Papers and Originals

Autoimmune Haemolytic Anaemias*

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It is a great privilege to be asked to give this lecture in honour of the late Professor Kettle. His contributions to research and literature were made mainly in two areas of medical science—on tumours and on the effects of harmful industrial dusts on infection and resistance, while his knowledge of the histology of infective processes appears to have been unequalled in his time. In all his writings there is evidence of accurate observation and wise judgement; his experimental work was carefully planned, and he stressed the importance of correlating observations made in man with the results of experimental research.

In this lecture I shall perforce deal with a haematological topic. But the principles of pathology are nevertheless essentially the same irrespective of the organ or organs affected. In the autoimmune haemolytic anaemias we are dealing with problems of immunopathology and cytopathology and with the effect of pathological processes on the blood (haematology) and on the patient (clinical medicine). The subject is a very large one, and here I can deal only rather superficially with two of its aspects—namely, aetiology, which is a problem of immunopathology, and pathogenesis, essentially a problem of cytopathology.

Discussion and argument on the role of antibodies in cases of acquired haemolytic anaemia stretch back to the early years of this century, but it has been only in the last two decades, following the discovery by Boorman *et al.* (1946) that the red cells of patients with acquired haemolytic anaemia often gave positive antiglobulin or Coombs tests (while those of patients with congenital and hereditary haemolytic anaemias characteristically did not), that autoantibody development became firmly established as the prime cause of haemolysis in an important group of cases which we now call the autoimmune (or autoallergic) haemolytic anaemias. Much, too, has been learnt about the nature of the antibodies involved and their physical and chemical characteristics, their specificity for red-cell antigens, and their diversity and individual patient characteristics. Their main characteristics are summarized in Table I.

A point that should be made in relation at least to the IgG warm-type antibodies is that, though acting as autoantibodies, they are in other respects essentially the same as their more familiar isoantibody counterparts. It is the circumstances which surround their formation that are different; in the case, for instance, of Rh-specific antibodies such as anti-e they are formed not as the result of the transfusion of red cells carrying the e antigen into a recipient whose red cells lack that antigen, but occur as an apparent spontaneous development in an individual whose red cells carry that very antigen, thus constituting an apparent flagrant breaking of immune tolerance.

Theories on Formation of Red Cell Autoantibodies

Discussion on the problem of why an animal able to form antibodies against the red cells of a foreign species, or even against the red cells of another animal of the same species, appeared to be incapable of forming antibodies against its own red cells stems from the early years of this century. Ehrlich and Morgenroth's famous phrase "horror autotoxicus" occurs in a paper written in 1901. In Bolduan's (1906, p. 82) English translation of Ehrlich's *Collected Studies on Immunity* we read: "In the third communication, on isolysins, we pointed out that the organism possesses certain contrivances by means of which the immunity reaction, so easily produced by all kinds of cells, is prevented from acting against the organism's own elements and so give[s] rise to autotoxins. Further investigations made by us have confirmed this view, so that one might be justified in speaking of a 'horror autotoxicus' of the organism. These contrivances are naturally of the highest importance for the existence of the individual." Earlier, Ehrlich and Morgenroth, in referring to the possibilities of autointoxication occurring in man, made the point that: "Only when the internal regulating

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TABLE I.—*Characteristics of Red Cell Autoantibodies Giving Rise to Autoimmune Haemolytic Anaemias*

	Warm Antibodies	Cold Antibodies	Cold Antibodies—Donath–Landsteiner
Immunoglobulin type	Usually IgG, with or without IgM. May be IgM only; occasionally IgA with IgG	IgM	IgG
Light chain type	Usually kappa + lambda	Almost always kappa in chronic cold haemagglutinin disease. Kappa and lambda usually in transient antibody formation	Unknown
"Complete" or "incomplete"	Almost always "incomplete"; "complete" antibodies rare	"Complete"	"Complete"
Thermal range	React optimally at about 37°C.	Association to red cells increases from 28–32°C. to 0°C. High titres at low temperatures	Association to red cells increases from 15–20°C. (rarely higher) to 0°C. Titres at low temperatures seldom high
Fixation of complement	IgG antibodies almost always fail to fix complement; IgM probably always do; IgA do not	Always, but sera vary in ability to lyse red cells; often most lysis at acid *p*H (6·5–7·0)	Complement always fixed, resulting in major lysis of red cells
Specificity	IgG and IgA antibodies usually Rh specific; specificity of IgM antibodies uncertain	Usually anti-I; occasionally anti-i or outside Ii system	Anti-P; all human cells lysed except pp and P^k cells

