Autoimmune haematological disorders

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The nature of autoimmune disease

Autoimmune diseases affect around 5% of the population but until recently, although the pathological features of these disorders could be described, there was little idea about the aetiology. Through the development of animal models and the identification of target genes some insight is being gained into the pathogenetic basis of these complex diseases. Blood disorders in which autoantibodies are found include cytopenias (autoimmune haemolytic anaemia (AIHA), idiopathic thrombocytopenia purpura (ITP) and autoimmune neutropenia), in addition to coagulation disorders (eg acquired haemophilia).

This article will discuss the autoimmune process in general and concentrate on cytopenias.

The immune system

The two principal components of the immune system are:

1 The innate immune system comprising skin, mucous membranes, neutrophils, macrophages and other scavenging cells, in addition to the complement system.

2 The adaptive immune system which includes antibody and T cell receptors (TCR).

Generation of diversity in antigen receptor genes

There are two requirements for an effective immune system:

1 The ability to recognise millions of potential antigens.

2 Prevention of self-reacting lymphocytes from causing tissue damage.

The former is achieved through irreversible somatic recombination of immunoglobulin (Ig) and TCR genes generating many millions of different antibody and TCR molecules.

Generation of autoantibodies

Having such diversity ensures that there is an antibody for every potential antigen. The downside of this extreme diversity is that antibodies are generated that recognise self-antigens (autoantibodies). It is likely that in normal healthy subjects autoantibodies are generated against a wide variety of antigenic targets. Autoimmune disease is not common, so there must exist a mechanism for removing self-reacting antibodies. In effect, an immunological lack of responsiveness or tolerance must exist, whereby self-reactive cells are prevented from causing damage. Recent research has shown this to be the case[1].

The spectrum of autoimmune diseases

The spectrum of autoimmune diseases ranges from organ-specific autoimmune diseases (eg Hashimoto's thyroiditis) at one end to non-organ-specific autoimmune disease at the other (eg systemic lupus erythematosus (SLE)). Many diseases lie between these two extremes. Conditions in which there are circulating immune complexes tend to be systemic, whilst those associated with autoantibodies or autoreactive T cell responses are organ-specific[2].

Table 1. Factors that may play a role in autoimmune disease[2].

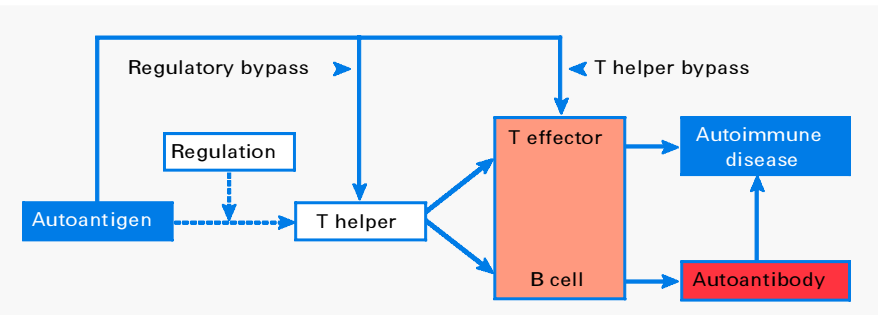
- Failure of tolerance to self-antigens
- Infection
- Tissue injury
- Abnormalities of antigen-presenting cells
- Imbalance between pro- and anti-inflammatory cytokines
- Genetic factors
- Variable effector mechanisms (eg production of immune complexes, autoantibodies or autoreactive T cells)

Factors that may play a role in autoimmune disease[2]

Table 1 lists the factors that may play a role in autoimmune disease. The way in which the normal mechanisms controlling autoreactivity are bypassed leading to T or B cell mediated autoimmune disease are shown in Fig 1[3].

