Autoimmune disease: Pathogenesis, Genetics, Immunotherapy, Microbial triggers, Prophylaxis

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Abstract

Pasteur’s correct Germ Theory of Disease led to discovery of the Immunity System that exists for defence against diseases caused by bacterial or viral infections. In order to cope with the huge diversity of microbial infections, the immune system needs a flexibility of immune response. This is provided by use of semi-random somatic gene mutations in lymphocytes, the cells that recognise invading bacteria and viruses.

Burnet realised that this flexibility leads to development of the Forbidden Clones of lymphocytes that cause the autoimmune diseases by accidentally being reactive with a host antigen instead of a microbial one. Ebringer has recently discovered the microbial triggers of rheumatoid arthritis and ankylosing spondylitis and has determined the amino acid sequences of the antigens on them that trigger the related autoimmune diseases. This has explained how histocompatibility antigens can predispose to autoimmune diseases. The Salk and Sabin anterior poliomyelitis vaccines have prevented the leg paralyses of the polio epidemics. We postulate that these paralyses were rare autoimmune complications of virtually universal poliovirus infection. As autoimmune diseases are triggered by microbial infections, we suggest that the triggering bacteria or viruses be sought, so that prophylaxis of the autoimmune diseases can be achieved by vaccination against their triggering microbes.

Keywords: Forbidden clone theory; H gene theory; Microbial triggers of autoimmune diseases; Poliomyelitis vaccines; Prophylaxis of poliomyelitis paralyses; Prophylaxis of autoimmune diseases

Introduction

Endemic goitre and its conquest

In mountainous parts of the world, such as Switzerland, the Himalayas, the Andes and New Zealand, Goiter [an enlargement of the thyroid gland] is common. This was discovered to be due to shortage in the soil of the trace element iodine [1]. Surprisingly, Kelly et al. [2], London scientists recruited by the Chilean government discovered that iodine in soil does not come from the weathering of rock. Iodine is dissolved in the sea from which it vaporizes by oxidation to be deposited in soils by rain. From the soil, iodine is taken up by plants from where it comes to man, directly in vegetables and fruit or indirectly through meat animals. Iodine takes a long time to build up in soils to the levels we need for manufacture of sufficient thyroid hormone.

Geologically old soils, like those of England and France and the Eastern United States, contain adequate iodine for human need but the new soils of Switzerland, the Himalayas, Chile and New Zealand, where there has been geologically recent up-thrust of mountains have not had time to accumulate adequate amounts of iodine to meet the needs for humans and other animals. This causes goitre, an enlargement of the thyroid gland, caused by increased secretion of thyroid stimulating hormone from the pituitary gland aimed at enabling an enlarged thyroid to obtain more iodine from the blood. In New Zealand, HD Purves [3], with brilliant studies, found that the amount of iodine needed to be added to the domestic salt was 1 part of potassium iodide per 20,000 parts of sodium chloride, 10 times more than the ineffectual level previously used. This abolished New Zealand’s endemic goitre [4].

As Professor of Public Health and Preventive Medicine at Otago Medical School, Charles Hercus followed classic academic practice in requiring his 5th year medical students to write a thesis, on any topic they chose, giving scope for expression of originality. Duncan Adams seized this opportunity to write a thesis on the aetiology of asthma, from which he himself had suffered severely, leading to an invitation by Sir Charles Hercus for him to join the research world via a Medical Research Council Fellowship. This led on to years of professional research and Directorship of the MRC Autoimmunity Research Unit (Figure 1).

Pathogenesis

The long mysterious pathogenesis and genetics of the autoimmune diseases is now solved [4,5]. The key to the pathogenesis is Jerne’s selection theory of antibody formation [6], which led to Burnet’s clonal selection theory of acquired immunity [7] and his forbidden clone theory of autoimmune disease [8]. This states that somatic gene mutations in multiplying lymphocytes produce the appropriately-named Forbidden Clones that cause the autoimmune diseases by reacting with a host antigen instead of a microbial one. Figure 2 illustrates clonal selection by antigenic stimulation and clonal diversification by somatic mutations in lymphocyte V genes. After discovery that the thyroid gland over-activity of Graves’ disease is caused by auto-antibodies that react with the thyroid’s receptor for thyroid-stimulating hormone from the pituitary gland (Figure
2) study of the properties of these auto-antibodies confirmed the **Forbidden Clone theory** by showing that they originate from single lymphocytes and show fine variation from patient to patient indicative of the random element in the mutations that produced them [9].