contrivances are no longer intact can great dangers arise" (Bolduan, 1906, p. 35). Their conclusions can hardly be faulted. The concept of "horror autotoxicus" still stands, and even if the internal regulating contrivances are still largely unknown the suggestion that their breakdown could lead to great dangers still holds.

It has to be admitted that the problem of why an individual, after perhaps many years of apparently good health, starts to form antibodies against antigens on his red cells that he has previously tolerated is still largely unsolved. In all probability there is no single or simple explanation. Four hypotheses are summarized in Table II. The third hypothesis does not stand by itself. But for either of the mechanisms envisaged under hypotheses 1 and 2 an enhanced ability to form antibodies has to be postulated. Possibly this hypothetical enhanced ability is genetically controlled.

TABLE II.—*Hypotheses for the Development of Autoantibodies in Autoimmune Haemolytic Anaemia.*

1. Modification of red-cell antigens leading to termination of immune tolerance
   ? by virus, drug, enzyme.
2. Formation of cross-reacting antibodies
   ? to exogenous antigens, virus, etc.
3. An enhanced ability to form antibodies
   ? autoantibodies as well as isoantibodies or heteroantibodies;
   ? a genetic effect.
4. The "spontaneous" appearance of non-malignant ("forbidden") antibody clones
   ? as the result of a failure of elimination at an early stage;
   ? failure of immune process.

Modification of Red-cell Antigens

The hypothesis that autoantibodies may be produced as the result of red-cell antigens being altered by the action of virus, drug, or enzyme has always been an attractive one, but difficult to prove or refute. Moreover, it has to be accepted that antibodies formed in this way—that is, against modified antigens—nevertheless can react with strictly normal red cells, that is if they bear the corresponding antigen. It is relevant perhaps that Weigle (1965), in experimental studies on acquired tolerance, has shown that tolerance to an exogenous antigen can be broken by injecting an antigen which is serologically related to that to which the animal had acquired tolerance. It is certainly conceivable that tolerance could be broken in man in a like manner, with virus or drug-modified red cells acting as the agent breaking the tolerance. The occurrence of haemolytic anaemia, or at least the development of warm anti-red-cell autoantibodies, in some patients receiving methyldopa (Aldomet) for hypertension at first sight seems to support this concept (Carstairs *et al.*, 1966; Worlledge *et al.*, 1966). There is as yet, however, no clear evidence that methlydopa does in fact modify red-cell antigens, and the same applies to other drugs which are thought to lead to autoimmunization and to the more speculative viruses and metabolites of endogenous origin.

I should like to emphasize here, however, a point of extraordinary interest This is that when methyldopa causes autoantibody formation the antibodies are directed against antigens within the Rh system. This is the specificity, too, of most of the antibodies met with in so-called "idiopathic" warm antibody cases. Why autoantibodies are rarely directed against other identifiable antigens is an intriguing mystery. Their predilection for Rh antigens is equally mysterious, and one can only speculate that tolerance to these antigens is perhaps less firmly achieved and may in consequence be relatively easily broken.

Formation of Cross-reacting Antibodies

The transient occurrence of anti-red-cell autoantibodies following *Mycoplasma pneumoniae* infection, or rarely following virus infections such as infectious mononucleosis, measles, or varicella, is very suggestive of the development of cross-reacting antibodies as part of the immune response to the infective agent, and there is experimental evidence that this is so, at least with respect to *Myco. pneumoniae* (Costea *et al.*, 1969). These antibodies, however, are characteristically cold ones of I or i specificity, or rarely Donath–Landsteiner in type, and there appears in fact to be no evidence which suggests that the common and usually very persistent warm autoantibodies of Rh specificity are ever formed as cross-reacting antibodies to exogenous antigens.