Genetic factors. It has long been recognised that genetic factors are involved in autoimmune disease, with the strongest correlation with major histocompatibility complex (MHC) genes, especially MHC

Fig 1. Loss of regulation of autoreactivity. The normal mechanisms controlling autoreactivity are bypassed leading to T or B cell mediated autoimmune disease (reproduced, with permission, from Roitt 1997[3], *Essential immunology*, Blackwell Science Ltd).



class II. Insulin-dependent diabetes mellitus (IDDM) is a prime example where multiple genes are implicated. For example, 95% of Caucasians with IDDM possess HLA-DR3, HLA-DR4 or both (vs 40% in normal subjects), and 40–50% of patients are heterozygous for HLA-DR3/-DR4 (vs 5% of normal subjects). Other non-HLA genes are also implicated in IDDM, including interleukin-2 polymorphism and another region that maps close to CTLA-4[4].

Infection. Pathogens may be able to induce autoimmunity through several mechanisms:

1 *Production of local inflammation:* local inflammation can expose co-stimulatory molecules on antigen-presenting cells and lead to breakdown of T cell anergy and the development of autoimmune disease.
2 *Production of neoantigens:* tissue injury may result in the production of neoantigens to which an autoantibody may have specificity.
3 *Molecular mimicry* in which antigens on microorganisms may resemble those on the host tissues such that antibodies produced against the pathogen will cross-react with the host tissue[5]. Examples may include multiple sclerosis, IDDM and childhood acute ITP, if the antibody produced in a childhood viral infection by chance cross-reacts with antigen(s) on the platelet surface. Although elegant, there is little apart from circumstantial evidence at present to support the existence of molecular mimicry in man[5].

The multifactorial nature of autoimmune disease

Autoimmune disease is therefore highly complex, involving genetic and environmental factors. Possibly a combination of the 'wrong genes' and the 'wrong environment' leads to the development of autoimmune disease in those unfortunate enough to suffer with these conditions (Fig 2)[6].

Idiopathic thrombocytopenic purpura

In ITP, platelets are coated (opsonised) with anti-platelet autoantibodies and removed prematurely by the reticuloendothelial system (RES), leading to a reduced peripheral blood platelet count. The aetiology of ITP is unknown and the clinical course is variable and unpredictable. ITP has an incidence of 5.8–6.6 new cases per million population per year in the US[7].

Childhood idiopathic thrombocytopenic purpura

ITP in childhood is generally termed 'acute' since the illness is seasonal, typically follows a trivial viral infection or vaccination, and in most cases is transient requiring no treatment. There is spontaneous recovery in 80% of cases.

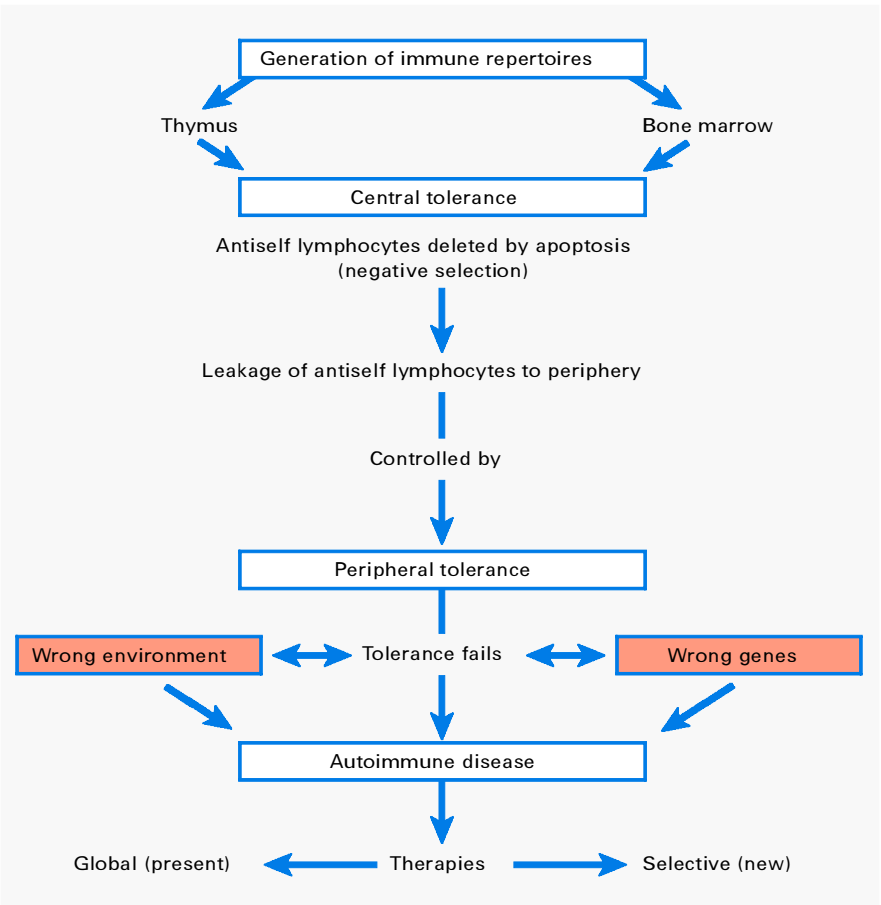
Adult idiopathic thrombocytopenic purpura

