Historical Review

THE IMMUNE HAEMOLYTIC ANAEMIAS: A CENTURY OF EXCITING PROGRESS IN UNDERSTANDING

The growth in knowledge of the scientific basis of disease and consequent advances in the practice of medicine that have taken place in the past century have been truly remarkable. This is certainly true of the haemolytic anaemias, which have been a main interest of the author since the mid-1930s. At that time, the cause and mechanism of important disorders such as the acquired antibody-determined (immune) haemolytic anaemias, haemolytic disease of the newborn, hereditary spherocytosis and paroxysmal nocturnal haemoglobinuria were unknown or but partially understood. The purpose of this short review is to record and highlight some of the most significant advances in knowledge of the acquired immune haemolytic anaemias that have been made between the first decade and the end of the twentieth century.

First, however, a diversion. According to Crosby (1952), William Hunter of London, in an article on pernicious anaemia published in 1888, was the first to use the term ‘haemolytic’ to denote an anaemia caused by excessive blood destruction. By the turn of the century, the term was being widely used in clinical literature, e.g. by Widal et al (1908a,b) and Micheli (1911), and in experimental studies, e.g. by Cantacuzene (1900). At that time, however, and for a decade or so later, no reliable data were available as to the life-span of the erythrocyte in health. Peyton Rous, in his comprehensive review ‘Destruction of the red blood corpuscles in health and disease’ (Rous, 1923), concluded that the generally held view in the early 1930s was that about one-fifteenth of the erythrocyte mass was destroyed daily. Rous was aware of the pioneer work of Winifred Ashby (1919a,b), who, by following the survival of serologically distinct but compatible transfused erythrocytes, had found that normal erythrocytes might live for up to 100 d in the recipients’ circulation. The difficulty in accepting Ashby’s conclusions without question was that she was unable, using her technique, to measure the life-span of the experimental subjects’ cells in their own environment. Subsequent work using radioactive chromium (51Cr) as an erythrocyte label, showed that Ashby’s data and conclusions were in fact correct, i.e. that normal erythrocytes in health circulate in the peripheral blood for approximately 110 d.

Both Ashby’s differential agglutination method and 51Cr labelling have been used with brilliant effect in the investigation of patients suffering from many types of increased haemolysis. Thus, in contrast to the finding in hereditary haemolytic anaemias, e.g. hereditary spherocytosis (HS), that the life-span of transfused normal erythrocytes is normal, that of normal erythrocytes transfused to patients with an acquired immune haemolytic anaemia is characteristically shortened (Brown et al, 1944; Mollison, 1947). Erythrocyte labelling with 51Cr also had a further advantage over the Ashby method in addition to enabling the life-span of the patients’ erythrocytes to be assessed in his or her own circulation, namely, that it was possible, by surface counting, to detect and measure the accumulation of radioactivity in the spleen and liver, i.e. the presence in the organ of the labelled erythrocytes, and thereby assess the organs’ role in haemolysis (Szur, 1970).

THE IMMUNE AUTO-ANTIBODY HAEMOLYTIC ANAEMIAS: EARLY DESCRIPTIONS

It was in France in the first decade of the twentieth century that Widal et al (1908a) and Le Gendre & Brulé (1909) reported that autohaemagglutination was a striking finding in some cases of icteré hémolytique acquis. At about the same time, Chauffard & Trosier (1908) and Chauffard & Vincent (1909) had described the presence of haemolysins in the serum of patients suffering from intense haemolysis. These observations suggested that abnormal immune processes, i.e. the development of auto-antibodies damaging the patients’ own erythrocytes, might play a part in the genesis of some cases of acquired haemolytic anaemia. That this might be so had in fact been antedated by the classic observations of Donath & Landsteiner (1904) and Eason (1906) on the mechanism of haemolysis in paroxysmal cold haemoglobinuria, the clinical aspects of which had been well described in the 19th century.

COLD AUTOHAEMAGGLUTINATION

That blood might auto-agglutinate when chilled had been described by Landsteiner (1903) and that an unusual degree of the phenomenon might complicate some types of respiratory disease was reported by Clough & Richter (1918) and later by Wheeler et al (1939). It was not until a few years later, however, that it was reported by Peterson et al (1943) and Horstmann & Tatlock (1943) that cold auto-agglutinins at high titres were frequently to be found in the serum of patients who had suffered from the then so-called primary atypical pneumonia. Significantly, two of their patients had developed acute haemolytic anaemia.
Earlier, in the 1930s, a chronic syndrome of unknown origin, not associated with respiratory disease, had been recognized in which a markedly raised titre of cold agglutinins associated with intravascular haemolysis and haemoglobinuria were prominent features (Ernstene & Gardner, 1935; Roth, 1935; Salén, 1935).

Stats & Wasserman’s (1943) review on cold haemagglutination was a valuable contribution to contemporary knowledge. They listed in a table as many as 94 references to papers published between 1890 and 1943 in which cold haemagglutination had been described. In 32 of the papers the patients referred to had suffered from increased haemolysis.

Recognition that cold auto-antibodies played an important role in the pathogenesis of some cases of haemolytic anaemia led to the concept that auto-immune haemolytic anaemia (AIMA) might usefully be classified into warm-antibody or cold-antibody types, according to whether the patient is forming (warm) antibodies which react (perhaps optimally) at body temperature or (cold) antibodies which react strongly at low temperatures (e.g. 4°C) but progressively less well as the temperature is raised and are perhaps inactive at 37°C. The clinical syndrome suffered by the patient would depend not only on the amount of antibody produced but also on its temperature requirement.