Enhanced Ability to Form Antibodies

An unusual or enhanced ability to form antibodies by patients who develop autoimmune haemolytic anaemia has often been postulated, and there is some evidence for this. Thus it is well known that patients who have systemic lupus erythematosus have developed an unusual number of types of immune isoantibody following transfusion (Callender and Race, 1946), and the same is probably true of patients who suffer from other types of autoimmune haemolytic anaemia (Cleghorn, 1959). It is well known, too, that apparently healthy individuals differ widely in their isoantibody responses to experimental inoculation with incompatible blood cells. It has to be admitted, however, that the hypothesis that an increased capacity to respond to specific stimulation with exogenous antigens increases the proneness of the individual to develop *auto*antibodies is unproved.

Appearance of "Forbidden" Clones

The association of autoimmune haemolytic anaemia with lymphomas, particularly chronic lymphocytic leukaemia, fits in well with Burnet's (1969) clonal selection theory of immunity as applied to autoantibody formation. On this hypothesis clones of immunocytes having the ability to react with self-antigens appear and are not eliminated, perhaps in much the same way and for the same reasons as the malignant clone or clones producing lymphomas are not eliminated. Several assumptions have to be made: that the clones have an affinity for, and can eventually form antibodies against, self-antigens; that they have the ability to proliferate; and that they cannot be eliminated though not overtly malignant. Analogies can be drawn between the development of non-malignant "forbidden clones" and what appears to happen in graft-host disease when parental lymphoid cells from an inbred strain of mouse are inoculated into F1 hybrids (Oliner *et al.*, 1961). The parental cells cannot be eliminated by the hybrids, but they can and do react against the antigens provided by the other parent of the hybrid.

In relation to human chronic lymphocytic leukaemia a point that should be made is that autoimmune haemolytic anaemia appears to be able to develop at almost any stage of the disease; certainly it can appear long after the leukaemia has become well established. It seems probable, therefore, that the anti-red-cell autoantibodies derive from new clones of immunocytes and that the cells of the malignant lymphoma are generally not themselves antibody-forming. In chronic lymphocytic leukaemia it seems likely, nevertheless, that the autoantibodies derive from clones of immunocytes the mechanism of development of which is similar to, if not identical with, that of the main clone or clones of lymphoma cells. The body cannot eliminate the antibody-forming clone(s) any more than it can the malignant clones. As an extension of this idea, it is certainly possible that many cases of "idiopathic" autoimmune haemolytic anaemia have an analogous origin—that is, the autoantibodies stem from a clone or clones of lymphoid cells which, though not malignant, cannot be eliminated and which, as opposed to most of the clones in chronic lymphocytic leukaemia, are capable of acting as immunocytes. It is an interesting and significant fact that autoimmune haemolytic anaemia is very seldom met in

association with other types of malignant disease, for instance, carcinoma. This negative fact increases the significance of the association already mentioned with malignancies of the lymphoreticular system.

Disorders of Immunoglobulin Synthesis

A recently recognized association not yet mentioned is the occurrence of autoimmune haemolytic anaemia in patients suffering from disorders of immunoglobulin synthesis. Several instances have been reported in patients with various types of hypogammaglobulinaemia or dysgammaglobulinaemia (Hinz and Boyer, 1963; Pirofsky, 1969, p. 339), and it has been suggested that it is the immune deficiency which allows the hypothetically abnormal clones of immunocytes to proliferate (Pirofsky, 1969, p. 370). Similarly, the development of a malignant lymphoma in an immune-deficient individual may also be looked on as a consequence of the failure to eliminate undesirable clones at an early stage of their development (Pirofsky, 1968a). The known association between hypogammaglobulinaemia, malignancy, and autoimmune haemolytic anaemia has prompted my colleagues and myself to measure serum immunoglobulins in as many patients with autoimmune haemolytic anaemia as possible. These studies have shown that immunoglobulin deficiencies are in fact relatively frequent (Blajchman *et al.*, 1969). Thus, slightly more than half of the 48 patients who had the "idiopathic" type of autoimmune haemolytic anaemia had minor (or occasionally major) deficiencies in their serum globulins. This was particularly true of IgA.

