Immune Suppression in Auto-Immune Hemolytic Anemia*

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The acquired auto-immune hemolytic anemias represent a diversity of disease states in which the most constant immunologic finding is a positive direct anti-human globulin test (van Loghem, 1965; Swisher et al, 1965). This is true of the symptomatic variety of acquired hemolytic anemia as well as the idiopathic form. The positive Coomb's test has been seen in association with primary atypical pneumonias, occasionally in favism, in some bacterial and drug induced hemolytic anemias, in patients with malignancies of the lymphoid tissues, and in collagen-vascular disorders—chiefly SLE.

The original hope that the direct Coomb's test would be negative in all forms of hereditary hemolytic disease and thus differentiate them from acquired forms of hemolytic anemia has not been borne out.

Acquired auto-immune hemolytic anemias vary widely in the severity of the hemolysis, in the association of additional pathologic states and in the comparative clinical influence of these factors. Those cases of auto-immune hemolytic anemia that are associated with self-limiting viral diseases such as infectious mononucleosis, measles or bacterial infections, usually subside with the primary disease. Forms of Coomb's positive hemolytic anemias associated with drug therapy can usually be eliminated by withholding the offending drug. In instances where the Coomb's positive hemolytic anemia is associated with benign or malignant tumors or cysts, surgical correction of the abnormality may result in permanent hematologic and serologic remission. It has also been observed that a positive Coomb's test may occur in association with pernicious anemia, folic acid deficiency and iron deficiency anemia. In these circumstances, replacement of the specifically deficient material may result in complete remission of the hematologic abnormality. This discussion will concern itself primarily with the idiopathic disease, since immunosuppression is not necessary or desirable in most of the symptomatic varieties.

Since the diagnosis of an auto-immune hemolytic anemia of the idiopathic variety is tied to the exclusion of recognizable causative disease, the frequency with which the diagnosis is made will first depend on the aggressiveness of the attending physician in looking for secondary causes and secondly, the nature of the patient population. Since the disease has a peak incidence in the fourth through seventh decades, a large pediatric population would diminish the incidence. Likewise, since about 60 percent of the patients in reported series are women, a largely male clinic or hospital population would have a similar effect. No racial discrepancies have been noted to date. In Dacie's series (1969) followed over a 20 year period, 111/210 or 52 percent adult patients with a positive direct Coomb's test were classified as idiopathic, whereas only 18.2 percent of Pirofsky's 234 cases were idiopathic (1969). A positive direct Coomb's test is, of course, central to the diagnosis of auto-immune hemolytic anemia although a positive direct Coomb's may be present for years with hemolysis.

The demonstration that the globulins coating the red cells are "true" antibodies is now generally accepted with the proviso that the coating of the red cells with a protein giving rise to a positive antiglobulin reaction does not necessarily mean that the reacting substance is an erythrocyte antibody. It may even be complement absorbed as a result of antibody activity. By performing a gamma globulin neutralization test one can elucidate whether the coating globulin of the erythrocyte is a gamma globulin, complement or mixed type. Further evaluation of the protein can be done with antiglobulin sera specific for IgG, IgM, or IgA. The great majority of auto-immune hemolytic anemias will have IgG antibody.

The cardinal points which identify the coating globulins as antibodies are their transferability to normal red cells; the frequent presence in serum of similar globulins which can be absorbed by normal cells; and in many instances, their ability to react with specifically identifiable red cell antigens. For instance, in patients who form warm antibodies, it is often possible to demonstrate a clear specificity for one or more Rh

antigens, but the nature of specificity of other antibodies which do not have affinity with Rh antibodies is yet unknown. It appears probable that an antibody reacting indifferently with all human cells may be involved. Although the etiology of the auto-immune hemolytic anemias remains obscure, current theory (Parker and Vavra, 1969) surmises that somatic mutation leads to the development of forbidden clones of antibody forming cells which are not susceptible to, and escape from, the normal homeostatic mechanisms which prevent auto-antibody formation. The association of auto-immune hemolytic anemias with other diseases thought to be of an auto-immune nature, such as disseminated lupus erythematosis and ulcerative colitis, lends unity to this concept. Chronic lymphocytic leukemia and lymphosarcoma may represent malignancies of potential antibody forming cells in which the tumor cells, in some instances, retain their ability to form antibodies, particularly abnormal ones which react with erythrocytes. Another possibility is that the antibodies primarily formed against the abnormal neoplastic lymphoid cells act as antigens; the antibodies which cross-react with red cells. It also seems conceivable that the malignant lymphomas represent an enormous monoclonal proliferation of lymphoid cells which displace the immunologically competent cells and interfere with the delicate balance between the two. This allows the development of a clone of auto-antibody forming cells. Whichever of these mechanisms ultimately proves to be true, the current therapy for auto-immune hemolytic anemias of a sufficient degree of severity to require treatment involves attempts at immunological manipulation.