Another important advance in understanding has been the realization that both types of AIHA could develop in association with a wide range of underlying disorders (secondary AIHA) as well as ‘idiopathically’, i.e. for no obvious cause (primary AIHA). Published data on the relative proportion of primary to secondary cases, based on large numbers of patients, date from the 1950s. For instance, Lal & Speiser (1957) reported that 48 out of 97 cases of warm-antibody AIHA had been secondary cases and van Loghem et al (1958) reported 60 out of 122 cases. Subsequently, somewhat higher proportions of secondary cases were reported, e.g. by Videbaek (1962a), 36 out of 41 cases, Homberg et al (1967), 60 out of 95 cases, and Pirofsky (1969), 190 out of 234 cases. The author’s own experience was summarized in a review (Dacie & Worlledge, 1969): 99 out of 210 cases of warm AIHA were judged to be secondary as were 39 out of 85 cases of cold AIHA. Petz & Garratty (1980), summarized the data from six centres: 55% out of a total of 656 cases had been reported as secondary. They listed the disorders with which warm-antibody AIHA had been associated as chronic lymphocytic leukaemia, Hodgkin’s disease, non-Hodgkin’s lymphomas, thymomas, multiple myeloma, Waldenström’s macroglobulinaemia, systemic lupus erythematosus, scleroderma, rheumatoid arthritis, infectious disease/childhood viral disorders, hypogammaglobulinaemia, dysglobulinaemias, other immune deficiency syndromes, ulcerative colitis and ovarian dermoid cysts.

Conley (1981), in an interesting review of warm-antibody AIHA patients seen at the Johns Hopkins Hospital, emphasized how important it was to carry out a careful enquiry into the patient’s past history and also to undertake a prolonged follow-up. He stated that a retrospective review of 33 patients whose illnesses ‘in the past have been designated ‘idiopathic’ had revealed an associated immunologically related disorder in 19 of them. An additional three patients had developed a lymphoma 2–10 years after they had developed AIHA.

SECONDARY WARM-ANTIBODY AIHA

As already referred to, warm-antibody AIHA is now known to complicate a wide range of underlying diseases, particularly malignant lymphoproliferative disorders, other auto-immune disorders and immune deficiency syndromes.

AIHA IN MALIGNANT LYMPHOPROLIFERATIVE DISORDERS

There is an extensive literature. Reports of the occurrence of haemolytic anaemia in chronic lymphocytic leukaemia (CLL) and Hodgkin’s disease date from the 1920s and 1930s (Netousek, 1922–23; Holler & Paschkis, 1927; Paschkis, 1927), but whether auto-antibodies played a part in the haemolysis in their patients is unknown. Reports of patients in whom the direct antiglobulin test (DAT) had been carried out and found to be positive date from the early 1950s (Hutt, 1950; Dameshek et al, 1951). It was not long before it was reported that in some patients the haemolytic anaemia had appeared to antedate the development of the lymphoma; in other patients, however, the reverse appeared to be true (Rosenthal et al, 1955). Most of the patients who had developed AIHA appear to have suffered from CLL. Pirofsky (1968a, 1969) reported that, of 113 personally observed patients, 48 had had CLL, 10 acute lymphocytic leukaemia (ALL), 13 Hodgkin’s disease, seven lymphosarcoma, one giant-follicle lymphoma and four reticulum-cell sarcoma. What proportion of patients suffering from a lymphoproliferative disorder develop AIHA is an interesting question. Dührsen et al (1987) stated that this had occurred in 12 out of 637 patients. It has also been reported (D. Catovsky, personal communication) that the DAT had been positive in 57 out of 788 CLL patients at the time their leukaemia was diagnosed.

AIHA IN OTHER AUTO-IMMUNE DISORDERS

Systemic lupus erythematosus (SLE)

AIHA is well known to develop in a minority of patients suffering from SLE. The first reports date from the late 1940s and early 1950s (Aegerter & Long, 1949; Zoutendyk & Gear, 1950). Occasionally, haemolytic anaemia dominates the clinical picture: it may even antedate the development of overt SLE by many months or even years (Michael et al, 1951; Wasserman et al, 1955; Videbaek, 1962b). Early data on the incidence of a positive DAT in SLE were provided by Harvey et al (1954) – in six out of 34 patients tested the DAT had been positive. Later, Mongan et al (1967), who had studied a large number of patients suffering from a variety of connective tissue disorders, reported that the DAT had been positive in 15 out of 23 patients with SLE, none of whom, however, had suffered from overt haemolytic anaemia.
Rheumatoid arthritis
AIHA has been recorded; the incidence, however, is low. de Gruchy (1954) and Evans & Weiser (1957) recorded single cases of the association. The author (Dacie, 1967) reported that two out of 250 patients with AIHA had given a history of rheumatoid arthritis and Pirofsky (1969) reported five out of 234 patients.

Ulcerative colitis
Rather rarely, AIHA complicates ulcerative colitis. Lorber et al (1955) referred to four such patients. The author (Dacie, 1967) reported that two out of about 250 patients with AIHA he had seen between 1947 and 1965 had ulcerative colitis and that the DAT had been positive in the absence of clear evidence of haemolysis in a third patient.

AIHA IN IMMUNE DEFICIENCY SYNDROMES
It has been realized since the 1960s that warm-antibody AIHA may develop in patients suffering from a variety of immune deficiency syndromes, both congenital and acquired. The author (Dacie, 1992) listed 25 papers published between 1961 and 1988 in which 33 such patients were described. Earlier reports of splenomegaly and hypersplenism in association with agammaglobulinaemia had been reviewed by Citron (1957) and Prasad et al (1957).

AIHA IN ASSOCIATION WITH OVARIAN DERMOID CYSTS OR TERATOMATA
The rare occurrence of generally severe haemolytic anaemia in patients with ovarian dermoid cysts or teratomata has been recognized since the late 1930s (West-Watson & Young, 1938; Singer & Dameshek, 1941). A remarkable feature of these early reports was that haemolysis had subsided following removal of the tumour. This has been true in similar patients described in later reports. The DAT has been found to be positive in almost all the patients in which the test has been carried out; typically, the test has become negative 3–6 months after surgery. The haematological and serological findings have been indistinguishable from those of ‘idiopathic’ AIHA.

DRUG-INDUCED AIHA: α-METHYLDOPA
It was in the mid-1960s that it was realized that, in a significant proportion of patients thought to have ‘idiopathic’ warm-antibody AIHA, the development of the causal auto-antibodies had been triggered in some way by a drug the patient was taking. The first drug implicated was the antihypertensive drug α-methyldopa (Aldomet) (Carstairs et al, 1966a,b). According to Worlledge et al (1966), who had investigated 40 patients, the clinical, haematological and serological findings were indistinguishable from those of ‘idiopathic’ AIHA. An interesting question was the proportion of hypertensive patients being treated with α-methyldopa (who were not suffering from overt haemolytic anaemia) in whom the DAT was positive. The author (Dacie, 1999) listed 22 reports published between 1966 and 1972. Remarkably, the incidence of positive DATs varied widely – from 0% to 27%. There were hints of a possible genetic influence, for the lowest percentages (0–4%) were found in African, coloured, Indian and Chinese patients (it is possible, however, that these patients had been treated with lower doses of the drug or had failed to take their medicine regularly).