We do not know whether the deficiencies that have been found are congenital or acquired. We suspect that some at least are congenital. None of the patients had received any immunosuppressive drug other than adrenocorticosteroids and none had undergone splenectomy before the measurements were made. In fact, in many instances the low globulin levels were found in serum specimens withdrawn from patients before steroid treatment. There seem to be two types of possible explanation for an acquired deficiency—namely, failure of synthesis (by an as yet unexplained mechanism) or the development of antibodies directed against immunoglobulins—that is, anti-antibodies. There seems at the moment to be no evidence for or against either hypothesis. It is certainly logical to believe that undesirable antibody clones persist in autoimmune haemolytic anaemia because of the individual patient's inability to eliminate them. Whether this can in any way be associated with defects in immunoglobulin synthesis or cellular immunity has not yet been proved.

Association with Other Disorders

The association of autoimmune haemolytic anaemia with other disorders thought to be of autoimmune origin must now be commented on. This has been particularly stressed by Pirofsky (1969). In his view autoimmune haemolytic anaemia almost always occurs as part of what he refers to as "diffuse autoimmune" or "diffuse immunologic" disease, one or more aspects of which may be dominant. He concludes in fact that the concept of "idiopathic" cases (of autoimmune haemolytic anaemia) may be illusory. The point he is making is that in his experience the abnormal immune reaction leading to autoimmunization rarely, if ever, affects the red cells alone. This is, I feel, a rather extreme view, for in about half of our patients no underlying disease of any sort—autoimmune affecting any tissue other than the blood, inflammatory, or neoplastic—has been demonstrable, and the red cells (but sometimes the platelets, too) alone have appeared to be affected. Of course the longer a patient is followed the greater is the likelihood of the appearance of some additional condition.

Systemic lupus erythematosus is probably the best known autoimmune disease with which autoimmune haemolytic anaemia may be associated; indeed haemolytic anaemia is sometimes the presenting and dominant manifestation (Lee and Davis, 1959).

Other probable or possible autoimmune diseases which may be complicated by autoimmune haemolytic anaemia include rheumatoid arthritis, scleroderma, polyarteritis, ulcerative colitis, and thyrotoxicosis. I should like to stress, however, that these latter associations are rare. Possibly all these occurrences are linked together by a factor common to all the disorders mentioned—namely, an aberrant or defective immune apparatus—and in line with this concept it was especially interesting to find in a recent patient of Dr. Michael Brain, a young boy with thyrotoxicosis and autoimmune haemolytic anaemia, persistent deficiencies of IgG, IgA, and IgM.

Genetic Influences

I shall now say something more on genetic influences in relation to autoimmunization. The discovery by Bielschowsky *et al.* (1959) of the regular occurrence of autoimmune haemolytic anaemia in NZB/BL hybrid mice was an important event in the history of our subject. Even so, despite much experimental work, the exact mechanism for the formation of the anti-red-cell autoantibodies remains a mystery. Some members of the mouse strain, at least, carry a virus (East *et al.*, 1967), and it is possible but unproved that viruses play a part in the causation of the auto-immunization, perhaps by one of the mechanisms already mentioned.

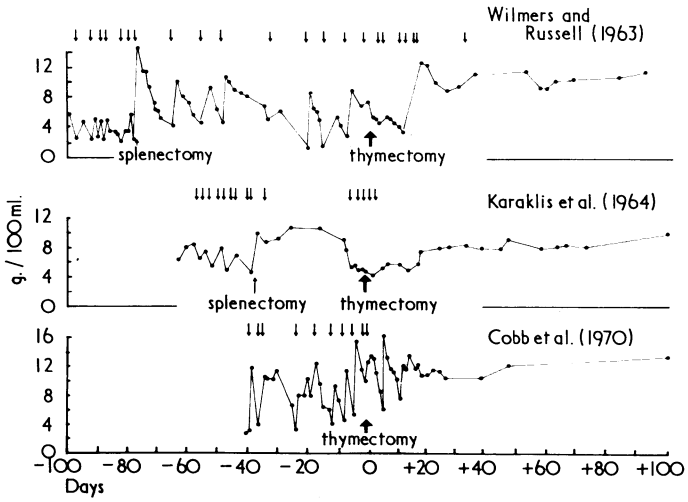
In man genetic influences seem also likely to be important. There is in fact a small but growing literature on the occurrence of autoimmune haemolytic anaemia in more than one member of a family (Pirofsky, 1968b, 1969). Such occurrences are, however, rare and we have seen only one unequivocal example of family incidence in more than 100 warm-type cases.