Current Concept of the Immune Response

The current concept of the immune response (Fig 1) is divided into afferent, recognition, stimulatory and effector phases. All phases of this scheme can be manipulated to produce immunologic tolerance, as we shall see.

(1) In the afferent phase it is believed that the antigen is processed by macrophages into highly immunogenic complexes with RA and other cellular constituents.

(2) In the recognition phase the macrophage transmits messenger RNA to "antigen sensitive cells" which have the morphologic appearance of a small lymphocyte. These cells arise from stem cells in the marrow
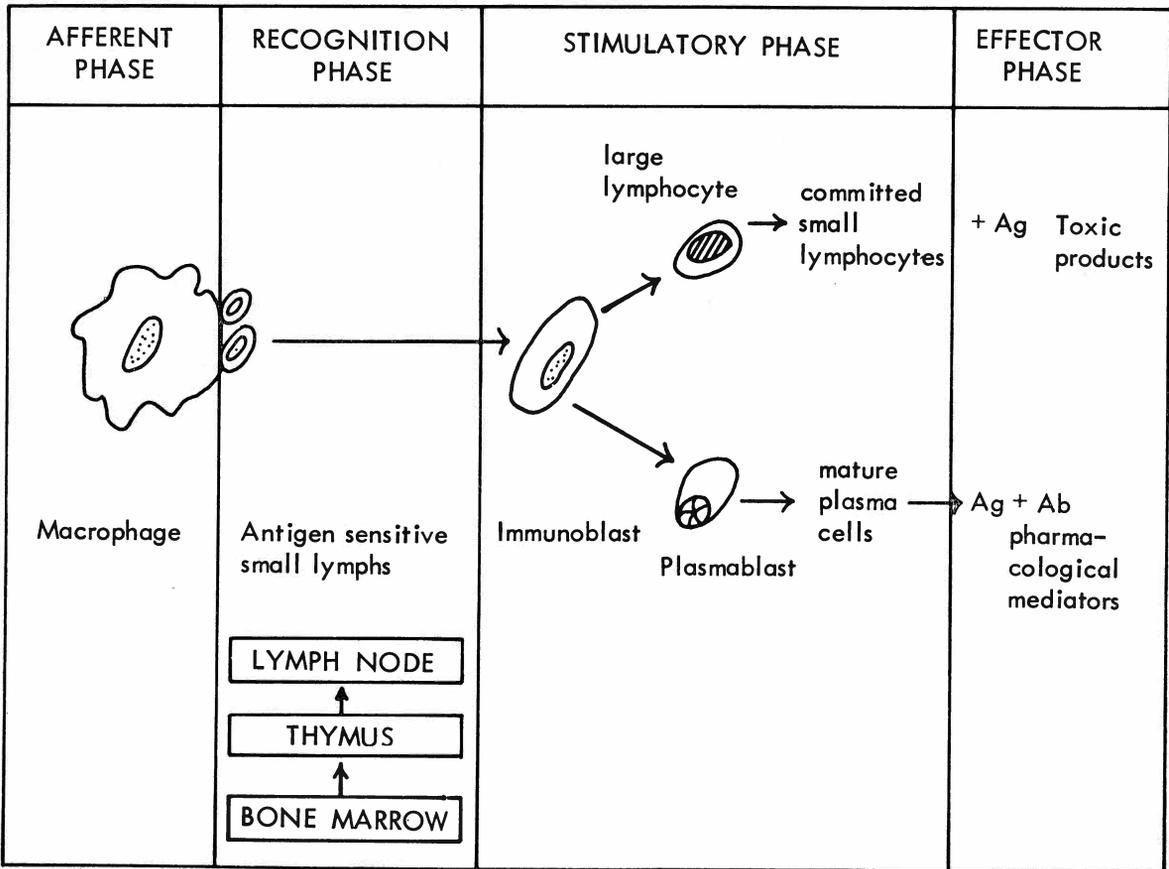
Fig 1—The four phases of the immune response (modified from Davis, et al; Microbiology, 1967).

where they are incapable of responding to antigen. Upon leaving the marrow they migrate to the thymus where partial maturation takes place and then seed peripheral lymphoid tissue where they acquire immunologic competence. Stimulation of these "antigen sensitive cells" appears to be confined to lymphoid organs. They do not in themselves produce antibody, but they give rise to clones of antibody producing cells as well as to additional antigen sensitive cells.