DRUG-DEPENDENT IMMUNE HAEMOLYTIC ANAEMIAS
An interesting development in the history of the immune haemolytic anaemias was the realization in the mid-1950s that, rather rarely, haemolysis was brought about by the patient developing antibodies that were directed against a drug the patient had been taking and that the erythrocytes were in some way secondarily involved. The first drug to be implicated was Fuadin (stibophen), which had been used to treat a patient with schistosomiasis (Harris, 1954, 1956). The patient’s serum contained an antibody that agglutinated his own or normal erythrocytes and/or sensitized them to agglutination by antiglobulin sera; however, this occurred only in the presence of the drug. Other drugs, e.g.
quindine, phenacetin and penicillin, were later reported to be a cause of haemolytic anaemia by a similar mechanism. The author (Dacie, 1999) listed 12 drugs that had been implicated between 1954 and 1961. It is now realized in fact that a wide range of drugs are capable of causing the DAT to be positive in susceptible recipients and, occasionally, causing clinically significant haemolysis. Habibi (1987) listed as many as 78 drugs and Garranty (1994) lists 71 drugs. Remarkably, some patients have been reported to have formed both drug-induced auto-antibodies and drug-dependant antibodies (Habibi, 1985).

AIHA: THE ROLE OF GENETIC FACTORS

The importance of patient individuality, i.e. genetic factors, has been repeatedly referred to in the foregoing brief descriptions of the clinical syndromes associated with AIHA. The occurrence of more than one case of AIHA in the same family is, however, most unusual, but it has happened. The author (Dacie, 1969) reported that he had encountered only one example – a sister and brother – out of more than 100 cases of AIHA seen up to that time. Writing in 1992, he was, however, able to list 20 published descriptions of the occurrence of AIHA in more than one family member, included in the relationships were four pairs of twins. The reported occurrence of an immune-mediated disorder, other than AIHA, in the family of a patient with AIHA is, however, not rare. Dreyfus (1964), in an important review, mentioned the occurrence of thrombocytopenia, polyarteritis nodosa, rheumatoid arthritis, pernicious anaemia and hypogammaglobulinaemia. Pirofsky (1968b, 1969) stated that one or more relatives of eight out of 43 patients had suffered from a wide range of possible or probable auto-immune disorders. Conley (1981) reported an even higher incidence – 14 out of 33 patients.

ASSOCIATION OF THROMBOCYTOPENIA WITH WARM-ANTIBODY AIHA (THE EVANS SYNDROME)

In the late 1940s, several accounts of patients with AIHA who had persistently low platelet counts were published, e.g. Fisher (1947) and Evans & Duane (1949); and it was suggested that the patients might have been forming auto-antibodies directed against platelets. This concept was further developed by Evans et al (1951). Eight out of their 18 patients with AIHA were thrombocytopenic; four had clinically obvious purpura. Evans et al (1951) suggested that there exists ‘a spectrum-like relationship between acquired haemolytic anaemia and thrombocytopenic purpura’: also that ‘on the one hand, acquired haemolytic anaemia with sensitization of the red cells is often accompanied with thrombocytopenia, while, on the other hand, primary thrombocytopenic purpura is frequently accompanied with red cell sensitization with or without haemolytic anaemia’. Many further case reports of AIHA accompanied by severe thrombocytopenia have since been published, e.g. in the later 1950s by Crosby & Rappaport (1957) and Dausset & Colombani (1959). AIHA was even reported to have developed in patients who had previously undergone splenectomy for thrombocytopenic purpura. Earlier, Waugh (1932), had described a possible example. The author (Dacie, 1954, 1962) described two similar occurrences. Some patients, too, have suffered from granulocytopenia as well as thrombocytopenia and haemolytic anaemia, e.g. as reported by Fisher (1947) and Evans et al (1951). Later descriptions include reports of the presence of antibodies against platelets and leucocytes in such cases, e.g. in patients described by Baumgartner (1956) and Müller & Weinreich (1956) (who coined the descriptive term ‘Immunopancytopenien’).

WARM-ANTIBODY AUTO-IMMUNE HAEMOLYTIC ANAEMIA (AIHA): THE BLOOD PICTURE

There are two features in the blood film of a patient with an acquired haemolytic anaemia which indicate that he or she is suffering from AIHA: one is auto-agglutination, the other is erythrophagocytosis. Spherocytosis, although often present to a marked degree, is of course found in other types of haemolytic anaemia.

Auto-agglutination

The early descriptions by French authors published in the first decade of the century have already been referred to. Noteworthy descriptions published in the 1940s of patients suffering from acute haemolysis in which auto-agglutination was conspicuous include those of Reisner & Kalkstein (1942), Evans (1943), Renner & McShane (1947) and Hahn & Lüttgens (1949).

Erythrophagocytosis

Phagocytosis by monocytes circulating in the peripheral blood has been frequently reported. It is most easily seen in buffy-coat preparations (Zinkham & Diamond, 1952; de Gruchy, 1954). Hargraves et al (1941) had earlier described a patient suffering from acute haemolysis in whom both monocytes and neutrophils were acting as erythrophages.

Spherocytosis

It was not until the late 1930s that spherocytosis was recognized to be a common phenomenon in acquired haemolytic anaemias, in addition to being a characteristic finding in congenital acholuric jaundice (hereditary spherocytosis). Small darkly staining round microcytes had, however, been noticed much earlier than this as a feature of the blood picture in experimental antibody-produced haemolytic anaemia in animals, e.g. by Christophers & Bentley (1908, 1909) who described the cells as spherocytes, and Muir & McNee (1912). The ‘rediscovery’ of spherocytes by Dameshek & Schwartz (1938a, 1940) in the blood of animals (guinea-pigs) to which immune sera had been administered and their report of similar cells in the blood of human patients suffering from acute haemolytic anaemia proved to be a most important contribution to knowledge (Dameshek & Schwartz, 1938a,b; 1940). Up to that time, the current teaching, in the United Kingdom at least, was that many, perhaps most, of the cases of acquired haemolytic anaemia with spherocytes in the peripheral
blood were in reality cases of previously symptomless congenital acholuric jaundice (Dawson, 1931; Vaughan, 1936; Israëls & Wilkinson, 1938).