In the adult (chronic) form there is usually no obvious antecedent illness, most patients have chronic thrombocytopenia and spontaneous recovery is uncommon[8]. In most cases of adult ITP the antigenic target is a platelet glycoprotein (GP), GP IIb/IIIa or GP Ib/IX[9].

Clinical features

Patients may be asymptomatic or have purpura, bruising or mucosal bleeding (including gum bleeding), retinal haemorrhage, epistaxis, melaena or menorrhagia. The degree of bleeding largely

Fig 2. The multifactorial nature of autoimmune disease. How tolerance is established and may fail. A combination of the wrong genes and the wrong environment may lie behind the development of autoimmunity in some cases (reproduced, with permission, from Mackay 2000[6], *British Medical Journal*; **321**:93–6, BMJ Publishing Group).



depends on the platelet count, and patients with platelet counts below 10×10^9/l are at greatest risk of bleeding. Splenomegaly is not a feature of ITP; if present, it tends to suggest an alternative diagnosis.

Diagnosis

The diagnosis of ITP remains clinical and one of exclusion. Secondary causes include SLE, lymphoproliferative disease and HIV infection. Standard investigations include:

- full blood count (isolated thrombocytopenia)
- blood film (to ensure no red cell fragments, leukaemia, parasitic infections)
- autoimmune profile (to exclude lupus).

A bone marrow biopsy is often performed in adults, but not usually in children. It typically shows normal or increased megakaryocytes in an otherwise normal marrow. Immunological assays have been devised, including platelet-associated IgG or IgM and monoclonal antibody immobilisation of platelet antigens, but these do not alter management and are of debatable value.

Standard first-line therapy

There is a lack of clinical trial data to help guide treatment. Efforts should now be focused on constructing high quality randomised trials to determine the most effective therapy in this disorder.

Table 2. Investigations for autoimmune haemolytic anaemia.

Investigation	Result
Full blood count	Reduced haemoglobin ± – MCV (reticulocytosis)
Reticulocytes	Increased
Blood film	Spherocytes, polychromasia
Serology	Red cells are coated with IgG or complement or both (detected by direct antiglobulin test)
Biochemistry	– Lactate dehydrogenase
Serum haptoglobins	↓ or absent
Bone marrow	Increase in erythropoiesis but should be no evidence of lymphoma or other bone marrow pathology
Autoimmune screen	Negative (to exclude secondary autoimmune or connective tissue disorders)

Ig = immunoglobulin; MCV = mean corpuscular volume.