(3) In the stimulatory phase exposure to antigen initiates an immunologic response after a latent period of four to 24 hours. Mature antibody processing cells appear to arise from a large immunoblast (hemacytoblast) and reproduce rapidly. Serum antibody arises logarithmically and gradually declines. Small lymphocytes are also formed, committed to react specifically with antigen. They seem to be the primary mediators of cellular immunity and probably carry long term immunologic memory.

(4) In the effector phase the small lymphocytes are responsible for such forms of hypersensitivity as allograft rejection, and contact skin sensitivity.

Responses attributable to serum antibody include local and systemic anaphylaxis, Arthus phenomenon, hemolytic anemia and thrombopenia.

Approaches to Immunosuppression

In clinical immunosuppression the number of options available to the clinician is somewhat restricted by the clinical setting. Since the immune response in auto-immune hemolytic anemia is mediated by humoral antibody, attempts at immunosuppression should be so directed.

Table I shows the theoretical areas where immunosuppression might be possible in immuno hemolytic anemia. Administration of antigen might be considered as analogous to desensitization of patients with allergic asthma. Since the nature of the erythrocyte antigen is unknown, this approach does not seem feasible at present. Administration of specific antibody as a form of immunosuppression has received renewed interest as a result of the successful use of Rh antibody in the prophylaxis of erythroblastosis. It seems dubious that such an approach would prove successful in auto-immune hemolytic anemia for a variety of reasons.

I would like to discuss the types of immunosuppression which have been attempted in auto-immune hemolytic anemia, paying particular attention to those methods which are most commonly used in treatment today (Table II).

Surgical Ablation of Lymphoid Tissue

One of the four major approaches to immunosuppression has been that of surgical ablation. Splenectomy, of course, is the oldest form of immunological manipulation that has been attempted in autoimmune

TABLE I
Approaches to immunosuppression

1. Administration of antigen
2. Administration of specific antibody
3. Administration of antilymphocyte serum
4. Cytotoxic drugs
5. Radiation
6. Surgical ablation of lymphoid tissue

TABLE II
Approaches to immunosuppression in idiopathic auto-immune hemolytic anemia

I. Surgical ablation of lymphoid tissue
   A. Splenectomy
   B. Thymectomy
   C. Thoracic duct drainage
II. Administration of cytotoxic drugs
   A. Corticosteroids
   B. Alkylating agents
   C. Purine antagonists
III. Radiation
   A. Total body
   B. Local
      1. spleen
      2. Lymph nodes

hemolytic anemias. The rational behind its first application in 1911 by Michelle was a result of Banti's work incriminating the spleen as a primary site of blood destruction. The mechanistic concept of its function in auto-immune hemolytic anemia is probably still a prime reason for its removal; but it is included in the list of immunological manipulations because of the evidence showing splenic hyperplasia, particularly an increased production of lymphocytes and plasma cells, in the spleen. This suggests that hemolysis can potentiate the ability of the spleen to engage in antibody production. Jandl (1965) speculated that the enhanced immunologic reactivity of the hyperplastic spleen could take two pathways. In one it could initiate immunologic responses directed against related or coincidental antigens of the erythrocyte; in the other it could convert reactions which were intially nonimmune into immune forms. The latter mechanism could possibly permit metabolically modified antigenic determinants to be recognized as foreign. In this manner, it could supply the background to the auto-immune state described as an enhanced sensitivity of antibody forming tissues. Splenic hyperplasia creating these two antibody producing states could result in an immune relationship which is auto-catalytic and which would appear as an auto-immune hemolytic anemia.

The beneficial effects of splenectomy have been amply documented in several series reported since 1911. Therapeutic response has been noted in about 70 percent of the patients treated with half of the patients apparently being cured of their disease (Allgood and Chaplin, 1967). The mortality rate, however, of splenectomy in patients with auto-immune hemolytic disease is high and averages about 17 percent in the series reported. There is a further increase in mortality if splenectomy is delayed for over a year after the onset of the disease. This high surgical mortality rate has tempered the enthusiasm for splenectomy in most clinics.