WARM-ANTIBODY AIHA: SEROLOGICAL FINDINGS

The pioneer French observations on auto-agglutination already referred to were generally overlooked until the late 1930s, and serological studies seem seldom to have been undertaken until the publication of Dameshek & Schwartz’s (1938b) report in which they described the presence of ‘haemolysins’ in cases of acute apparently acquired haemolytic anaemia. Dameshek & Schwartz (1940) summarized contemporary knowledge in an extensive review. They concluded that it was not improbable that haemolysins of various types and ‘dosages’ were in fact responsible for many cases of human haemolytic anaemias, including congenital haemolytic anaemia, which they suggested might be caused by the ‘more or less continued action of an haemolysin’.

Dameshek and Schwartz’s concept of the important role of haemolysins in haemolytic anaemia was viewed with some scepticism by their contemporaries, a major difficulty in its acceptance being that, in the great majority of cases, the presence of a haemolysin could not be demonstrated by techniques that were then available. Six years were to pass before the concept that an abnormal immune mechanism played a decisive role in some cases of acquired haemolytic anaemia was clearly demonstrated by Boorman et al (1946), who reported that the erythrocytes of five patients with acquired acholuric jaundice had been agglutinated by an antiglobulin serum, i.e. that the newly described antiglobulin reaction or Coombs test (Coombs et al, 1945) was positive, while the test had been negative in 28 patients suffering from congenital acholuric jaundice. This work aroused great interest and was soon confirmed and extended by other workers throughout the world. Early reports included those of Denys & van den Broucke (1947), who demonstrated the presence of free antibody globulin in their patients’ sera by means of an indirect Coombs test and also that normal cells varied in their sensitivity to the antibody. Sturgeon (1947) reported that direct and indirect tests were positive in three patients and that antibody could be eluted from washed patients’ erythrocytes.

Evans et al (1947) also showed that globulin could be eluted from washed suspensions of patients’ cells by heating at 56°C and that the eluted material could be transferred to normal cells. They showed, too, that their patients’ cells auto-agglutinated when suspended in 30% bovine albumin, and they drew attention to the similarity in behaviour between the presumed antibody of haemolytic anaemia and the antibodies of various types and ‘dosages’ were in fact responsible for many cases of human haemolytic anaemias, including congenital haemolytic anaemia, which they suggested might be caused by the ‘more or less continued action of an haemolysin’.

In 1951, the author had reported that the effect on the agglutination of erythrocytes from patients with AIHA of adding human γ-globulin to antiglobulin sera was inconsistent (Dacie, 1951). When the test was carried out with the erythrocytes of two patients with warm-antibody AIHA, the reaction was inhibited by very small amounts of γ-globulin; in contrast, much more γ-globulin was required to inhibit the reaction in two patients whose serum contained high-titre cold agglutinins.

These findings aroused considerable interest. In particular, interest was focused on the nature of the material coating erythrocytes that were agglutinated by antiglobulin sera to which large amounts of γ-globulin had been added – a so-called non-γ reaction. Were the cells coated by antibody globulins other than γ-globulin, or was the reaction between absorbed complement components and anticomplement antibodies in the antiglobulin serum, or did proteins being adsorbed non-specifically to antibody-damaged cells? Subsequent work established that the non-γ reaction depended upon the adsorption of sublytic amounts of complement components (Dacie et al, 1957; Rosenfield et al, 1960).

Much subsequent work has been devoted to identifying the complement components involved. According to Lachmann et al (1983), using monoclonal antibodies, the erythrocytes of patients with the cold-haemagglutinin disease (CHAD), which give a strong non-γ-antiglobulin reaction, are coated with C3d, g (an α-2D globulin). Voak et al (1983) also demonstrated that CHAD erythrocytes were agglutinated by both anti-C3g and anti-C3d sera.

SPECIFICITY OF THE AUTO-ANTIBODIES

Until the 1950s, the auto-antibodies responsible for AIHA were generally concluded to be ‘non-specific’. According to Wiener et al (1953), ‘Red cell auto-antibodies react not only with the individual’s own red cells but also with the erythrocytes of all other human beings. The substances on the red blood cell envelope with which the auto-antibodies combine are agglutinogens like the A–B–O, M–N and Rh–Hr systems, except that, in the former case, the blood factors with which the auto-antibodies react are not type specific but are shared by all human beings.’ They suggested that the auto-antibodies might be directed to the ‘nucleus of the Rh–Hr substance’. Earlier work had, however, indicated that the specificity of normal group-compatible erythrocytes to a patient’s auto-antibody might vary considerably (Denys & van den Broucke, 1947; Kuhns & Wagley, 1949).

That auto-antibodies might have a clearly defined Rh specificity, e.g. anti-e, was described by Race & Sanger (1954) in the second edition of their book. Referring to Wiener et al (1953), they wrote: ‘This beautifully clear investigation made the present authors realize that a curious result obtained by one of them (Ruth Sanger) in 1953 in Australia had after all been true; the serum of a
man who had died of a haemolytic anaemia 3000 miles away contained anti-e: his cells were clearly CDe-cde. A similar finding, i.e. an auto-anti-e, was described by Weiner et al (1953). Holländer (1953), too, reported that he had identified a specific auto-antibody in a case of AIHA: the patient’s probable genotype was CDe/cde; anti-e was identified in serum and anti-c and a ‘non-specific’ component in erythrocyte eluates.

Dacie (1953) and Dacie & Cutbush (1954) were able to report on the specificity of the auto-antibodies formed by 10 patients with warm-antibody AIHA. Nine of the patients had developed antibodies that appeared to be ‘non-specific’. However, three of them were also forming specific Rh antibodies, namely anti-e, and one further patient had formed anti-e and anti-D at different times. As the probable genotype of these patients was CDe/cde, it was clear that the specific antibodies were reacting with auto-antibodies. The ‘non-specific’ antibodies were interesting too: in three patients the antibodies appeared to have two components – a component reacting with all the cells tested and a component reacting with all the cells tested except those of D–/D– genotype. The results of studies carried out by van Loghem & van der Hart (1954a, b) were equally interesting. Specific auto-antibodies were identified in five out of six patients: namely, anti-D, anti-c, anti-C + e (two patients) and anti-Jkβ.

