Significance of a Positive DAT

The direct antiglobulin test (DAT) is generally used to determine if red cells have been coated, in vivo, with immunoglobulin, complement, or both. A positive DAT, with or without shortened red cell survival, may result from:

1. Autoantibodies to intrinsic red cell antigens.
2. Alloantibodies in a recipient’s circulation, reacting with antigens on recently transfused donor red cells.
3. Alloantibodies in donor plasma, plasma derivatives, or blood fractions that react with antigens on the red cells of a transfusion recipient.
4. Alloantibodies in maternal circulation that cross the placenta and coat fetal red cells.
5. Antibodies directed against certain drugs that bind to red cell membranes (eg, penicillin).
6. Adsorbed proteins, including immunoglobulins, that attach to abnormal membranes or red cells modified by therapy with certain drugs, notably those of the cephalosporin group.
7. Complement components or, rarely, IgG bound to red cells after the administration of drugs such as quinidine and phenacetin has induced a drug/anti-drug interaction.
8. Nonantibody immunoglobulins associated with red cells in patients with hypergammaglobulinemia or recipients of high-dose intravenous gammaglobulin.

A positive DAT does not necessarily mean that a person’s red cells have a shortened survival. Small amounts of both IgG and complement appear to be present on all red cells. A range of 5-90 molecules of IgG/red cell and 5-97 C3d molecules/red cell (possibly up to 500 or more) appears to be normal on the cells of healthy individuals.

The DAT, as routinely performed, can detect a level of 100-500 molecules of IgG/red cell and 400-1100 molecules of...
C3d/red cell, although newer reagents and more sensitive test methods may detect lower levels of these proteins. As many as 15% of hospital patients, and between 1 in 1000 and 1 in 14,000 blood donors, have a positive DAT without clinical manifestations of immune-mediated red cell destruction.

Most blood donors with positive DATs appear to be perfectly healthy, although some may show signs of increased cell destruction or develop autoimmune hemolytic anemia (AIHA) over time. Elevated levels of IgG or complement have been noted on the red cells of patients with sickle cell disease, β-thalassemia, renal disease, multiple myeloma, autoimmune disorders (including systemic lupus erythematosus), AIDS, and other diseases with elevated serum globulin or blood urea nitrogen (BUN) levels with no clear correlation between positive DAT and anemia. Interpretation of positive DATs must include the patient's history, clinical data, and the results of other laboratory tests.

The Direct Antiglobulin Test

The principles of the DAT are discussed in Chapter 11. Although any red cells may be tested, anticoagulated samples are preferred to avoid in-vitro uptake of complement; EDTA is the anticoagulant most frequently recommended. If red cells from a clotted blood sample have a positive DAT, the results should be confirmed on cells from a freshly collected EDTA-anticoagulated specimen.

Most DATs are initially performed with a polyspecific reagent capable of detecting both IgG and C3d (see Method 3.6). If positive, tests with more specific anti-IgG and anti-complement reagents may be appropriate. Occasionally, polyspecific antiglobulin reagents react with cell-bound proteins other than IgG or C3d (eg, IgM, IgA or other complement components); specific reagents to distinguish these proteins are not readily available in most laboratories.

The Pretransfusion DAT and the Autologous Control

Neither the AABB Standards for Blood Banks and Transfusion Services nor any other accrediting agency requires a DAT or an autologous control (autocontrol) as part of pretransfusion testing, but many workers advocate this practice. Several studies, however, have shown that eliminating the DAT/autocontrol portion of routine pretransfusion testing carries minimal risk.

Evaluation of a Positive DAT

Extent of Testing

Clinical considerations should dictate the extent to which a positive DAT is evaluated. Dialogue with the attending physician is important. Interpretation of the significance of serologic findings requires knowledge of the patient's diagnosis; recent drug, pregnancy, and transfusion history; and information on the presence of acquired or unexplained hemolytic anemia. The results of serologic tests are not diagnostic; their significance must be assessed in conjunction with clinical information and such laboratory data as hematocrit, bilirubin, haptoglobin, and reticulocyte count.