In addition to the immediate surgical mortality of splenectomy, the predisposition of patients with auto-immune hemolytic anemia to thrombosis may be enhanced by the thrombocytosis occurring after the spleen is removed. In younger patients, such a thrombocytosis is usually well tolerated, but in the older age groups with co-existing atherosclerosis, it may constitute a major hazard to the patient's survival. The increased frequency of overwhelming infections in infancy following splenectomy seems to be real, but since auto-immune hemolytic anemia occurs very rarely in this age group, it is seldom a consideration. A more serious difficulty following splenectomy is the hyperplasia which sometimes occurs within the reticulo-endothelial cells in the liver leading to progressive hepatomegaly, liver dysfunction and death. Responses to splenectomy in patients with auto-immune hemolytic anemias secondary to reticulo-endothelial neoplasm seem to be somewhat less frequent than in the idiopathic form of disease. Splenectomy may be of value after patients have failed on corticosteroids, and Crosby's data suggest that the remission rates following splenectomy in patients who are unresponsive to steroids may be as good as those treated *de novo* with splenectomy. Several authors have emphasized criteria which may enable one to select candidates for splenectomy. Among these are the patients with warm acting, incomplete erythrocyte auto-antibodies and documentation of splenic sequestration. Several methods of determining significant splenic sequestration of Cr^{51} labeled red cells are in use including those devised by Korst (1955), Jandl (1956), and McCurdy and Roth (1958). All suffer the same defect, ie, they are not entirely reliable in predicting either response to splenectomy or failure of response.

Goldberg and his associates have suggested that a reduced Chromium 51-T ½ may be of importance in selecting candidates for splenectomy; this seemed to be the case in their series of 13 patients in whom 11 had good results. It is difficult to know what this means since all patients with auto-immune hemolytic anemia have reduced Cr^{51} red cell survival times and the response rate is what one would expect. Evidence of sequestration of red cells in the liver is a poor prognostic sign and suggests that splenectomy will offer little benefit.

Thymectomy is an attractive approach to immunosuppression in anto-immune hemolytic anemia because of the central role of the thymus in the maturation of immunologically competent cells. It is also technically easier than splenectomy and should carry a lower mortality rate. No data are available on adults treated in this manner, but in infants there have been two remissions induced with this procedure and one failure. Thymectomy appears to be of no value when thymoma is associated with auto-immune hemolytic anemia.

Thoracic duct drainage seems potentially useful because primary antigen sensitive cells and immunologic memory cells can be removed. Unfortunately, immunologic impairment is very short lived.

Cytotoxic Drugs

The administration of cytotoxic drugs is a second approach to immunosuppression. The immunosuppressive effects of corticosteroids resemble those of x-irradiation in that they produce lymphocytolysis particulary in the germinal centers of lymph nodes and spleen with resultant lymphocytopenia. The mechanism by which the cell damage is effected is unknown. They are also active in suppression of delayed hypersensitivity but their effect on serum antibody synthesis is less impressive.

Because of the high surgical mortality associated with splenectomy in auto-immune hemolytic disease, the observed lympholytic activity of adrenocorticoids was investigated in 1951 by Dameshek in auto-immune hemolytic anemias.

In a series reported in 1956, Dameshek and Komninos claimed that 90 percent of the cases of auto-immune hemolytic anemia would show an initial therapeutic response to corticosteroids. Of these, 65 percent were complete remissions and in an additional 25 percent a definite response was obtained. Two-thirds of the cases, however, relapsed when corticosteroids were discontinued. Subsequent series have shown similar results. Horster reported a 6.8 percent cure rate with corticosteroids, and remissions lasting over a year in about a third of the cases after discontinuation of corticosteroid therapy. About half of the patients could be maintained in remission only while corticosteroids were continued. Pirofsky's data suggests that the corticosteroids, too, are less effective in the auto-immune hemolytic anemias associated with reticulo-endothelial malignancy. The dosage of corticosteroids necessary to induce remission has been variable in the series reported but in the average adult, 300 mg of cortisone per day or it's equilvalent of prednisone, triamcinolone, or dexamethasone, appear to be an effective dose level. If using corticosteroids is effective, time of onset of the evidence for reduced

hemolysis is rapid. Response is usually clearly apparent within the first one to two weeks, although a few patients will respond as long as 21 days after therapy is begun. In the patients who respond slowly, the response tends to be less good and it is difficult to remove such patients from steroid coverage.