The reports summarized above established without doubt that many of the auto-antibodies demonstrable in warm-antibody AIHA were reacting with specific antigens, in nearly all cases within the Rh system. The relative proportions of specific to apparently ‘non-specific’ antibodies was, however, uncertain, as cases in which a definite specificity had been demonstrated were more likely to be recorded than those in which no specificity had been established. The data available up to 1960 suggested, in fact, that auto-antibodies of demonstrable specificity were being formed by almost one-third of patients.

MORE RECENT STUDIES ON SPECIFICITY

The studies referred to above were greatly expanded and elaborated. A complicated picture emerged. It has been established, for instance, in relation to Rh antigens that several antibodies of different specificities may be present at the same time and that some antibodies, while reacting with normal erythrocytes, fail to react with cells in which certain Rh antigens have been partially or completely deleted. In relation to apparently ‘non-specific’ antibodies, it has been shown in some cases that the antibodies have in fact been reacting with certain common antigens outside the Rh system, e.g. that the antibodies had the specificity anti-Weβ, anti-Enα, anti-LW or anti-U. Some important studies are referred to below.

Weiner & Vos (1963) reported that they had investigated the specificity of the antibodies they had eluted from 56 patients with AIHA and from 10 clinically normal blood donors in whom the direct antiglobulin test (DAT) had been positive. They had used cells of common Rh phenotypes as test erythrocytes as well as cells of the rare deleted genotypes Dc–/Dc–, D–/D– and −−/−−. Six eluates contained specific Rh antibodies; the remaining 60 eluates contained panagglutinating antibodies which reacted with cells of all the common Rh phenotypes. Nine of the above eluates, however, failed to react with D–/D– cells and −−/−− cells; these antibodies were designated anti-nl (normal). Other eluates reacted with deleted cells, but less strongly than with cells of common Rh phenotypes- anti-pdl (partly deleted) antibodies. Those antibodies that reacted with cells of normal phenotypes and also with the deleted cells were designated anti-dl (deleted) antibodies.

Issitt et al (1976) reported on the specificity of the auto-antibodies formed by 87 patients with warm-antibody AIHA. Sixty-four had formed anti-dl antibodies: two of these were identified as anti-Wrβ, and a further 32 patients had formed anti-Wrβ as well as antibodies of other specificities. In all, antibodies of more than one specificity were present in 63 of the 87 patients. Only four patients had formed specific anti-Rh antibodies of their own. Sokol et al (1981) summarized the results of studies that had been carried out on 573 patients with warm-antibody AIHA between 1961 and 1980: 71 had formed anti-e, seven anti-c and other anti-Rh antibodies, and 29 anti-Rh antibodies in the absence of anti-e.

A further development in the unravelling of a complicated story was the realization that some of the antibodies which appeared to be specific were reacting with more basic antigens, although showing a preference for specific antigens, i.e. some specific auto-antibodies appeared to be less specific than their allo-antibody counterparts. Moreover, some antibodies, reacting with specific antigens, have been shown to be partially or completely absorbable by antigen-negative cells. Issitt (1986), in his Emily Cooley Lecture, reported that his data indicated that about 70% of warm-antibody AIHA patients had been forming what he termed ‘mimicking antibodies’. He suggested in the case of anti-e auto-antibodies that there was about an 80% chance that the antibodies were directed against basic Rh antigens such as Hr or Hrα, but with a preference for e-positive cells.

‘NON-SPECIFIC’ WARM AUTO-ANTIBODIES

As already mentioned, many apparently ‘non-specific’ anti-dl antibodies have been shown to be not strictly ‘non-specific’ but to react with antigens of very high frequency, e.g. to be anti-Wrβ, anti-Enα, anti-LW or anti-U. Issitt et al (1980) listed six additional very common antigens that had been identified as targets for anti-dl auto-antibodies, i.e. Hr, Hrα, Rh34, Rh29, Kpβ and K13.

SPECIFIC WARM AUTO-ANTIBODIES OF UNUSUAL SPECIFICITY

The author (Dacie, 1992) referred to a small number of case reports in which anti-Fy (Duffy), anti-Ge (Gerbich), anti-Jk (Kidd), anti-Lu (Lutheran), anti-S, anti-Sc3 (Scianna), anti-Vel and anti-Xgα antibodies had been identified.

SPECIFICITY OF COLD AUTO-ANTIBODIES: ANTI-I AND ANTI-i

As already referred to, haemolytic anaemia, often accompanied by haemoglobinuria, and sometimes acrocyanosis,
occurs in association with the presence of high-titre, high-thermal-amplitude, cold auto-antibodies developing as a complication of a variety of disorders, e.g. mycoplasma pneumonia, infectious mononucleosis, lymphomas or, for unknown reasons, in the cold-haemagglutinin disease (CHAD).

An interesting question was the specificity of the antibodies. Early work, e.g. by Mino (1924), was thought to indicate that cold agglutinins were non-specific. Amzel & Hirschfeld (1925) concluded, in contrast, that human erythrocytes did in fact differ in their agglutinability, an observation confirmed by later workers. However, no association with known blood groups could be established (Crookston et al., 1956). Earlier, however, Unger et al. (1952) had reported that fetal erythrocytes might be poorly agglutinated. Wiener et al. (1956) described some seminal observations they had made while testing a serum obtained from a patient suffering from CHAD. Out of 22,964 erythrocyte samples, five were not agglutinated at room temperature. The insensitive cells were designated I-negative or i, the normally reacting cells I and the cold agglutinin anti-I. The rare occurrence of (adult) I-negative cells was again described by Jenkins et al. (1960) and Tippett et al. (1960). Issitt (1967) reported that the frequency of the i (I-negative) phenotype in adults was, according to different reports, somewhere between 1 in 3000 and 1 in 17,000.

The next exciting development was the discovery that certain cold agglutinins acted as anti-i sera. Marsh & Jenkins (1960) reported that two patients, suffering from reticulum-cell sarcoma and a reticulosarcoma, respectively, had developed sera that agglutinated I-negative adult cells and fetal cells strongly but normal adult cells weakly. Marsh (1961) subsequently reported that infant erythrocytes react at birth as i cells and that their phenotype normally changes to I by the time the infant is 18-months-old.