Thymectomy

I must now mention the possibility that abnormalities in thymus function can lead to autoimmune haemolytic anaemia in man. This has been described in a few patients who have had thymomas, with or without myasthenia gravis (Halperin *et al.*, 1966; Mongan *et al.*, 1966). But the nature of the association remains obscure. In infants under 1 year of age—in whom autoimmune haemolytic anaemia, though rare, is well recognized—thymectomy has been carried out on at least four occasions with an apparently successful outcome in three (Wilmers and Russell, 1963; Karaklis *et al.*, 1964; Cobb *et al.*, 1970). All three infants who seemed to respond had severe autoimmune haemolytic anaemia refractory to adrenocorticosteroid therapy (and in two instances also to splenectomy). In each case the thymus was removed, not because it was known to be pathological, but as a last desperate therapeutic resort. All three went into remission two to three weeks after operation. Whether this was due to removal of the thymus is, of course, unproved, but the time-sequence of the remission suggests that it was (see Chart).

The history of the infant who failed to respond to thymectomy was reported by Oski and Abelson (1965). At the early age of 6 weeks she developed autoimmune haemolytic anaemia of severity at least equal to that of the other three infants. Thymectomy was carried out when she was 14 weeks old, and, because of no apparent benefit, splenectomy was undertaken two weeks later. About 10 days after splenectomy—that is, about 24 days after thymectomy—the infant went into remission and the haemoglobin gradually rose to 12-13 g. Fifty-three days later she appeared to suffer an

acute relapse for she was found dead in bed and profoundly anaemic. It seems impossible to say whether thymectomy had contributed to the infant's temporary remission.



Effect of thymectomy on three infants suffering from autoimmune haemolytic anaemia; redrawn from the reports of Wilmers and Russell (1963), Karaklis *et al.* (1964), and Cobb *et al.* (1970). The points represent measurements of haemoglobin, the small arrows blood transfusions. The time scale represents days before and days after thymectomy.

In all four infants sections of the thymus showed no very obvious pathological features; the glands were atrophic, and active lymphoid foci were not present, though plasmablasts were present in certain areas in one of the glands (Karaklis *et al.*, 1964). All the infants had, however, received large doses of adrenocorticosteroids. If, nevertheless, it is assumed that the autoimmune process in the infants who responded was in fact dependent on the presence of thymic tissue and capable of being interrupted by thymectomy, then the relatively rapid cessation of antibody formation suggests that the clones of cells forming the autoantibodies were short-lived and not renewed or activated once the thymus had been removed. The responses made by these infants certainly suggest that the thymus may sometimes play an unexpectedly important part in relation to autoimmune haemolytic anaemia in infants. Whether this may also be true of the disease in older children or in adults seems at present to be unknown. In my view thymectomy would be justified in them if all else had failed. The difficulty is to know whether or not the disease in infants should be regarded as having a special and perhaps unique aetiology different from that of the disease in older children or adults.

The foregoing thoughts on the aetiology of autoimmune haemolytic anaemia can be summarized as follows: (1) There is no single cause. (2) In patients who develop autoimmune haemolytic anaemia there may be a genetically determined proneness to form autoantibodies, which may in some cases be associated with failure to form immunoglobulins. (3) On the above background a number of causes may lead to the development and persistence of abnormal ("forbidden") clones of immunocytes which form antibodies against certain red-cell antigens—that is, to the breaking of tolerance. Methyldopa may be one such cause; virus infection can be suspected as another possible cause. (4) Autoantibodies against red cells are formed particularly frequently in patients who suffer from other types of autoimmune disorder or who form autoantibodies against other tissue antigens. (5) Autoimmune haemolytic anaemia is also particularly frequent in patients who suffer from malignant lympho-proliferative diseases. In both autoimmune haemolytic anaemia and in the malignant lymphomas there may be an abnormal proneness to develop mutant clones of lymphoid cells which the patient is unable to eliminate. (6) Further information concerning the normal processes of immune tolerance is required before the riddle of the aetiology of autoimmune haemolytic anaemia can be solved.