### Classification of immune reactions causing disease

This is shown in (Table 1), where allergy and anaphylaxis and serum sickness are distinguished from the two types of autoimmunity, that caused by B cell forbidden clones and that caused by T cell forbidden clones.

#### Graves' disease, a paradigm for autoimmune disease

In 1986, at the University of Pisa, Professor Aldo Pinchera et al. led an International Symposium on Thyroid Autoimmunity [23], at which **Graves' disease** was described as a paradigm of autoimmune disease [15] and Duncan Adams (Figure 4) was awarded a Medal for Fundamental Contributions to Biomedical Science, namely discovery of the thyroid-stimulating auto-antibodies, first called the long-acting thyroid stimulator (LATS).

<table>
<thead>
<tr>
<th>Table 1: Classification of immune reactions causing disease.</th>
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<tr>
<td><strong>Type I. Allergy and Anaphylaxis.</strong> Gut worm-defence mechanism reacting to non-worm antigens [10,11]. Fault: a B lymphocyte IgE clone reactive with an allergen. e.g. hay fever, anaphylaxis, gut allergy, skin allergy.</td>
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<tr>
<td><strong>Type II. Serum Sickness and Immune Complex Disease.</strong> Fault: excessive quantity of antigen. This swamps complement-neutralising mechanisms, leading to complement-mediated damage. Anti-microbial immune defense is designed to cope with picogram quantities of antigen, not milligrams of horse serum protein nor micrograms of released intra-cellular protein, such as nuclei [12,13]. e.g. serum sickness following passive immunization against diphtheria toxin with horse serum, systemic lupus erythematous, lupus nephritis.</td>
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<tr>
<td><strong>Type III. Autoimmunity.</strong> Fault: forbidden clones, which are anti-microbial lymphocyte clones with accidental host-antigen specificity, arising from unlucky somatic mutations in their lymphocyte V genes [9-14].</td>
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<tr>
<td><strong>Type III B. Diseases caused by B lymphocyte forbidden clones:</strong> e.g. Graves' disease[15], myasthenia gravis [16], rheumatoid arthritis [17].</td>
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<tr>
<td><strong>Type III T. Diseases caused by T lymphocyte forbidden clones:</strong> e.g. Diabetes Type 1 [18,19], diabetic retinopathy [20,21], experimental autoimmune encephalomyelitis[22] and presumptively Addison's disease, hypoparathyroidism, and other autoimmune diseases with specific parenchymal cell destruction[14].</td>
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This came from the experimental advantages provided by the presence of iodine in thyroid hormone, the hormone receptor nature of the auto-antigen and the control of thyroid activity by the pituitary gland.

#### Genetics

**The familial aggregation**

Studies of families, including twins, show that autoimmune diseases are weakly inherited, with disease specificity. The Mendelian pattern is that of multiple co-dominant genes with incomplete penetrance [14]. What genes are these? Before discussing this we need to describe the histocompatibility System.

#### The histocompatibility system

This system is essential for defense against virus infection, prevents allo-transplantation, and influences risk of autoimmune disease. Unlike the blood group antigens, A, B, O, on red blood cells, important for blood transfusions, the histocompatibility antigens are on the surface of all nucleated cells, including the white blood cells, the leucocytes, where they were discovered, and named by Dausset and Svejgard, the human leucocyte antigens (HLA) [24].
Involvement of the MHC in autoimmune disease

Vladutiu and Rose [25] discovered involvement in autoimmune disease of the major histocompatibility gene complex (MHC), which had been discovered in the field of surgical transplantation [26]. In man, a collection of genes on chromosome 6 code for peptides expressed on the surface of all nucleated cells. In the mouse a similar collection is on chromosome 17. Why does the MHC exist?

Functions of the MHC

First function: defense against virus disease: One of the classical experiments of recent times is that of Zinkernagel, et al. [27], who found that virus-infected cells extrude peptides from the virus into the Bjorkman Groove of their major histocompatibility antigens, this combined viral-histocompatibility antigen on the cell surface being the target for attack by the defensive cytotoxic T-cells. Adams [28] realized that the explosive speed of viral replication [29] necessitates this histocompatibility antigen involvement, which directs the cytotoxic T-cell attack on to the surface of the infected cell, destroying the virus factory, rather than ineffectively being muffled by the myriad numbers of free virions, as shown in (Table 2 [A,B,C]). This explains the Simonsen phenomenon [30], our having huge clones of cytotoxic T-cells reactive with allo-histocompatibility antigens, which our immune system mistakes for viral peptides on host histocompatibility antigens. The explosive speed of viral replication makes this mechanism necessary to prevent swift death of the virus-infected animal [28]. Polly Matzinger’s “Danger Theory” [31] is in full accord with Simonsen’s discovery that the immune system is far less concerned with things that are foreign than those that are dangerous.