Hematologic remission may occur while the direct anti-globulin tests remain positive. In idiopathic auto-immune hemolytic anemias, there may be reduction in spleen size, although this effect is not as marked in the symptomatic varieties of the disease. Since the side-effects of steroids are not inconsiderable, reductions from the high dose levels initially used, should be made as soon as possible, and the minimally effective dose of corticosteroid should be used as the maintenance dose. Even with maintenance, a significant number of relapses will occur within the first six months, although the relapse is usually of gradual onset. If the patient is being seen at frequent intervals, there is normally sufficient warning to increase the amount of steroid. In a few patients, prednisone may gradually be discontinued over a period of five or six months and the patients will remain in remission.

Alkylating agents include such compounds as the nitrogen mustards, sulfur mustards, sulfonate esters and ethylenimines. They are chemically highly reactive and are capable of combining irreversibly with DNA, proteins and other essential macromolecules in the cell.

Cyclophosphamide, a latent compound activated *in vivo* after tissue phosphorylases cleave the cyclic phosphamide moiety to expose the alkylating radicals, has been shown to be a very promising immunosuppressive agent. It appears to be capable of reducing the antigen sensitive cell population, blocking cellular proliferation during the inductive phase and is ever active in reducing antibody synthesis. Hersh found it to be the most effective immunosuppressive agent among the various alkylating agents. I am unaware of any published reports on its effectiveness in auto-immune hemolytic anemias, although there are isolated reports of remission induced by other alkylating agents (Taylor, 1963).

Purine and pyrimidine antagonists are another approach included among the cytotoxic drugs. In 1957, Stertzle and Holub suggested that 6-Mercapto-purine (6-MP) might interfere with antibody synthesis, but were unable to confirm it in the test system that they were using. This was independently confirmed in extensive studies by Schwartz and Dameshek (Schwartz and Dameshek, 1959; Dameshek and Schwartz, 1960; Schwartz, Eisner and Dameshek, 1959; Schwartz and Dameshek, 1960) who demonstrated that 6-MP could suppress antibody formation in rabbits immunized with Bovine albumin. In addition, 6-MP apparently could induce a state of immunologic unresponsiveness in adult rabbits and could suppress transplantation rejec-

tion of skin graft although not completely at tolerated doses. Further studies on the mechanism by which 6-MP was capable of inducing this effect led to the conclusion that the drug had its primary effect on the lymphoid hemocytoblast (immunoblast) and morphologic studies of lymph nodes subsequent to 6-MP after a homograft administration revealed extensive disruption of the lymphoid follicles (Andre et al, 1962). Borel also showed suppression of IgG response with prolonged administration. Studies were extended (Schwartz and Dameshek, 1962) to include cases of human auto-immune hemolytic anemias treated with 6-MP or Thioguanine with a favorable effect in three of six cases. 6-MP and thioguanine have been sporadically reported since (Demis, Brown and Crosby, 1964; Shearn, 1965; Hitzig and Massimo, 1966) to cause remissions in auto-immune hemolytic anemia. The primary limiting factors to the administration of these agents is bone marrow toxicity (Demis, Brown and Crosby, 1964). Azothiaprine (an imidazole subsituted 6-MP) is converted to 6-MP *in vivo*. It has been used in the auto-immune hemolytic anemias alone and in combination with corticosteroids with some success. Its spectrum of immunosuppression is similar to 6-MP but it appears to have a better therapeutic ratio (Frisch and Davis, 1962; Frisch, Davis and Milstein, 1962) and produces less bone marrow suppression. It also produces fewer G.I. symptoms when given orally.

It seems probable that these agents do not show cross resistance with corticosteroids, and failure on the latter does not necessarily militate against induction of remission with the former. Response rates are difficult to access on the basis of the limited number of cases reported, but the drugs are somewhat less effective than corticosteroids. This may merely reflect the fact that the patients are further advanced in their disease.