Therapy is seldom necessary for patients whose platelet counts exceed $20–30 \times 10^9$/l and in whom there are few spontaneous bleeding episodes[10], unless they are undergoing a procedure likely to induce blood loss[11]. Standard treatments, including oral prednisolone[8], intravenous (iv) Ig and splenectomy, will elevate the platelet count sufficiently in most adults. However, some 20–25% of adults with ITP are refractory to first-line therapy.

Chronic refractory idiopathic thrombocytopenic purpura

Those patients who fail to respond to first-line treatment or require unacceptably high doses of corticosteroids to maintain a safe platelet count are termed 'chronic refractory'. A number of agents have been used as second-line therapy for ITP, including high-dose steroids, high-dose iv Ig, iv anti-D, vinca alkaloids, danazol, azathioprine, combination chemotherapy and dapsone. (An excellent summary is provided in Ref 7.)

Experimental therapies

For those who fail to respond to standard first- and second-line therapy and who require treatment the options are limited and include:

- interferon-α[8]
- cyclosporin A
- CAMPATH 1H
- protein A columns[12].

Key Points

The immune system is able to generate billions of different antibodies, and by chance, some of these will recognise host antigens

Autoimmune disease is multifactorial involving genetic and environmental factors

Current treatment for autoimmune disease is largely non-specific but novel targeted therapies are currently being developed

Despite advances in molecular immunological techniques the diagnosis of autoimmune cytopenias remains one of exclusion

Treatment of autoimmune cytopenias is not evidence based and quality randomised trials are needed in order to determine the optimal therapy for these disorders

Autoimmune red cell disorders

In AIHA, red cell autoantibodies react with the patient's red cells, causing their premature destruction by the RES. If the red cell destruction is incomplete, spherical red cells are formed (spherocytes). Warm (IgG mainly) AIHAs may be secondary to drugs or lymphoproliferative disorders but most are idiopathic[13].

The clinical features are variable and many patients have few symptoms. Mild jaundice and splenomegaly are common. The investigations for AIHA are listed in Table 2. A typical AIHA blood film is shown in Fig 3[14].

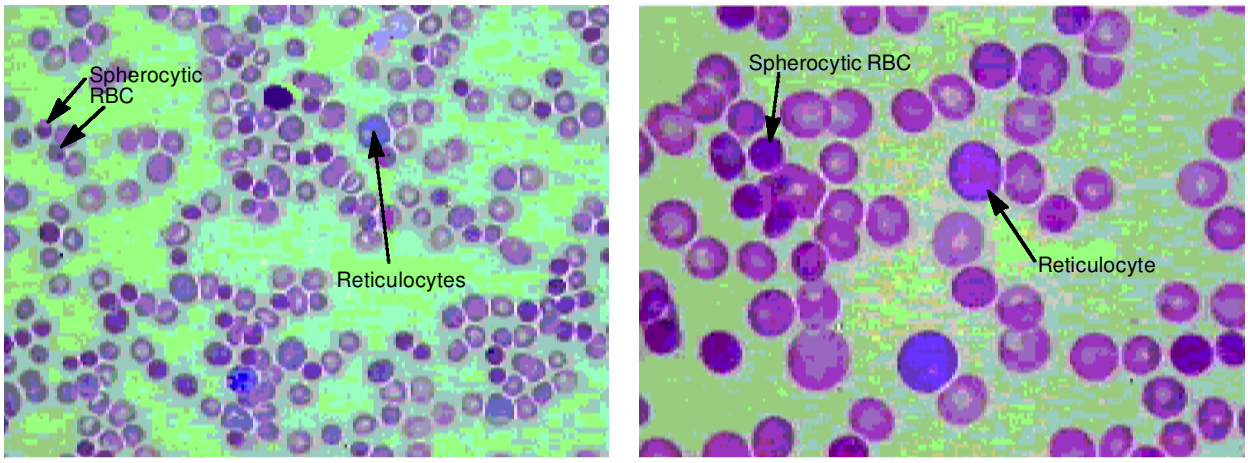


Fig 3. Peripheral blood film features in autoimmune haemolytic anaemia (AIHA). Low and high power views of peripheral blood in warm AIHA. Note the numerous spherocytes and reticulocytes (reproduced, with permission, from Provan and Weatherall 2000[14], Red cells II: acquired anaemias and polycythaemia. Review, *Lancet*; **355**: 1260–8. © by *The Lancet* Ltd).