Second function: Imposition of polymorphism on the immune repertoire. This diversity usually enables some members of a population to survive an epidemic infection, preventing whole populations from being wiped out.

Third function: Imperfect defense against autoimmune disease. [4].

Fourth function: Provision of a gene haven for MHC Class-III gene products [4].

The H gene Theory of Inheritance of Autoimmune disease.

Building on the Bielschowsky’s discovery [32] of autoimmune anemia in their NZB/BL strain of inbred mice, Howie and Helyer discovered that F1 hybrids of this strain with the healthy NZW strain unexpectedly develop the autoimmune kidney disease, lupus nephritis [33]. The occurrence of this disease in a hybrid, both of whose parents lack it, shows that at least one gene from each of the two parental strains causes the lupus nephritis. Back-cross and linkage studies showed that three genes contribute to the lupus nephritis [34], one from the NZB mice and two from the NZW mice. This has been confirmed, corrected and extended by Drake et al. [35] and Kono, et al. [36], using the wonderfully detailed microsatellite gene markers.

In all, Knight and Adams found four genes, with linkage information coding for autoimmune disease in mice [37]. None were the expected V genes, one was in the MHC and two appeared to be in the neighbourhood of the minor histocompatibility antigens, Hh and H-18 [37]. The linkage studies show that these are not the immunoglobulin V genes that were expected. Einstein [38] stated.

“The history of scientific and technical discovery teaches us that the human race is poor in independent thinking and creative imagination. Even when the external and scientific requirements for the birth of an idea have long been present, it generally needs an external stimulus to make it actually happen; man has, so to speak, to stumble right up against the thing before the idea comes.”

In an example of creative imagination, Adams and Knight put together the research fields of autoimmunity and transplantation, arriving at the H-Gene Theory of inheritance of autoimmune disease [39]. This states that histocompatibility antigen genes, major, minor and HY (the male sex antigen), together with the V (variable region) genes coding for antigen receptors on B and T-lymphocytes, are the germ-line immune response genes, the genes that influence the risk of autoimmune disease. The H-Gene Theory received general acceptance and wide admiration when delivered by John Knight to a distinguished audience at a Ciba Foundation Symposium in London in 1982 [40].

Immune Response Genes [14].

Table 3 lists the genes that govern the specificity of immune responses, indicating how they work. Section-A shows the germ-line variable region (V) genes, it provide the repertoire by coding for antigen receptors on B-cell and T-cell lymphocytes. For B-cell antigen receptors, the heavy chains are coded on chromosome1q, the kappa light chains on chromosome 2p and the lambda light chains on chromosome 22q. There are separate genes for the T-cell antigen receptors, which are of two types, αβ and γδ, coded for by genes on chromosomes 14q and 7q as shown in the Table 3. Section-B shows that new clones are added to the repertoire by somatic mutations in the V-genes of multiplying lymphocytes. Section-C shows that clones are subtracted from the repertoire by the H (histocompatibility antigen) genes which delete nascent complementary clones.
The Class-I major histocompatibility antigens are on all nucleated cells but the Class II are only on B-lymphocytes and professional antigen presenting cells such as macrophage and dendritic cells.

Females have two X-chromosomes; males have an X-chromosome and a Y-chromosome which confers masculinity.

**Aire (Autoimmune regulator) [41]**

Mutations in the transcriptional regulator, Aire, cause APECED, a poly-glandular autoimmune disease. Animal models of APECED have revealed that Aire plays an important role in T cell tolerance induction in the thymus, mainly by promoting ectopic expression of a large repertoire of transcripts encoding proteins normally restricted to differentiated organs residing in the periphery. The absence of Aire results in impaired clonal deletion of self-reactive thymocytes, which escape into the periphery and attack a variety of organs.

The significance of this phenomenon for human autoimmune disease is most intriguing.

**Microbial triggers**

Autoimmune diseases are caused by malfunction of the immunity system, being consequences of infection by bacteria or viruses [41,42].

