clone.

Immunotherapy of rheumatoid arthritis

Calpains are intra-articular proteolytic enzymes that disolve detritis in the joint space.
Calpastatin neutralises calpains, preventing them from damaging the synovium.
Menard & El-Amine have found autoantibodies to calpastatin in patients with rheumatoid arthritis and postulated that they cause the joint lesions by impairing calpastatin's protective function.
Immunology Today 1996; 17: 545.
In support of this postulate, depletion of B lymphocytes gives sustaimed improvement in rheumatoid arthritis.
Edwards & Cambridge 2001 Rheumatology; 40: 202.
Hence, with inadequate existing therapy and availabity of the probable autoantigen, calpastatin, rheumatoid arthritis seems suitable for trial of cure by manufacture and use of a **cytotoxic calpastatin molecular complex** for destroying the pathogenic forbidden clone of lymphocytes.

A 4th gene category for McKusick's catalogue of Mendelian Inheritance in Man

Johns Hopkins University Press, 1986.

1. Dominant: presence of the gene causes disease.
2. Recessive: absence of the gene causes disease.
3. X-linked: sex-linked, gene on X chromosome.
4. Autoimmune: Multiple codominant genes (V genes and H genes) with incomplete penetrance (due to need for antigenic triggers and somatic gene mutations in lymphocytes).

Adams, Knight, Ebringer. Autoimmunity Rev 2010; 9: 525-30.

Professor Alan Ebringer, BSc, MD, FRCP, FRACP

- Discovered that *Proteus mirabilis* in the upper urinary tract triggers rheumatoid arthritis.
- Discovered that *Klebsiella pneumoniae* in the gut triggers ankylosing spondylitis.
- Confirmed the H Gene Theory by finding two antigens on *Proteus*, one resembling HLA-DR1/4 the predisposing HLA antigen, one resembling the autoantigen attacked.