Mechanisms of Haemolysis

The way in which antibodies damage red cells and shorten their life-span has been the subject of speculation and experiment since the early years of this century. Of the early publications, those of Christophers and Bentley (1908), Muir and McNee (1911-12), and Banti (1913) were of outstanding importance.

Christophers and Bentley were employed by the Government of India and published their paper in Calcutta as one of a new series of Scientific Memoirs by Officers of the Medical and Sanitary Departments. Their work was undertaken in an attempt to obtain insight into haemolytic mechanisms; in addition to observations on cases of blackwater fever, it consisted of an extensive haematological study of the effects of the injection into dogs of antisera against dog red cells prepared in goats. Christophers and Bentley's contribution was a remarkable one, and they incidentally seem to have been the first authors to coin and use the word spherocyte. Among many other prescient observations they recognized that haemoglobinaemia resulted from intravascular haemolysis, in contradistinction to red-cell destruction outside the blood stream, which they attributed to erythrophagocytosis without solution of haemoglobin. Increased osmotic fragility was correlated with the presence of darkly staining spherocytes. Such cells were noted to be inconspicuous in the initial stages of lysis, but might, they reported, be present in blood from viscera. They stated: "The groups of agglutinated cells in hepatic vein blood are all spherocytes" and noted that such cells were often arranged around one or two leucocytes.

Muir and McNee (1911-12), working with rabbits, and Banti (1913), working with rabbits and dogs, covered much the same ground as did Christophers and Bentley in similarly extensive series of experiments. All three groups of workers stressed the apparent lack of correlation between the effects of haemolytic agents in vitro and in vivo. Banti concluded that animals possessed or developed what he referred to as a "fragilizing activity," which potentiated the effects of immune sera in vivo, and concluded that the severe anaemia which follows the injection of immune serum was in large part due to the haemolytic activity or potentiality of the animal itself. Banti considered where the hypothetical haemolytic potentiality was likely to be located and concluded that the spleen, which invariably became engorged with blood and contained many erythrophages, was the organ which particularly potentiated the effect of the immune serum. He also pointed out, however, that if the dose of serum was increased the liver showed changes somewhat similar to those in the spleen—the sinuses became packed with red cells and the Kupffer cells functioned as macrophages.

Present Knowledge

The following account attempts to summarize what is now known of how antibodies bring about haemolysis in vivo. In man, as already indicated, most anti-red-cell autoantibodies are IgG and are not complement-fixing; they are of the so-called incomplete type and do not agglutinate red cells in saline suspension. It is now realized that this type of antibody leads to the preferential sequestration of red cells in the

red pulp of the spleen. How this is brought about is, however, still not exactly known. There are several possible mechanisms, which may perhaps act in combination. One is that in the spleen pulp, where the circulation is slow, red cells coated with incomplete antibodies adhere one to the other, and it is possible that this autoagglutination, even if readily broken up in circulating blood, may be sufficient to impede the cells from passing readily through the slit-like stomata which control the exit of cells from the spleen pulp into the splenic sinuses.

Another possibility is that red cells coated with incomplete antibodies may be less deformable than normal (Teitel, 1967), and in consequence pass through the stomata less readily than normal because of loss of plasticity, even if unagglutinated. At all events the spleen acts as a fine filter and tends to retain the antibody-coated cells. This retention probably facilitates phagocytosis by reticuloendothelial cells, including the blood monocytes, by a remarkable mechanism which includes the adherence of IgG on the red-cell surface to specific receptors on the surface of the phagocytic cells (LoBuglio *et al.*, 1967). The spherocytosis which is so characteristic of this type of antibody-induced haemolytic anaemia, particularly when severe, is probably caused by the presence of cells in the circulating blood which have previously been in contact with phagocytes but have broken free; particularly small spherocytes may in fact be the rounded off remains of partially phagocytosed cells.

Warm-type antibodies which act as agglutinins in vitro are much less commonly formed than are incomplete antibodies. Autoagglutination is firmer and may persist in circulating blood and the agglutinates are probably larger than those produced by incomplete antibodies. At any rate the splenic fine-filter mechanism is reinforced by the coarser and less sensitive filter of the liver (Jandl and Kaplan, 1960). The presence of this type of agglutinating antibody is associated with very pronounced spherocytosis, and, as may be imagined, haemolysis in vivo is clinically severe (Dacie, 1962, p. 610).