A. **Rheumatic Carditis and Streptococci.**

Before the advent of penicillin, rheumatic fever, with crippling or fatal lesions of the heart, was a frequent consequence of infection by β−haemolytic streptococci of Lancefield Group A. This was because of an antigenic similarity between a component of these streptococci and heart tissue, discovered by Kaplan and Meyerserian [43]. Today, with such infection therapeutically aborted by penicillin, rheumatic heart disease, once common, has become rare.

B. **Glomerulonephritis and Streptococci.**

Post-infective glomerulonephritis follows infection by Group-A streptococci of multiple M types. This disease is also less frequent due to use of antibiotics.

C. **Reactive Arthritis.**

This has been observed after enteric infection with *Shigella, Salmonella, Versinia, Campylobacter* and genital infection with *Neisseria gonorrhea.*

D. **Rheumatoid Arthritis (RA) and Proteus mirabilis [44].**

Multiple studies over three decades have found high titres of antibodies against this bacterium in a total of 1375 RA patients, but not in other diseases or healthy controls in studies by independent groups in 15 different countries. There was no such elevation in antibodies against 27 other microbial agents. There is evidence that the upper urinary tract is the main site of *Proteus* infection in RA.

E. **Ankylosing Spondylitis (AS) and Klebsiella [45].**

In worldwide studies involving 1330 AS patients and 1191 healthy controls, the AS patients showed significantly increased antibody titres to *Klebsiella.* There is evidence that the gut is the...
Figure 4: Clonal selection by antigenic stimulation and clonal diversification by somatic mutation. Concept of Jerne [6] and Burnet [7].

Figure 5: Professor Alan Ebringer, BSc, MD, FRCP, FRACP.
1. Discovered that Proteus mirabilis in the upper urinary tract triggers rheumatoid arthritis
2. Discovered that Klebsiella pneumoniae in the gut triggers ankylosing spondylitis
3. 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.
4. Showed how HLA-B27 predisposes, 69-fold, to ankylosing spondylitis.

Figure 6: Molecular similarity between histocompatibility antigen HLA-DR1/4 and Proteus haemolysin, preventing immune reaction against this bacterial antigen.

main site of Klebsiella infection in AS.

F. Type 1 Diabetes and Coxsackie virus.

Richter and Horowitz [46] present the considerable evidence that Coxsackie viruses, especially B4, may trigger type 1 diabetes, including the discovery that there are shared regions of homology between the Coxsackie virus protein PC-2 and the islet antigen GAD65.

G. Systemic Sclerosis and Infections.
Randone, et al. [47] describe the evidence suggesting that Parvovirus B19, Cytomegalovirus, Eblstein-Bar virus, Endogenous retrovirus, or Helicobacter pylori infection might trigger systemic sclerosis, where it has been postulated that fibroblast-stimulating forbidden clones, probably of B cell origin, are the immunological agent [8].

H. Schizophrenia and virus infection.

Acute schizophrenia has been observed to follow upper respiratory tract virus infection. Knight et al have assembled much evidence indicating that schizophrenia is an autoimmune disease caused by auto-antibodies that react with neuronal receptors influencing the limbic system [48-50]. Recently, Fabienne Brilot [51], a Paediatrician, has discovered dopamine-2 receptor antibodies in children with encephalitis. If these auto-antibodies are found in cases of acute schizophrenia, it will confirm the autoimmune basis of this disease.

Information from Sequencing Antigens on Triggering Bacteria

a. Basic books

Details of the development of the methods used for successful determination of the amino acid sequences of antigens on the rheumatoid arthritis-triggering bacteria, Proteus mirabilis are described in the book, "Rheumatoid arthritis and Proteus" by Ebringer [52] (Figure 5).

Similarly, the book "Ankylosing spondylitis and Klebsiella", also by Ebringer [53], describes how the amino acid sequences of antigens on Klebsiella pneumoniae, that enormously increase the risk of ankylosing spondylitis, were determined, and how they exert their effect.

b. Confirmation of the H Gene theory

This research provides experimental confirmation, at the molecular level, of the H-Gene Theory of the inheritance of the autoimmune diseases, described above, in confirming the speculated presence of multiple antigens on triggering bacteria and alternative clonal development causing development of the forbidden clones that cause the associated autoimmune diseases.

c. How HLA-DR1/4 predisposes to Rheumatoid Arthritis [54]

Figure 6 shows space-filling models of the amino acid sequences of the histocompatibility antigen HLA-DR1/4 and the Proteus mirabilis haemolysin antigen. Close structural similarity is apparent. This means the immune tolerance imposed by the histocompatibility antigen will extend to this Proteus antigen, preventing immune reaction with it.