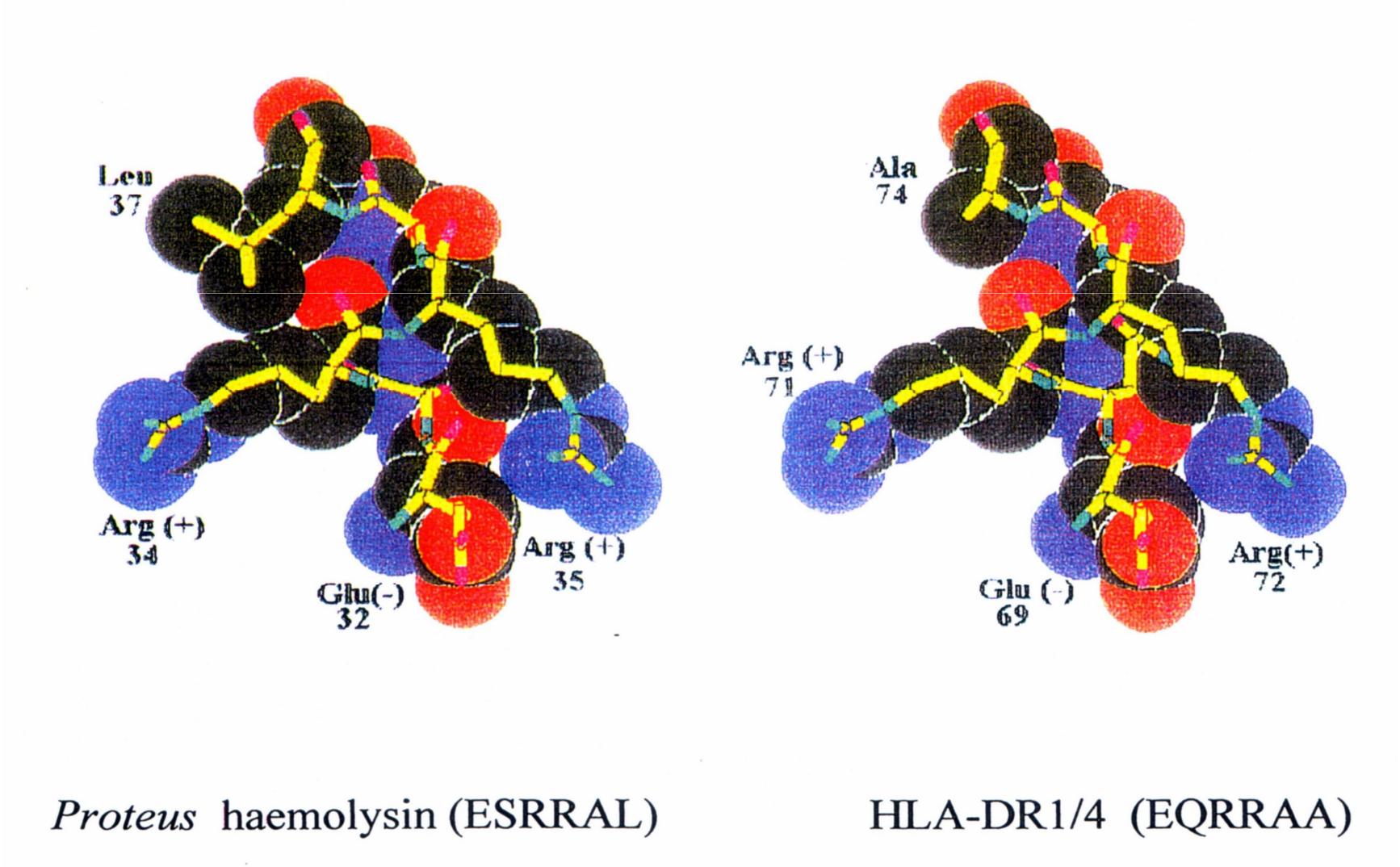


Molecular confirmation of the H Gene Theory

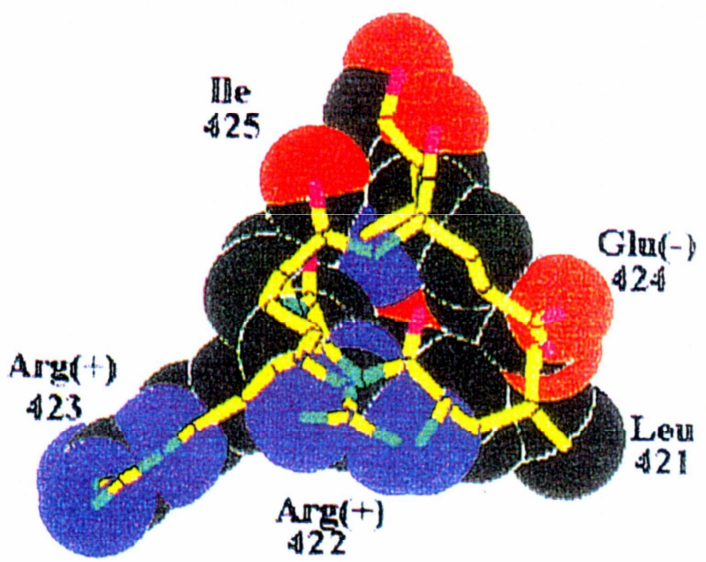
How can a Histocompatibity antigen, by deleting complementary clones, predispose to an autoimmune disease? The H gene Theory postulates **alternative clonal development**, and this has been demonstrated at the molecular level by Ebringer and colleagues for **Rheumatoid Arthritis**, where *Proteus mirabilis,* with a causative association, has two antigens, one similar to the predisposing HLA-DR1/4 antigen and another similar to the autoantigen attacked. (Wilson, Ebringer et al, *Ann. Rheumat. Diseases 1995; 54: 216-20.)*

Similarly, in **Ankylosing Spondylitis,** *Klebsiella pneumoniae* with a causative association, has two antigens, one similar to the predisposing HLA-B27 and one different and therefore able to stimulate a reactive clone towards mutation into the pathogenic forbidden clone. (Ebringer, Rashid et al, *Current Rheumatology Revs 2006; 2: 55-68.)*

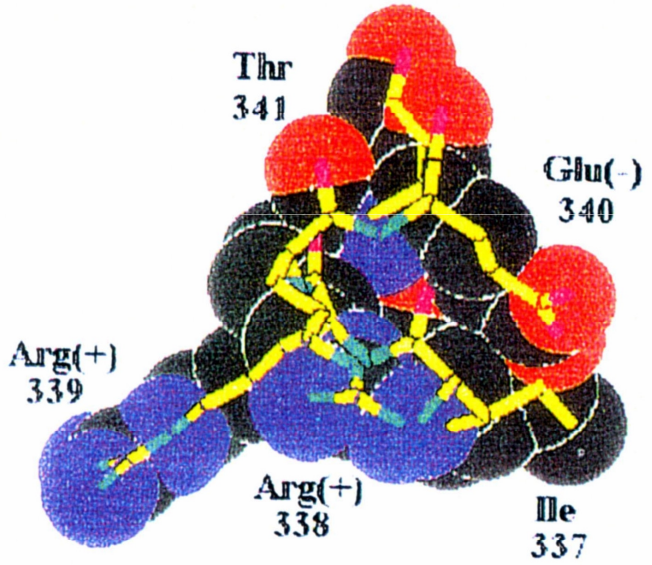
Proteus haemolysin resembles HLA-DR1/4, preventing immune reaction against this antigen.