Answers to the following questions may help decide what investigations are appropriate:

1. Is there any evidence of in-vivo red cell destruction?

Reticulocytosis, hemoglobinemia, hemoglobinuria, decreased serum haptoglobin, and elevated levels of serum unconjugated bilirubin or lactate dehydrogenase (LD), especially LD1, may be associated with increased red cell de-
struction. If an anemic patient with a positive DAT does show evidence of hemolysis, a workup to evaluate a possible immune etiology is appropriate. IF THERE IS NO EVIDENCE OF INCREASED RED CELL DESTRUCTION, NO FURTHER STUDIES ARE NECESSARY, unless the patient needs transfusion and the serum contains incompletely identified unexpected antibodies to red cell antigens.

2. Has the patient been recently transfused?

Many workers routinely attempt to determine the cause of a positive DAT when the patient has received transfusions within the previous 3 months, because the first indication of a developing immune response may be the attachment of antibody to recently transfused red cells. Mixed-field reactivity is the classical observation but may be difficult to observe. Antibody may appear as early as 7-10 days after transfusion in primary immunization and within 2-7 days in a secondary response; these alloantibodies could shorten the survival of cells already transfused or given in later transfusions.

Antibodies coating the patient’s red cells and/or the transfused red cells may also be present in serum. Elution done to evaluate a positive DAT often concentrates antibody activity and may facilitate identification of weakly reactive serum antibodies. If the serum contains multiple antibodies, the eluate may contain only one or a few, and this may clarify antibody identification.

If a non-group O patient has received plasma containing anti-A or anti-B (as in transfusion of group O platelets), ABO antibodies may be detected on the recipient’s red cells without necessarily implying significantly accelerated red cell destruction. If, however, the recipient of incompatible plasma does appear to have immune hemolysis, the eluate can, initially, be tested against A and/or B cells; if predicted ABO antibodies are not detected, other causes of the positive DAT should be sought.

Intravenous immunoglobulin (IVIG) may contain ABO antibodies, anti-D or, sometimes, other antibodies. Significant red cell destruction due to passive transfer of antibodies from IVIG is rare.

3. Is the patient receiving any drugs, such as procaainamide, α-methyl-dopa, or intravenous penicillin?

Approximately 3% of patients receiving intravenous penicillin, usually at very high doses, and 15-20% of patients receiving α-methyldopa will develop a positive DAT, but fewer than 1% of those patients who develop a positive DAT have hemolytic anemia. Procaainamide therapy is also associated with positive DATs. Cephalosporins also are associated with positive DATs but less often with immune red cell destruction. Positive DATs associated with other drugs are rare. If a positive DAT is found in a patient receiving such drugs, the attending physician should be alerted so that appropriate surveillance for red cell destruction can be maintained. If red cell survival is not shortened, no further studies are necessary.

4. Has the patient received a bone marrow or organ transplant?

Passenger lymphocytes of donor origin produce antibodies directed against ABO or other antigens on the recipient’s cells, causing a positive DAT.

5. Is the patient receiving antilymphocyte globulin (ALG) or antithymocyte globulin (ATG)?

Patients receiving ALG or ATG of animal origin may develop a positive DAT within a few days, apparently related to high-titer heterophile antibodies in these products and the presence of corresponding antibodies in animal-derived antihuman globulin sera. Free, unbound ALG/ATG in the circulation may cause positive results when the patient’s serum is used in indirect antiglobulin
testing. Problems due to heterophile antibodies can usually be circumvented by neutralization or by use of the low ionic polycation technique (see Method 3.2.5).

**Serologic Studies**

Three investigative approaches are helpful in the evaluation of a positive DAT:

1. Test the DAT-positive cells with anti-IgG and anti-C3d antoglobulin reagents to characterize the types of proteins coating the red cells.
2. Test the serum/plasma to detect and identify clinically significant antibodies to red cell antigens.
3. Test an eluate (see Methods 5.1-5.6) prepared from the coated red cells against a panel of antigen-typed red cells to define whether the coating protein has alloantibody activity. When the only coating protein is complement, eluates are frequently nonreactive.

Results of these tests, combined with the patient’s history and clinical data, should assist in classification of the problems involved.