Subsequent discoveries of great interest were the finding that anti-i cold agglutinins were in fact developed not uncommonly in patients with lymphomas and also in infectious mononucleosis. Jenkins et al. (1965) reported that the serum of an 18-year-old girl with infectious mononucleosis, who had developed a severe haemolytic anaemia, agglutinated cord-blood cells more strongly than adult cells. Subsequent tests revealed the presence of anti-i at low titres in seven out of 85 patients with uncomplicated infectious mononucleosis. Later, Horwitz et al. (1977) reported the results of tests carried out on the sera of 157 patients with infectious mononucleosis: 32% of the sera contained anti-i agglutinins compared with 0-3% of 1022 controls.

Studies on the specificity of cold auto-antibodies that have been undertaken subsequently to those referred to above have revealed that the Ii system is remarkably complex. There is apparently a close relationship between the chemistry of the Ii antigens and ABH(O) antigens, and serological studies have demonstrated antibodies of apparent anti-HI, anti-AI and anti-BI specificity in some individuals. The relevant literature is now extensive. Early reports on the specificity of the antibodies and the chemical nature of the corresponding antigens include those of Voak (1964) and Bird (1966). Later important reviews include those of Roelcke (1974) and Feizi (1981). Roelcke (1989) listed and named 12 distinct Ii antigens against which antibodies had been developed.

Anti-i\(^T\)

An apparent variant of anti-I, referred to as anti-i\(^T\) (T for transitional), was first described in the sera of certain Melanesian individuals. Subsequently, a similar antibody – reacting with fetal erythrocytes but only weakly with adult I and i cells – was identified in the sera of a small number of patients with Hodgkin’s disease (Garratty et al., 1972, 1974).

Chemical nature of Ii auto-antibodies

Studies first undertaken in the 1940s and 1950s established that the cold agglutinins present in the serum of patients suffering from chronic cold-haemagglutinin disease were 19S(γM) macroglobulins and that, if in sufficient concentration, their presence resulted in an abnormal (γ1 globulin) peak on electrophoresis (Stats et al., 1943; Gordon, 1953).

SPECIFICITY OF THE DONATH–LANDSTEINER (D–L) ANTIBODY OF PAROXYSMAL COLD HAEMOGLOBINURIA (PCH)

Early studies had shown significant differences in the sensitivity of adult erythrocytes to lysis by sera containing D–L antibodies (van Loghem et al., 1952; Schubothe, 1958). However, no clear association with known human blood-group antigens could be established. However, Levine et al. (1963), who had studied six patients with PCH, reported that the antibodies in their sera appeared to have the specificity anti-P + P1 (anti-Tj\(^a\)). Chemical studies showed that D–L antibodies are 7S(IgG) globulins (Hinz, 1963).

IMMUNE HAEMOLYTIC ANAEMIAS: AETIOLOGY

What are the causes and origins of the immune haemolytic anaemias? These are the questions that the author attempted to answer in a chapter of 28 double-column pages (Dacie, 1992), based on almost 200 references stretching back to the experimental studies of Ehrlich & Morgenroth (1900) and their concept of 'horror autotoxicus'. In relation to human acquired haemolytic anaemia, the discovery in the late 1940s and 1950s that many cases were apparently brought about by the development of damaging anti-erythrocyte antibodies led to intense interest and speculation into the why and how of auto-antibody formation. Of seminal importance at the time were the experiments and theoretical arguments of Burnet (Burnet & Fenner, 1949; Burnet, 1957, 1959, 1972) and the studies on transplantation immunity of Medawar (Billingham et al., 1953; Medawar, 1961). Of particular interest, too, was the report by Bielschowsky et al. (1959) of the occurrence of AIHA in an inbred strain of mice – the NZB/BL strain. Remarkably, by the time the mice were 9-months-old the DAT was positive in almost every mouse. Burnet (1963) referred to the gift of the mice to the Walter and Eliza Hall...
Institute of Medical Research, Melbourne as ‘the finest gift the Institute has ever received’.

The following three paragraphs summarize the author’s conclusions as to the views on aetiology that were current at the time he wrote the above-mentioned chapter.

**Acute transient AIHA**

This was thought to result from anti-erythrocyte antibodies developing as the result of microbial infection in a patient who had an unusual ability to develop antibodies, immunological self-tolerance being perhaps broken as the result of the invading organisms modifying the antigenicity of erythrocyte antigens or by unmasking antigens that were not normally available.

**Chronic AIHA**

Self-tolerance was thought to be broken, perhaps by a failure of T-lymphocyte surveillance or an abnormal T-suppressor to T-helper ratio. Immunoglobulin abnormalities, particularly IgA deficiency, appeared to play a part in the breakdown of tolerance. Genetic factors were considered to be particularly significant, leading perhaps to an unusual propensity to form antibodies.

**Chronic cold-haemagglutinin disease (CHAD)**

This was regarded as a clonal lymphoproliferative disorder, with somatic mutation, of unknown causation, resulting in the unrestrained proliferation of immunocytes dedicated to the production of cold (anti-I) antibodies.

**IMMUNE HAEMOLYTIC ANAEMIA: PATHOGENESIS**

Exactly how is it that auto-antibodies reacting with an erythrocyte surface antigen result in the cell’s premature destruction? The possible role of auto-agglutination in bringing about haemolysis was emphasized by Castle and colleagues as the result of a series of studies carried out in the 1940s and 1950s. As summarized by Castle et al. (1950), an antibody which appears to be incapable of causing lysis in vitro might bring about the following sequence of events in vivo. (1) Red cell agglutination in the peripheral blood; (2) red cell sequestration and separation from plasma in tissue capillaries; (3) ischaemic injury of tissue cells with release of substances that increase the osmotic and mechanical fragilities of red cells locally; (4) local osmotic lysis of red cells or subsequent escape of mechanically fragile red cells into the blood stream where the traumatic motion of the circulation causes their destruction.

That incomplete auto-antibodies might bring about agglutination in a medium of high protein content, but fail to do this in saline, had earlier been demonstrated by Wiener (1945) and Wagley et al. (1948). Later, Jandl & Castle (1956) reported on detailed studies on the phenomenon of agglutination in colloidal media of cells coated by incomplete antibodies.