Proteus urease resembles Type XI collagen, the autoantigen attacked

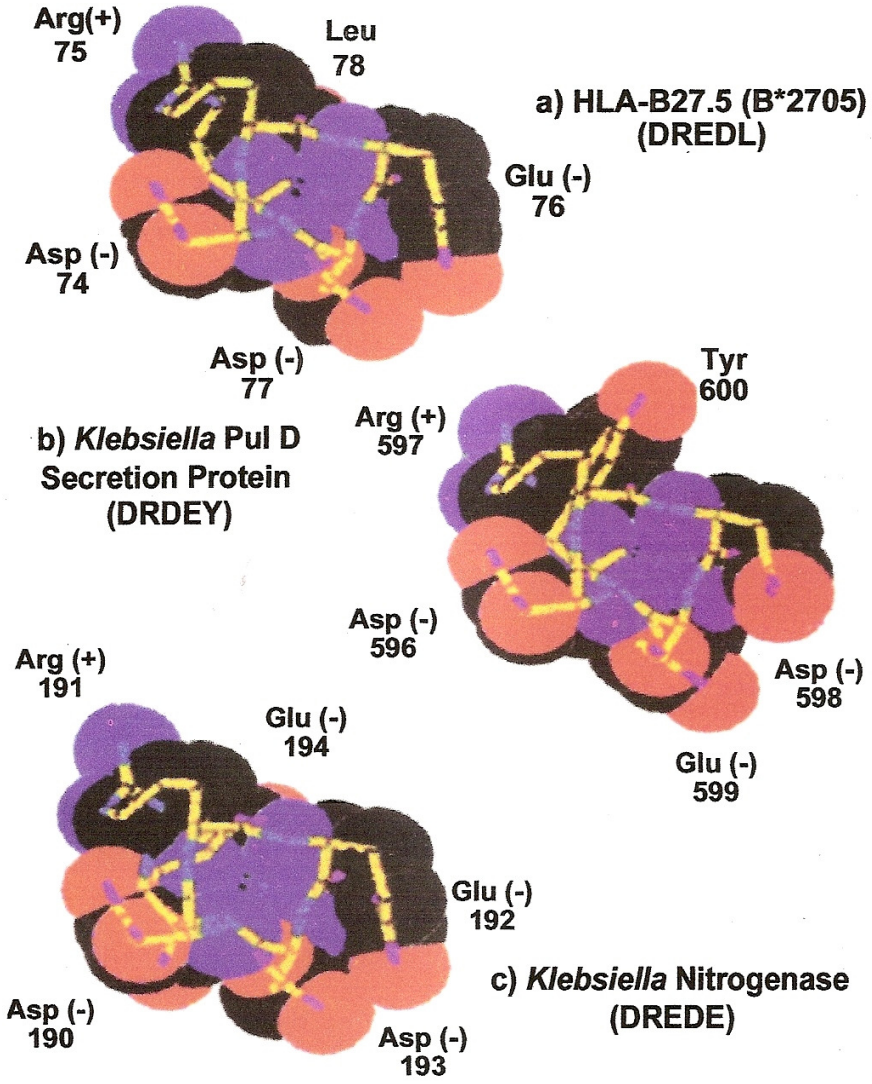


Type XI collagen (LRREI)



Proteus urease (IRRET)

Molecular similarity between HLA-B27 and the nitrogenase antigenic peptide of *Klebsiella pneumoniae,* preventing immune reaction, and dissimilarity with the pullanase antigenic peptide (Pul D), which is free to stimulate an immune reaction. These amino acid sequences, obtained by Ebringer's team, explain how HLA-B27 increases, 69-fold, the risk of ankylosing spondylitis, discovered by Schlosstein and Terasaki, N Engl J Med 1973; 288: 704-6.



Prophylaxis of Autoimmune Diseases.
1. Abortion of microbial trigger by penicillin.

The classic autoimmune disease triggered by microbial infection is **rheumatic carditis**. It is triggered by Type A beta-haemolytic streptococcal infections of the throat. Many people died and many had their lives ruined by this autoimmune complication of the streptococcal infection.. Kaplan and Meyeserian found a cross- reaction between these bacteria and heart tissue (Lancet 1962), explaining the aetiology. Fortunately, the use of penicillin, discovered by Flemming and made available by Florey, has aborted the streptococcal infections, making rheunatic carditis a rarity.

Prophylaxis of Autoimmune Diseases.
2. Vaccination against microbial triggers

Ebringer's discovery that *Proteus mirabilis* triggers rheumatoid arthritis and *Klebsiella pneumoniae* triggers ankylosing spondylitis, shows how microbial triggers can be found. Once this is achieved, prophylaxis by vaccination against the trigger will be easy. A particularly urgent clinical need is to prevent schizophrenia in this way.

The success of the Salk and Sabin anti-poliomyelitis vaccines in preventing the leg paralyses, which must have been rare autoimmune complications of vrtually universal polyomyelitis virus infection, sets the examlple.

B27 males and ankylosing spondylitis

There is 69-fold increased risk of ankylosing spondylitis in males with HLA-B27 (Schlosstein and Terasaki, N Engl J Med 1973;288: 704-6). Ebringer's discovery that the bacterium *Klebsiella pneumoniae* triggers ankylosing spondylitis offers prevention of this disease by vaccination of HLA-B27 positive boys with *Klebsiella pneumoniae*. This could initiate a new form of prophylaxis, which could culminate in prevention of schizophrenia, once its triggering microbe (probably viral) has been identified.