**Elution**

Elution frees antibody from sensitized red cells and recovers it in a usable form. Details of eluate preparation are given in Chapter 16 and in Methods 5.1-5.6. Table 18-1 lists the advantages and disadvantages of several common elution methods; no single elution method is ideal in

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat (56 C)</td>
<td>Good for ABO-HDN; quick and easy method</td>
<td>Poor recovery of other blood group allo- and autoantibodies</td>
</tr>
<tr>
<td>Freeze-Thaw</td>
<td>Good for ABO-HDN; quick and easy method; requires small volume of cells</td>
<td>Poor recovery of other blood group allo- and autoantibodies</td>
</tr>
<tr>
<td>Cold Acid</td>
<td>Quick and easy method; sensitivity comparable to digitonin-acid</td>
<td>Less sensitive for warm auto- and alloantibodies</td>
</tr>
<tr>
<td>Digitonin Acid</td>
<td>Nonhazardous; good recovery of most antibodies; available in commercial kit</td>
<td>Time-consuming washing of stroma; less sensitive for Kidd antibodies</td>
</tr>
<tr>
<td>Dichloromethane (DCM)</td>
<td>Good for anti-K; nonflammable</td>
<td>Toxic; poor recovery of anti-Fy^b</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Rapid method; nonflammable; recovers most blood group antibodies</td>
<td>Carcinogenic, toxic, narcotic; less sensitive than xylene or ether</td>
</tr>
<tr>
<td>Xylene</td>
<td>Recovers most blood group antibodies</td>
<td>Flammable, carcinogenic, toxic, narcotic; hemolysis of test cells if residual xylene not removed</td>
</tr>
<tr>
<td>Ether</td>
<td>Excellent recovery of most blood group antibodies</td>
<td>Highly flammable, explosive, toxic, narcotic; requires special storage; poor recovery of anti-S, -s</td>
</tr>
</tbody>
</table>

*Modified from Judd and Sigmund.*

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all situations. Although many elution methods damage or destroy the red cells, certain techniques (see Methods 2.11, 2.13, 2.14) remove antibody but leave the cells sufficiently intact that they can be typed for various antigens or used for adsorption purposes. Some antigens may be altered by elution, however, and appropriate controls are essential.

When the cause of the positive DAT is unknown, an eluate may be prepared and tested against a panel of red cells. In cases of hemolytic disease of the newborn (HDN) or hemolytic transfusion reactions, specific antibody (or antibodies) is usually detected in the eluate. Usually the same specificity can be detected in the patient’s (or, in HDN, the mother’s) serum, although eluates may help in antibody identification when serum reactions are weak. When the eluate reacts with all cells tested, autoantibody is the most likely explanation, especially if the patient has not been recently transfused. WHEN NO UNEXPECTED ANTIBODIES ARE PRESENT IN THE SERUM, AND IF THE PATIENT HAS NOT BEEN RECENTLY TRANSFUSED, NO FURTHER SEROLOGIC TESTING OF AN ISOLATED AUTOANTIBODY IS NECESSARY.

Sometimes no reactivity is detected in the eluate, despite reactivity of the cells with specific anti-IgG. The cause may be that the eluate was not tested against cells positive for the corresponding antigen, notably group A or group B cells. Antigens of low incidence are also absent from most reagent cell panels. It may be appropriate to test the eluate against red cells from recently transfused donor units, which could have stimulated an alloantibody to a rare antigen, or, in HDN, against cells from the father, from whom the infant may have inherited a rare gene.

Reactivity of eluates can be enhanced by testing against enzyme-treated cells or by the use of solid-phase or other enhancement techniques, such as polyethylene glycol (PEG). Antibody reactivity can be increased by the use of a concentrated eluate, either by alteration of the fluid-to-cell ratio or by use of commercial concentration devices. Washing the red cells with low ionic strength saline (LISS), instead of normal saline, may prevent the loss of antibody while the cells are being prepared for elution.

Certain elution methods give poor results with certain antibodies. When eluates are nonreactive yet clinical signs of red cell destruction are present, elution by a different method may be helpful. If both serum and eluate are nonreactive at all test phases, and if the patient has received high-dose intravenous penicillin or other drug therapy, testing to demonstrate drug-related antibodies should be considered. Patients may have a positive DAT and nonreactive eluate with no evidence of hemolysis, and exhaustive pursuit of an explanation is not usually indicated.