Treatment

Treatment may not be required if the patient has well-compensated haemolysis. However, many patients require some form of therapy, most commonly oral prednisolone. For patients who relapse or fail to respond, other treatments include iv Ig and azathioprine. Splenectomy may be considered for patients who do not respond to standard treatment.

Cold antibody syndromes

Cold AIHA may occur after infections (eg *Mycoplasma pneumoniae* or Epstein-Barr virus). Haemolysis is usually mild, but may occasionally be severe. Idiopathic cold haemagglutinin disease affects elderly patients who develop acrocyanosis in cold weather. Occasionally, this picture may be seen in elderly patients with underlying B cell malignancies such as non-Hodgkin's lymphoma and chronic lymphoid leukaemia.

Drug-induced autoimmune haemolytic anaemia

About 12–18% of AIHA cases are drug-induced. Most drugs are too small to induce an immune response alone but may become immunogenic when bound to a larger protein (eg red cell membrane component):

- *Hapten mechanism* (drug absorption), where the drug interacts with a red cell membrane component, generating antigens that stimulate antibody production. Examples include penicillins.
- *Autoantibody-mediated haemolysis*, where a warm autoantibody is generated, for example, α-methyldopa – but note that, although direct antiglobulin test positivity is common in patients receiving α-methyldopa, frank haemolysis is not.
- *Innocent-bystander* (immune complex) mechanism where the drug forms an immune complex with antibody. This then attaches to the red cells, leading to complement fixation and red cell destruction. Examples include quinine and quinidine.
- *Miscellaneous*, for example reduction of red cell lifespan through interference with membrane lipids or oxidation of haemoglobin.

Table 3. Causes of neutropenia.

- Infections, especially viral
- Connective tissue disorders
- Haematinic deficiencies
- Marrow infiltrations
- Autoimmune
- Alloimmune (eg fetomaternal)
- Congenital
- Normal racial variants[15]

Autoimmune neutropenia

Neutropenia defines an absolute neutrophil count more than two standard deviations below the normal mean. In practical terms, patients with neutrophils below 2.0×10^9/l are neutropenic and are often referred for investigation. The laboratory assessment of neutropenia is unsatisfactory and, apart from cases in which a drug is clearly implicated or there is marrow infiltration by haematological or other malignancy, often no firm diagnosis is reached. The causes of neutropenia are listed in Table 3.

Autoimmune neutropenia may be idiopathic or secondary to other autoimmune diseases. The autoantibody may be directed against neutrophil precursors or mature neutrophils[16]. A bone marrow biopsy is often carried out, especially if the neutrophil count is below 1.0×10^9/l, to exclude serious underlying pathologies such as leukaemias, lymphomas or other bone marrow infiltrations. Most patients require simple expectant treatment only, and have a benign course[17]. Corticosteroids will elevate the neutrophil count in 50% of those with recurrent infections.

Specialised investigations

Immunological assays, similar to those used for the diagnosis of ITP, are available (for review, see Mollison *et al.*[18]).

Future prospects

With greater understanding of the mechanisms of the immune response and availability of sequence data for cytokine and other immune system genes, we are beginning to understand the mechanisms involved in autoimmune disorders. This may enable patients to be stratified, aid in the choice of treatments, and predict which patients are likely to have mild, and which severe, forms of autoimmune disease. Promising experimental targeted immunotherapies, for example CTLA-4-Ig (which down-regulates activated T cells)[19], anti-CD20, monoclonal antibodies against tumour necrosis factor (for rheumatoid arthritis) and others should mean that patients are no longer subjected to the global immunosuppression that has been used for decades.

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