Fig 2 shows the clinical course of a 79-year old white man, who was first seen here in August 1964 for treatment of progressive anemia of four months' duration. Physical examination was essentially unremarkable. His laboratory data revealed a hemoglobin of 7.8 gm percent, reticulocyte count 29 percent, MCV 96, MCH 32, MCHC 34, and Bilirubin 2.2 mg percent with 0.3 mg percent direct reacting fraction. Coomb's test was weakly positive. Cold agglutinins were positive in a titre of 1:64. Chromium[51] red cell survival was 7 ½ days, and the spleen to liver ratio was 0.72. This patient was started on prednisone 40 mgs per day with a prompt rise in his hemoglobin concentration, as shown in Fig 2. His hemoglobin was maintained well until steroid toxicity forced the reduction of his dose of prednisone. Three months later his hemoglobin gradually fell to levels of seven to eight grams percent. He required an interim hospitalization in September 1966, at which time his

Fig 2—The clinical course of a 79-year old white man being treated for progressive anemia.

hemoglobin was 8.5 gm percent, reticulocyte count was 40 percent, cold agglutinins were positive at a titre of 1:1, 280, and Coomb's was strongly positive. He was seen again in consultation and the recommendation was made that he be offered splenectomy. Because of his age and the patient's wishes this was not done, and he was instead started on 6-MP in a dose of 25 mg per day, which was continued uninterruptedly until the time of his terminal hospitalization in August 1969. We have only random blood counts available for this period of almost five years, because he was being followed elsewhere. He was admitted in August 1969 because of massive G.I. bleeding. At that time his hemoglobin concentration was 5.5 gm percent with a reticulocyte count of 16 percent. Prednisone, which had been continued at a low dose level of 5 mg per day was increased because of the uncertainty as to whether the marked fall in his hemoglobin concentration was the result of bleeding or an accelerated rate of hemolysis. His upper G.I. series was normal. His barium enema revealed some diverticula in the colon. His Coomb's test was strongly positive

and his cold agglutinin titre was too high to read. Serum protein electrophoretic pattern was essentially normal, except for a questionable blip in the slow gamma region. Immunoelectrophoretic assay of his serum revealed an IgM level of 1,168. IgG and IgA levels were normal. He was started on leukeran, 12 mg per day on September 11, 1969, which was continued through October 5, 1969, when it had to be discontinued because of leukopenia of moderate degree. His G.I. bleeding continued throughout his hospital illness, and he was transfused several times when his hemoglobin concentration fell to dangerous levels. It was not apparent, in this particular patient, who had what we would classify as cold agglutinin disease, that his immunologic process was altered either by the long-term administration of small doses of 6-MP or by the relatively aggressive therapy with chlorambucil at the time of his terminal illness.

Radiation

The primary effect of total body irradiation appears to be on DNA of lymphoid and other sensitive cells.

Unfortunately, when used alone, high doses are required which are lethal in themselves. At present there seems to be no place for total body irradiation in the clinical context of auto-immune hemolytic anemias.

Local irradiation has proven to be a more effective approach to immunosuppression. Since splenectomy is an efficient form of treatment for autoimmune hemolytic anemia it has seemed natural to try to circumvent the surgical mortality by splenic irradiation. Results have generally been poor. However, some transient responses have been obtained in secondary forms of auto-immune hemolytic anemias, particularly those associated with malignancy of the reticuloendothelial tissues.

As far as the lymph node is concerned, nodal irradiation seems to offer little promise in auto-immune hemolytic anemia but there are scattered reports by Wasserman, Brown, Eisner (Eisner, Ley and Mayer, 1967), and others of a beneficial effect on symptomatic hemolytic anemias from Hodgkin's disease to radiation of local tumor masses.

Administration of Anti-Lymphocyte Serum

Anti-lymphocyte serum has been shown to be of value in the treatment of the allograft rejection response but its primary effect seems to be in suppressing cellular immunity rather than humoral. There is some evidence that it may abolish immunologic memory and thus might be of value in auto-immune hemolytic anemia. We are unaware of any attempts to use the material in the clinical setting of auto-immune hemolytic anemia.

Prognosis

The outlook in auto-immune hemolytic anemia is unpredictable but a large number of the patients can be controlled by judicious therapy with corticosteroids. Despite the improvement in mortality rates since the advent of steroids (46 percent Dacie's series) the mortality rates are still high—31 percent Dausset and Columbani and 28.2 percent Allgood. If the disease cannot be controlled with low doses of steroid which produce minimal toxicity, splenectomy should be done. The time at which this should be performed will require individualization for each patient but, in general, a failure of corticosteroid therapy at acceptable levels of toxicity will require splenectomy at four to six months.

If splenectomy fails to halt the immune process or cannot be done because of the patient's poor condition, a trial of other immunosuppressive agents may be attempted or thymectomy may be resorted to. Information at present is not firm enough to recommend a specific line of approach. On the basis of the experimental data, drug therapy with either cyclophosphamide or azothiaprine would be my personal choice.

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