Immune-Mediated Hemolysis

Immune-mediated hemolysis (immune hemolysis) is the shortening of red cell survival by the product(s) of an immune response. If bone marrow compensation is adequate, the reduced red cell survival may not result in anemia. Immune hemolysis is only one cause of anemia, and many causes of hemolysis are unrelated to immune reactions. The serologic investigations carried out in the blood bank do not determine whether a patient has a “hemolytic” anemia. The diagnosis of hemolytic anemia rests on clinical findings and such laboratory data as hemoglobin or hematocrit values; reticulocyte count; red cell morphology; bilirubin, haptoglobin, and LD levels; and, sometimes, red cell survival.
studies. The serologic findings help determine whether the hemolysis has an immune basis and, if so, what type of immune hemolytic anemia is present. This is important since the treatment for each type is different.

The terms hemolysis and hemolytic, frequently used to indicate both intravascular and extravascular red cell destruction, may be misleading. As a description of in-vitro antibody reactivity, hemolysis—lysis of the red cells with release of free hemoglobin to the surrounding media—is both obvious and rare. In-vivo lysis of cells and release of free hemoglobin within the intravascular compartment is uncommon and, often, dramatic. Most immune red cell destruction occurs extravascularly, with little or no escaping hemoglobin, even in the immune “hemolytic anemias.”

Immune hemolytic anemias can be classified in various ways. One classification system is shown in Table 18-2. Autoimmune hemolytic anemias (AIHAs) are subdivided into four major types: warm antibody AIHA (WAIHA), cold agglutinin syndrome (CAS), mixed-type AIHA, and paroxysmal cold hemoglobinuria (PCH). Not all cases fit neatly

### Table 18-2. Classification of Immune Hemolytic Anemias

<table>
<thead>
<tr>
<th>Autoimmune Hemolytic Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Warm Autoimmune Hemolytic Anemia</td>
</tr>
<tr>
<td>a. primary (idiopathic)</td>
</tr>
<tr>
<td>b. secondary (to such conditions as lymphoma, systemic lupus erythematosus (SLE), carcinoma, or to drug therapy)</td>
</tr>
<tr>
<td>2. Cold Agglutinin Syndrome</td>
</tr>
<tr>
<td>a. primary (idiopathic)</td>
</tr>
<tr>
<td>b. secondary (to such conditions as lymphoma, mycoplasma pneumonia, infectious mononucleosis)</td>
</tr>
<tr>
<td>3. Mixed-Type AIHA</td>
</tr>
<tr>
<td>a. primary (idiopathic)</td>
</tr>
<tr>
<td>b. secondary (to such conditions as SLE, lymphoma)</td>
</tr>
<tr>
<td>4. Paroxysmal Cold Hemoglobinuria</td>
</tr>
<tr>
<td>a. primary (idiopathic)</td>
</tr>
<tr>
<td>b. secondary (to such conditions as syphilis, viral infections)</td>
</tr>
<tr>
<td>5. DAT-Negative AIHA</td>
</tr>
<tr>
<td>a. primary (idiopathic)</td>
</tr>
<tr>
<td>b. secondary (to such conditions as lymphoma, SLE)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug-Induced Hemolytic Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hemolytic disease of the newborn</td>
</tr>
<tr>
<td>2. Hemolytic transfusion reaction</td>
</tr>
</tbody>
</table>
into these categories. Immune hemolysis may also be induced by drugs (discussed in a later section of this chapter). The prevalence of each type can vary depending on the patient population studied.4-11 (See Table 18-3.)

Findings in a DAT performed with anti-IgG and anti-C3d may reduce the diagnostic possibilities; eg, the DAT in CAS is almost always positive using anti-C3d and negative using anti-IgG. Table 18-4 summarizes the expected DAT findings in the various autoimmune hemolytic anemias.

DATs performed with IgG- and C3-specific antiglobulin reagents, as well as the serum and eluate studies described earlier, can be used to classify AIHAs into one of the four major types (WAIHA, CAS, mixed-type AIHA, PCH). Two additional procedures may be useful: a diagnostic cold agglutinin titer (Method 6.2) and the Donath-Landsteiner test for PCH (Method 6.7). Table 18-4 summarizes the expected serologic results with the major types of AIHA.

