Immune haemolytic anaemia and thrombocytopenia: drugs and autoantibodies

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The study of the mechanisms involved in drug-induced autoimmune cytopenias may provide some information about the events involved in the generation of autoimmune disorders. There is still no definitive answer as to the initial events that trigger drug-induced autoimmune blood cell destruction but the spectrum of clinical conditions undoubtedly has a basis in the molecular interactions of drugs (or their metabolites) with blood cells. These may lead to production of antibodies that in turn may result in the destruction of a particular cellular population. What is clear is that in common with other autoimmune disorders, differences among individuals are important in determining susceptibility to drug-induced autoimmune cytopenia.

Drug-induced autoimmune thrombocytopenia has been reported in association with a few drugs: gold, methyldopa and levodopa [1]. These reports have been limited to case studies. Methyldopa and levodopa-induced thrombocytopenia can persist beyond elimination of the drug, and may be due to mechanisms similar to those involved in methyldopa-induced haemolysis. It is more difficult to use a parallel argument to support gold-induced autoantibodies as causing persistent thrombocytopenia. The problem is that gold remains in the body for many years following its discontinuation. Quinidine-induced thrombocytopenia is also well recognized (see [2] for review).

Drug-induced autoimmune haemolysis has been more extensively investigated. This disorder has been described in association with a number of drugs; methyldopa, levodopa, mefenamic acid, ibuprofen, phenacetin, chlorpromazine and procainamide. Of these methyldopa is by far the commonest cause, both in terms of total number of

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Abbreviations used: RBC, red blood cell; DAT, direct antiglobulin test.

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cases, and in terms of incidence in patients receiving a drug. Therefore, the rest of the review will concentrate on methyldopa-induced autoimmune disorders.

**Methyldopa-induced immune red cell disorders**

Methyldopa was introduced as an anti-hypertensive agent in 1960; its use has declined with the introduction of newer agents, though it is still widely used. Apart from autoimmune haemolysis, its use has been associated with several other unwanted immune effects including a lupus-like syndrome, autoantibodies against factor VIII:C, hepatitis, myocarditis and thrombocytopenia [3].

The drug causes two different, but very similar, clinical abnormalities of red blood cells (RBCs): autoimmune haemolytic anaemia and, more commonly, a positive direct antiglobulin test (DAT) without haemolysis. The clinical pictures caused by these two disorders are quite distinct: patients who present with positive DAT (indicating a high concentration of anti-RBC antibodies on the red cell surface) very rarely go on to develop haemolysis even if the drug is continued. This difference between haemolysis and anti-RBC antibodies without haemolysis also exists in non-drug-induced autoimmune red cell disorders, although in different proportions. That is, some patients have detectable amounts of spontaneous anti-RBC antibodies on the red cell surface, not associated with accelerated cell destruction; other patients with similar amounts of autoimmune anti-RBC antibody on their cells have haemolytic anaemia.

A recent study raises the interesting possibility that, for non-drug-induced autoimmune RBC disorders, the discriminating factors between haemolysis and no haemolysis seem to be the characteristics of the autoantibody and the absence of anti-idiotype antibodies [4] (Table 1); for drug-induced autoimmune RBC disorders the difference seems to lie in the effector cells of the reticuloendothelial system [5]. It must be stressed however, that the drug-induced and non-drug-induced anti-RBC autoimmune disorders have not been studied in a comparable manner.

**Methyldopa-induced anti-RBC autoantibody production**

Detectable anti-RBC antibodies arise in 10–20% of individuals who take the drug for longer than 3–6 months; less than 1% of those patients taking the drug develop haemolysis. The antibodies can persist for several months after the drug is stopped, but conversely they occasionally disappear if the drug is continued. The characteristics of the antibodies: class, subclass, complement-binding ability, antigen specificity and amount of antibody on the cell, show some differences between the two groups of antibody-developing patients (haemolysing and non-haemolysing) but not such as to explain the different clinical phenomena.

Antibody class is usually IgG, although the presence of IgM autoantibodies in association with

<table>
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<tr>
<th>Autoantibody</th>
<th>Non-drug induced</th>
<th>Drug-induced</th>
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<tr>
<td></td>
<td>Haemolysis</td>
<td>Positive DAT without haemolysis</td>
</tr>
<tr>
<td>Class</td>
<td>Usually IgG</td>
<td>Usually IgG</td>
</tr>
<tr>
<td>IgG subclass</td>
<td>1 + 3 [4]</td>
<td>1 (occasionally 4) [6]</td>
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<tr>
<td>Reticuloendothelial function</td>
<td>NR</td>
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Table 1

Laboratory findings in non-drug-induced and drug-induced anti-RBC autoimmune disorders

NR, not reported.
IgG may explain in part why some patients haemolyse, though not why most patients do not. In a study of 11 patients [8] with methyl dopa-induced anti-RBC autoantibodies, eight patients who had detectable IgM on the RBC surface had accelerated cell destruction; three patients with IgG only had no haemolysis. All four IgG subclasses are present on the RBCs of patients with methyl dopa-induced anti-RBC autoantibodies whether they haemolyse or not [8]. The anti-RBC autoantibodies do not induce the deposition of high concentrations of complement on the RBC surface. This is thought to be related to the frequent Rhesus specificity of these antibodies, but the exact reasons are unclear.