**Erythrophagocytosis (EP)**

Particular attention has been focused, since the early 1950s, on the part played by the class of antibody globulin coating erythrocytes and the role of complement in causing antibody-affected erythrocytes to adhere to phagocytes prior to being phagocytosed. Bonnin & Schwartz (1954), in in vitro studies, compared the ability of AIHA auto-antibodies to bring about EP with the ability of antibodies such as anti-A. Their work indicated that, in general, antibodies that fixed complement failed to do this. Later work established that phagocytic cells have receptors for the Fe fragment of IgG antibodies (LoBuglio et al. 1967) and that phagocytes have receptors, too, for the C3b component of complement (Huber et al. 1968).

**Role of the spleen and liver**

The availability of $^{51}$Cr in the 1950s provided a potent means of studying the relationship between the type of antibody and the sites of in vivo erythrocyte destruction. A series of important studies revealed that while strongly agglutinated erythrocytes were removed from the circulation rapidly, particularly by the liver, cells bearing incomplete antibodies were removed predominantly by the spleen (Jandl et al. 1957; Cuthbush & Mollison, 1958; Crome & Mollison, 1964). Why this should be has been a subject of much research and speculation. Ham & Castle (1940) had suggested that erythrostasis in the spleen was an important cause of destruction of antibody-coated and spherocytic erythrocytes.

**Spherocytosis**

It had been realized for many years that, while the injection of an anti-erythrocyte serum into an experimental animal would regularly result in spherocytosis, the phenomenon could not be produced using the admixture of erythrocytes and serum in vitro (Wasastjerna, 1948). Subsequent work established that spherocytes in the peripheral blood in the experimental animal and in human AIHA are erythrocytes coated with IgG and/or complement that have been damaged by contact with phagocytic cells, but that had broken free before being phagocytosed. Details of the process of EP and spherocyte transformation as viewed by cinematography and transmission and scanning electron microscopy were provided by Bessis & de Boisfleury (1970).

**AUTO-IMMUNE HAEMOLYTIC ANAEMIA: TREATMENT OF WARM-ANTIBODY AIHA**

**Adrenocorticosteroids**

The first reports of encouraging responses to treatment with adrenocorticotropic hormone (ACTH) or cortisone date from the early 1950s (Dameshek, 1950; Gardner, 1950; Dameshek et al., 1951; Davidson et al., 1951; Gardner et al., 1951). Reports on the value of prednisone came later (Sussman & Dordick, 1956). They were followed by many case reports and reviews that dealt with the relative value of ACTH and the corticosteroids and the appropriate dose regimes, e.g. those of Jandl (1963), Allgood & Chaplin (1967), Pirofsky & Bardana (1974), Petz & Garrantt (1980), Zupańska et al. (1981) and Rosse (1990). The general experience indicated that prednisone and prednisolone are valuable drugs and that up to 80–90% of patients achieve worthwhile
remissions. How to treat the patients who failed to respond to doses of the drugs that they could tolerate was (and still is) an important question. Therapies that have been tried include splenectomy, the use of immunosuppressive drugs, injections of heparin, exchange blood transfusion, plasmapheresis and plasma exchange, intravenous γ-globulin and, in infants, thymectomy.

**Splenectomy**

Early reports of the effects of splenectomy date from the second decade of the century (Micheli, 1911; Antonelli, 1913). According to Welch & Dameshek (1950), who reviewed the results of splenectomy in 34 cases, the operation had resulted in remission in about half of the patients so treated. Later reviews based on larger numbers of patients yielded rather similar remission rates, e.g. those of Goldberg et al (1966) (improvement in 52% of 182 patients) and Petz & Gerratty (1980) (benefit in about 60% of 316 patients). Pirofsky & Bardana (1974) highlighted the then two opposing views as to the value of splenectomy, i.e. (1) that it neither cured the disease nor affected its eventual outcome, and (2) that it might induce remission in up to 75% of patients who had failed to derive lasting benefit from treatment with corticosteroids.

**Immunosuppressive drugs**

Nitrogen mustard seems to have been the first drug used. Dameshek (1951) reported that he had administered it to four patients, one of whom had achieved a remission which lasted for at least 2 years. Following the synthesis of 6-mercaptopurine (6MP), 6-thioguanine (6TG) and azathioprine (Imuran), the more potentially dangerous nitrogen mustard was no longer used. Dameshek & Schwartz (1960) and Schwartz & Dameshek (1962) reported that nine out of 14 patients with AIHA had responded to treatment with 6MP or 6TG. Corley et al (1965), who reviewed the responses to treatment with azathioprine, stated that 15 out of 27 AIHA patients had responded favourably. In a later review, Skinner & Schwartz (1972) stated that 20 out of 42 AIHA patients, who had failed to respond adequately to corticosteroids, had derived benefit from being treated with immunosuppressive drugs.

Murphy & LoBuglio (1976), in their review of the current status of immunosuppressive therapy for AIHA, discussed the relative value and dangers of splenectomy or drug therapy for the 30–40% of patients who were unable to achieve long-term remission on corticosteroid therapy. They found it difficult to choose between azathioprine and cyclophosphamide.

**OTHER THERAPIES THAT HAVE BEEN TRIED**

**Heparin**

First used by Owren (1949), some later reports were encouraging. However, the high doses required and the consequent risk of haemorrhage were regarded as serious disadvantages.

**Vinca alkaloids**

Vinblastine and vincristine are thought to inhibit macrophage activity. This property was exploited by Ahn et al (1978, 1983) who infused AIHA patients with vinca-loaded platelets. Some benefit was recorded.

**Danazol**

This androgen was used by Ahn et al (1985) in conjunction with high doses of prednisone. The results were encouraging. Ahn (1990) reported that in 10 out of 13 ‘idiopathic’ AIHA patients the results of treatment had been scored as excellent or good.

**Thymectomy**

Wilmers & Russell (1963) reported that thymectomy had been carried out as a last resort on an infant aged 21/2 months who had been suffering from extremely severe AIHA. The infant went into remission approximately 1 month after the operation. Karaklis et al (1964) reported that a 10-month-old infant had responded similarly and Ducie & Worlledge (1969) showed the same good result in a 7-month-old infant. In complete contrast to these encouraging results was the experience of Johnson & Abilgaard (1976). Their patient, a 21/2-month-old infant, had been treated with hydrocortisone, prednisone and cyclophosphamide without benefit and had required numerous transfusions. Splenectomy had been carried out when he was 6 months of age, again without benefit. Thymectomy undertaken 1 month later likewise failed to help.