The liver, despite its bulk and the phagocytic potentiality of its Kupffer cells, is generally of less importance than is the spleen in the destruction of IgG-coated red cells. This may simply be due to the more rapid movement of blood through the liver sinuses preventing the ready adhesion of antibody-coated cells to Kupffer cells. The liver in fact seems to act only as an important haemolytic organ in relation to IgG antibodies when the red cells are very heavily coated by antibody.

Almost all the complement-fixing IgM antibodies which cause haemolytic anaemia in man are of the cold type. Their clinical importance depends on two factors: the temperature at which antibody can associate with antigen and the degree to which antibody-antigen interaction initiates complement fixation. As for temperature, most clinically important antibodies can be shown to agglutinate red cells up to about 28-32°C. (If the antibodies are inactive at this temperature, the patient will usually be found to be free from obvious haemolysis unless he exposes himself to an unusual degree of cold.)

In relation to antibody activity at 28-32°C., Barcroft and Edholm (1946) showed that the subcutaneous temperature of the bared forearm will readily fall to and below 30°C. if the forearm is exposed to air at rather cool room temperature—for example, 18·5°C. Thus transient antibody-antigen interaction can be expected to occur as blood circulates through areas of the body exposed to a cool environment. Autoagglutination may occur in peripheral small blood vessels to such an extent as to lead to acrocyanosis and Raynaud's phenomena or even gangrene of digits, toes, tip of the nose, and ears. Patients forming high-thermal-amplitude cold antibodies usually suffer from continuous haemolytic anaemia, and the association of this with Raynaud's phenomena on exposure to cold, and sometimes haemoglobinuria, is now widely referred to as the cold-haemagglutinin syndrome.

The mechanism of the haemolysis is a matter of considerable interest. The probability is that it is brought about almost entirely as the result of the fixation of complement to red cells and that the intermittent autoagglutination which occurs in peripheral blood vessels is of little or no consequence.

Role of Complement

Complement is now known to produce haemolysis in the experimental animal (and probably, too, in man) by two mechanisms. The first is by intravascular haemolysis which results from the completion of the complement sequence on the red-cell surface. The second is the less rapid extravascular lysis which stems from interaction between red cells and phagocytic cells. This follows from the presence of complement components on the surface of red cells which have not been lysed by the first mechanism. The first (and classical) mechanism of complement lysis is now known to involve the interplay of at least 11 serum proteins—C1q, r, and s and C2 to C9. The first four components (C1, 2, 3, and 4) constitute the activation mechanism, while C5, 6, 7, 8, and 9 are the attack mechanism (Müller-Eberhard, 1969). The final cytolysis is brought about, it is thought, by C8 and C9.

The occurrence in man of the second or extravascular mechanism of complement-mediated lysis is suggested by the fact that positive evidence for active intravascular haemolysis is not always forthcoming in anaemic patients suffering from the cold-haemagglutinin syndrome. Thus some patients never suffer from haemoglobinuria and raised plasma haemoglobin levels are not necessarily found. Moreover, another characteristic feature of the cold-haemagglutinin syndrome is the regular occurrence of strongly positive antiglobulin tests due to the presence of the complement components on the red cells. The remarkable thing is *not* that the red cells have fixed complement but rather that they have not undergone lysis.

The cause of resistance to complement lysis is not exactly known, but it appears to be a fact that the complement sequence can be activated at the surface of many red cells without their necessarily sustaining damage sufficient for lysis to occur. One probable reason is the short life of some of the complement components. Moreover, it is now well known that, as in the equally complicated coagulation system, the complement sequence has its own built-in series of inactivators. Whether lysis takes place depends, it seems, on the type of antibody, the number of antibody molecules, the spatial distribution of antigen sites on the red-cell surface, and unknown factors in the substrate (cell surface) with which the complement factors react. (Indirect evidence in favour of the importance of normality at the cell surface as a factor affecting complement lysis is provided by the greatly enhanced sensitivity of the abnormal red cells in paroxysmal nocturnal haemoglobinuria (Rosse and Dacie, 1966).) The positive antiglobulin tests given by circulating red cells after exposure to cold antibodies are now known to result from the interaction on the cell surface of C4 molecules (reacting with anti-β_1E) and C3 molecules (reacting with anti-β_1C). There is no reason to believe, however, that red cells giving positive tests necessarily have a shortened life-span.