Where it has been characterized, the most common specificity of the antibody is against antigens in the Rhesus system. This finding does not vary with the presence or absence of haemolysis. Anti-Rhesus system specificity is also common in healthy blood donors in whom anti-RBC autoantibodies without haemolysis are found. There is not a major quantitative difference in the amount of antibody on the RBCs of those who haemolysed compared with those who do not [3].

The aetiology of methyl dopa-induced anti-RBC autoantibody production

Three possibilities exist as to why anti-RBD autoantibodies seem to develop in a large proportion of patients taking methyl dopa: (1) the autoantibodies are not true autoantibodies, but are directed to a drug-induced neo-antigen on the RBC membrane; (2) the autoantibodies arise because of a perturbation of B cell function, allowing the proliferation of autoantibody producing clones; and (3) the drug impairs the immune system function in a more central fashion, resulting in failure of immune self-surveillance and an associated impairment of effector cell function.

(1) Methyl dopa-induced anti-red cell antibodies are not true autoantibodies

It has been proposed that methyl dopa and other drugs giving rise to apparently autoimmune cytopenias exert their effect by the formation of a neo-antigen on the cell surface. In this model the drug initially binds to and subsequently alters the red cell membrane producing changes in the antigenic nature of the membrane structure. These new antigens are then the target of the autoantibodies. Although one group of investigators has reported binding of methyl dopa to red cells [9], others were unable to do so [10]. It has also been demonstrated that the drugs glafenine and latamoxef induce autoantibody formation in association with drug-dependent antibody formation; the autoantibodies were directed against the drug-binding sites on the red cell membrane [11]. It has been argued [12] that these observations provide evidence that the antibodies involved are not true autoantibodies, and that perturbation of the immune system does not need to be invoked to explain drug-induced autoantibody production. Although these observations could explain some of the phenomena associated with methyl dopa-induced red cell immune diseases, they fail to explain the commonest phenomenon, namely antibody-coated RBCs that are not cleared by the reticuloendothelial system.

(2) The autoantibodies arise because the perturbation of B cell function allowing the proliferation of autoantibody producing clones

In studies in vitro, T cell proliferation was inhibited by methyl dopa. The effect was caused by a sustained elevation in cyclic AMP concentration in lymphocytes. This resulted in diminished suppressor cell maturation and activation. It was proposed that inhibition of suppressor T cell function resulted in unregulated autoantibody production by B cells [13]. While this observation could explain the appearance of the autoantibody, it too fails to explain why the great majority of patients taking methyl dopa who develop anti-RBC autoantibodies do not have haemolysis. In addition, other groups of investigators were unable to repeat these observations [14, 15].

(3) The drug impairs the immune system in a central fashion, resulting in failure of immune self-tolerance and in associated impairment of effector cell function

Several observations by independent groups have demonstrated that there is impaired reticuloendothelial cell function in patients taking methyl dopa who develop a positive DAT without haemolysis. In studies in vitro, monocytes from patients with haemolysis associated with methyl dopa-induced RBC autoantibodies phagocytosed antibody-coated RBCs; monocytes from non-haemolysing DAT-positive patients did not phagocytose the RBCs [16]. Extensive studies in vivo have confirmed and extended these observations [5]. Fc-dependent reticuloendothelial cell function in vivo was assessed by measuring the clearance of autologous ⁵¹Cr-labelled red cells sensitized with anti-D (antibodies against the Rh D antigenic determinant). Only Rh D-positive subjects were studied. Normal subjects cleared more than 40% of the labelled,
antibody-sensitized cells from the circulation within an hour of injection. Four patients with positive DAT without haemolysis in association with methyldopa therapy failed to clear the injected autologous cells normally. In addition, three of four other patients who were taking methyldopa but had no serological abnormalities also failed to clear the injected red cells. In contrast, a single patient with autoimmune haemolysis in association with methyldopa therapy cleared over 90% of the injected autologous red cells within an hour of injection.

These studies indicate that patients taking methyldopa therapy who develop a positive DAT without haemolysis have impaired reticuloendothelial cell function. In addition, it indicates that a considerable proportion of patients taking methyldopa therapy without any serological abnormality also have impairment of reticuloendothelial cell function. In contrast, when autoimmune haemolytic anaemia develops in association with methyldopa, reticuloendothelial cell clearance of antibody-labelled cells may be normal or increased.

It is interesting to speculate that the impairment of reticuloendothelial cell function and the emergence of anti-RBC autoantibodies are related phenomena in these patients. For example, study of the molecular mechanisms involved in impaired reticuloendothelial cell function might reveal the early effects induced by methyldopa on the immune system. It is not difficult to conceive that such information in turn could be exploited to explore the basis of concurrent or subsequent development of anti-RBC autoantibodies. In addition, it is possible that autoantibodies against other cells develop in these patients; in the absence of clinical disease their presence could remain unsuspected and unnoticed.

In summary, most patients on long-term methyldopa therapy develop impairment of reticuloendothelial cell function. Of these a sizeable proportion also develop detectable anti-RBC auto-antibodies. A small minority of patients taking the drug develop autoimmune haemolytic anaemia. The causes of these phenomena have not been clarified. This model of human autoimmune disease remains to be fully exploited.


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