**BLOOD TRANSFUSION IN AIHA**

The finding by means of the Ashby technique or labelling with 51Cr that transfused normal erythrocytes typically survived badly in AIHA patients provided a clear explanation for the limited and short-lived clinical value of blood transfusion. as did the demonstration that a patient’s auto-antibodies reacted with normal erythrocyte antigens in vitro tests. However, the specific nature of the antibodies suggested that it might be possible in some patients to avoid incompatibility by selecting cells for transfusions lacking the antigens corresponding to the specificity. That this was indeed possible was demonstrated in the 1950s, e.g. by Holländer (1954) and Mollison (1959). In practice, however, knowledge of the specificity of a patient’s auto-antibodies proved to be of less value than was at first anticipated. The reason for this was that, even when the specificity of a patient’s auto-antibody appeared to be anti-e, antibodies of a broader specificity outside the Rh system were probably present as well.

The possibility that blood transfusion might initiate remission in some patients with acquired haemolytic anaemia, in addition to providing haemoglobin, was raised by the reports of Lederer (1925, 1930). The general experience in the 1930s and 1940s failed, however, to support this contention. Dameshek & Rosenthal (1951) reported that, in their own experience, in only six out of 70 cases of mixed pathogenesis were transfusions followed by a remission. Nevertheless, at a time when transfusion and
splenectomy were the only possible remedies, the remote possibility that blood transfusion might initiate remission led to the drastic procedure of exchange (exsanguination) transfusion being attempted. e.g. by Pinney (1950) and Cattan et al (1951). Benefit was generally reported as being short-lived.

PLASMAPHERESIS AND PLASMA EXCHANGE

The realization that exchange blood transfusion could not only raise the haemoglobin but could also remove auto-antibodies circulating in the plasma in AIHA patients led to the introduction of plasmapheresis and plasma exchange as a means of treating severely ill patients (Branda et al, 1975; Isbister. 1979; Petz & Garratty, 1980).

SPECIFIC IMMUNOADSORPTION OF IgG

Besa et al (1981) described how they had managed to significantly reduce plasma IgG by extracorporeally exposing a patient’s plasma to a formalin-stabilized suspension of Staphylococcus aureus. Besa et al (1981) considered the procedure to be more efficient than plasmapheresis.

INTRAVENOUS INFUSION OF GAMMA GLOBULIN (IgG)

In the 1980s, following the use of intravenous IgG in the treatment of idiopathic thrombocytopenic purpura, intravenously administered IgG has been used quite extensively as treatment for patients seriously ill with AIHA (Bussel et al, 1983. 1986; Salama et al, 1984). Some favourable responses were recorded following 5-d courses at a dose range of 0·4–1·0 g/kg/d.

TREATMENT OF COLD-HAEMAGGLUTININ DISEASE (CHAD)

Alkylating drugs

The realization that CHAD is a monoclonal gammopathy logically led to attempts to treat the disorder with drugs that had been used successfully in the treatment of Waldenström’s macroglobulinaemia and the malignant lymphomas. Chlorambucil, in particular, has been reported to be helpful, if taken over long periods (Olesen, 1964; Worlledge et al, 1968; Hippe et al, 1970).

Mercaptanes

The discovery that mercaptanes can depolymerize macro-globulins (Deutsch & Morton, 1957) led to their use as possible treatments for CHAD. However, while their effect on cold-agglutinin activity was clearly demonstrable in in vitro experiments, e.g. by Fudenberg & Kunkel (1957), benefit from their use in vivo has been less clear-cut. While Ritzmann & Levin’s (1961) patient responded to orally administered penicillamine, the two patients reported by Lind et al (1963) failed to respond.

Blood transfusion

The rarity of adult I-negative (i) erythrocytes means that, for all practical purposes, seriously anaemic CHAD patients can only be transfused with (incompatible) I blood. Moreover, the enhancement of cold-agglutinin activity at low temperatures is an additional hazard. Rosenfield & Jagathambal (1976) memorably wrote: ‘The worst thing an attending physician can do to a patient who has either a severe postviral paroxysmal haemoglobinuria or florid cold agglutinin disease is to administer cold blood’. Petz & Gerratty (1980) suggested, however, that unwarmed blood could probably be transfused with safety, if given at a slow drip rate. They agreed with Rosenfield and Jagathambal that an on-line blood warmer could be useful.

Plasma exchange

This has been carried out on patients suffering from CHAD and has led to temporary improvement, as reported by Logue et al (1973) and Rosenfield & Jagathambal (1976). Rosse (1990) pointed out that the fairly rapid rate of IgM synthesis meant that any improvement induced by plasma exchange could only be temporary. In the patient whose history had been recorded by Logue et al (1973), the cold-agglutinin titre had, on two occasions, returned to its original level within 1 week of the exchanges.

THE IMMUNE HAEMOLYTIC ANAEMIAS: THE FUTURE

In the foregoing text, the author has attempted to describe the remarkable increases in knowledge as to the occurrence and mechanism of the immune (auto-antibody-induced) acquired haemolytic anaemias that have taken place in the twentieth century. However, despite these advances in knowledge, patients still develop immune haemolytic anaemias, and their treatment may present as an urgent and serious problem. What advances in knowledge of benefit to patients can be expected in the future? The essential problems remain to be fully solved. Why and how does self-tolerance to one’s own erythrocyte antigens break down and what can be done to contain the breakdown?

We can expect, as the years pass, that more and more will be known as to the intricate mechanisms that bring about self-tolerance and the mechanisms underlying the occurrence of auto-immune disorders in general, including the role of infectious agents, drugs and genetic factors. Patients with immune haemolytic anaemias can be expected to benefit from the new knowledge; for in parallel with a better understanding as to how immune self-tolerance breaks down will hopefully be the development of more effective drugs and therapies aimed at controlling the breakdown. A great deal will surely be accomplished in the forthcoming decade, to say nothing of the new knowledge that may be expected to accumulate during the whole of the twenty-first century.
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