Recent Experimental Work

The presence of active C3 on the red-cell surface can nevertheless be damaging even if the complement sequence fails to proceed to completion. It is now known that blood

monocytes (and also probably fixed phagocytes) have receptors on their surface not only for IgG but also for C3 molecules and that it is interaction between these receptors and C3 bound to the red-cell surface which leads to adhesion of red cells to monocytes and eventually to phagocytosis (Huber *et al.*, 1968). In so far unpublished studies carried out by Drs. Amiel Cooper and David Brown working in my Department, it has been possible to follow in the rabbit in some detail some of the steps in the processes outlined above, using as a source of antibody pure IgM cold antibodies prepared from the serum of patients who have the cold-haemagglutinin syndrome. Rabbit red cells carry an I-like antigen on their surface, and the cells react strongly with human anti-I sera: moreover, as the antigen-antibody reaction takes place at 37-40°C., the antibody can be used to bring about haemolysis in the rabbit in a way which it is possible closely to control.

The occurrence in rabbits of pronounced intravascular haemolysis leading to haemoglobinaemia and haemoglobinuria depends on the dose of anti-I antibody given, its ability to initiate complement activation, and the availability of complement. Through the courtesy of Dr. Peter Lachmann, Dr. Brown has been able to experiment with rabbits congenitally deficient in complement component 6 (C6) and this has enabled him to analyse in detail the effects of the administration of antibody to animals in which the final stages of the complement sequence cannot take place. It was particularly interesting to find in these rabbits that many of the antibody-sensitized cells nevertheless left the circulating blood only to return to the circulation later. A similar dose of antibody injected into rabbits in whom complement component 3 had been inactivated by a protein fraction obtained from cobra venom had no appreciable effects.

These experiments have also thrown some light on the leucopenia and thrombocytopenia which commonly accompany acute intravascular haemolysis. If antibody is administered to animals (with normal complement components) the resultant acute intravascular haemolysis is accompanied by neutropenia and thrombocytopenia and it seems likely that neutrophils and platelets adhere to red-cell-antibody complexes and are removed from the circulation as a result. Such adhesion may be temporary, for in the rabbit the neutrophil and platelet counts rapidly recover and in the case of isotope-labelled platelets most, though not all, of the platelet radioactivity returns to the peripheral blood. Platelets also undergo a temporary removal from the circulation in C6-deficient rabbits (in which intravascular lysis cannot take place). Here it seems that it is the coating of red cells by active C3 which results in immune adherence of platelets.

Similar experiments have been carried out with ^{51}Cr-labelled red cells sensitized with anti-I antibodies in vitro, exposed to C3- or C6-deficient serum, and then washed at 37°C. to remove the antibody before being injected back into rabbits. The cells exposed to C3-deficient serum and coated with C4 and C2 survived normally in C3-depleted rabbits, but most of the cells exposed to C6-deficient serum and coated with C3 left the circulation temporarily and were sequestered in the liver when injected into C6-deficient rabbits. There, histological examination showed some red cells phagocytosed within Kupffer cells; others were adherent to the surface of Kupffer cells, fixed, it is believed, as the result of C3–C3-receptor interaction. The subsequent return of most of these adherent cells to the circulation seems likely to be the result of inactivation of C3 by C3 inhibitor. Such cells are probably in much the same state as are the peripheral blood red cells of human patients who have the cold-haemagglutinin syndrome.

This lecture can be regarded only as a superficial account. I hope I have, nevertheless, succeeded in illustrating how a group of blood diseases which from the point of view of their effect in man can quite rightly be regarded as belonging to the province of clinical medicine, depends—as indeed does clinical medicine as a whole—for its real understanding on a deeper and deeper appreciation of the principles of pathology.

Only a few selected references have been given in the text of this paper. References to most of the relevant literature can be found in the monographs of Dacie (1962) and Pirofsky (1969).

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