Figure 7 shows space-filling models of the amino acid sequences of the Proteus mirabilis urease antigen and Type-11 collagen, an auto-antigen attacked in rheumatoid arthritis. The urease antigen is completely different from HLA-DR1-4, so will not be protected from immune reaction, being free to stimulate development of a forbidden clone reacting with the closely similar Type-11 collagen molecule, an auto-antigen attacked in rheumatoid arthritis.

d. How HLA-B27 predisposes to Ankylosing Spondylitis [55]

Figure 8 shows space-filling models of the amino acid sequences of the histocompatibility antigen HLA-B27 and two antigenic peptides on the bacterium Klebsiella pneumoniae. The Klebsiella nitrogenase antigen closely resembles HLA-B27, so will be covered by the tolerance induced by HLA-B27, but the bacterium’s pullulanase peptide is different and able to stimulate development of a forbidden clone attacking the spine to cause ankylosing spondylitis.

Prophylaxis of autoimmune diseases

a. The Poliomyelitis virus epidemics

A New Zealand example occurred in 1938, reported in the press and observed by Adams trapped in a boarding school in Masterton. An epidemic of leg paralyses occurred in Christchurch and spread progressively north, from town to town, to Picton, Wellington, Featherston, then Carterton, the town next to Masterton, engendering great fear. Then the boy in the bed next to Adams complained of a stiff neck, was taken away and reported to have polio. Six months later he returned with a paralyzed leg. Adams and his schoolmates were all unaffected.

Figure 7: Molecular similarity of Proteus urease with Type X1 collagen, an autoantigen attacked in rheumatoid arthritis.
b. Paralysis a rare autoimmune complication of universal virus infection?

I postulate that the leg paralyses of poliomyelitis were a rare autoimmune complication of virtually universal virus infection, the paralyses probably caused by forbidden clones of cytotoxic T-cells which attacked anterior horn neurons in the spine, hence the Pathologists’ appropriate name, “acute anterior poliomyelitis.”

c. The lead in prophylaxis given by the polio vaccines

The Salk (killed) and Sabin (attenuated) polio vaccines have been brilliantly successful in preventing the polio leg paralyses. This exemplifies how autoimmune diseases in general, can be prevented by finding and vaccinating against their microbial triggers.

d. Finding microbial triggers

Ebringer has succeeded in this with rheumatoid arthritis and ankylosing spondylitis. He has pioneered this new field of medical research, developing a whole new technology that needs to be copied in other diseases, especially schizophrenia. Systematic studies of autoimmune diseases, with collaboration between clinicians and microbiologists are needed. The American Academy of Microbiology would be an ideal organization for doing the research needed to provide this urgently-needed knowledge.

Discussion

Some disease associations are cross-tissue autoimmunity, for example the eye proptosis of Graves’ disease, caused by variants of the thyroid-stimulating auto-antibodies that react with receptors on orbital fat cells [56], and diabetic retinopathy [57], probably caused by destruction of retinal pericyte cells by antigenic variants of the T-cell forbidden clones that destroy the pancreatic islet β cells to cause Type-1 diabetes. Many autoimmune diseases, such as Graves’ disease, already have satisfactory therapy. Immunotherapy, by radiological or chemical immune ablation, with immune reconstitution by autologous bone marrow cells, pioneered by Tyndall [58], can be used to save the lives of patients with dangerous autoimmune diseases, such as systemic scleroderma [59].

Selective destruction of forbidden clones could be achieved by isolating their auto-antigen (such as the TSH receptor of Graves’ disease, cloned by Vassart and Dumont) [60] and attaching it to a cytotoxic moiety, such as bungarotoxin or 131I iodine (emitting short-range β particles), then administering the molecular complex intravenously to destroy the pathogenic clones of plasma cells.

When monoclonal antibody technology was discovered, it was mistakenly assumed that this would provide cures for the autoimmune diseases, a notion greatly encouraged by the drug companies. Struggling to help his patients get benefit from use of Rituximab, Dreyfus [61] envisages major progress from anti-viral therapies and ultimately virus vaccines. Prevention is better than cure, so finding and countering antigenic triggers of autoimmune diseases is the ideal. Recognition of the universality of microbial triggers of autoimmune diseases is a major advance, in showing how the diseases can be prevented by finding and vaccinating against their triggers. Ebringer has led the way by discovering two triggers and developing the technology for finding others.

References

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