The binding of antibody to red cells does not, in itself, damage the cells. It is the phenomena that the bound antibody-antigen complex promotes that may eventually damage cells. These include complement binding, adherence to Fc receptors on macrophages and monocytes, phagocytosis, and cytotoxic lysis. The IgG subclass of bound antibody may be significant. IgG1 is the subclass most commonly found, sometimes alone but often in combination with other subclasses. The other IgG subclasses occur more often in combination with other subclasses than alone. In general, IgG3 antibodies have the most distinctive effects, followed by IgG1. IgG2 antibodies are associated with less destruction and IgG4 with no or little destruction. Some monoclonal antiglobulin reagents may not detect IgG4.

The number of antibody molecules on the cells of apparently healthy blood donors with positive DATs (<1000, weakly positive) is far less than that usually seen in patients with AIHA.4 Some patients with apparent immune hemolysis may have negative findings on a routine DAT.

**Warm Antibody Autoimmune Hemolytic Anemia**

The most common type of AIHA is associated with warm-reactive (37°C) antibodies. Typical serologic findings are described below.

**DAT**

When IgG-specific and complement-specific antiglobulin reagents are used, three patterns of reactivity may be found: coating with IgG alone, with complement alone, or with both. In approximately 1% of cases the DAT will be positive with a polyspecific antihuman globulin reagent but negative with IgG- and complement-specific antiglobulin reagents. Some of these may be due to attachment of IgM or IgA alone.

**Serum**

Autoantibody in the serum typically is IgG and reacts by indirect antiglobulin testing against all cells tested. If the autoantibody has a high binding constant and has been adsorbed by the patient’s red cells in vivo, the serum may contain very little free antibody. The serum will contain antibody after all the specific antigen sites on the red cells have been occupied and no more antibody can be bound in vivo. The DAT in such cases is usually strongly positive. Approximately 50% of patients with WAIHA have serum antibodies that react with untreated red cells. With such methods as tests with PEG, enzyme-treated red cells, or solid phase methods,
over 90% of these sera can be shown to contain autoantibody. Autoantibodies that hemolyze untreated red cells in vitro at 37 C are rare but, if present, may be associated with significant in-vivo destruction of autologous and transfused cells. Approximately 15% of sera contain antibodies that hemolyze enzyme-treated red cells at 37 C. Approximately one-third of patients with warm-antibody AIHA have cold-reactive autoagglutinins demonstrable in tests at 20 C, but cold agglutinin titers at 4 C are normal; this does not necessarily mean the patient has CAS in addition to WAIHA (see mixed-type AIHA below).
Eluate

The presence of the IgG autoantibody on the red cells can be confirmed by elution (see Methods 5.1-5.6). Typically the eluate reacts with virtually all cells tested, with reactivity enhanced in tests against enzyme-treated cells or when PEG is used, but occasionally IgG is present at such low levels that the eluate is nonreactive. The eluate will usually have no serologic activity if the only protein coating the red cells are complement components. Occasionally antibody not detected by the DAT will be detected in the eluate, possibly due to the concentrating effect of eluate preparation.

Specificity of Autoantibody

The specificity of autoantibodies associated with WAIHA appears very complex. In routine tests, all cells tested are usually reactive. Some autoantibodies that have weaker or negative reactivity with cells of rare Rh phenotypes such as −D−, or Rhnull appear to have broad specificity in the Rh system. Apparent specificity for simple Rh antigens (D, C, E, c, e) is occasionally seen, either as the sole autoantibody or as a predominant portion, based on stronger reactivity with cells of certain phenotypes. Such reactivity is often termed a “relative” specificity. Such relative specificity in a serum may be mistaken for alloantibody, but cells negative for the apparent target antigen can adsorb and remove the “mimicking” specificity.

**Unusual Specificities**. Apart from Rh specificity, warm autoantibodies with many other specificities have been reported. When autoantibody specificity is to antigens in the Kell system (and perhaps others), the patient’s red cells may exhibit depressed expression of the antigen and the DAT may be negative (see below). Dilution and selective adsorption of eluates may uncover specificity or relative specificity of autoantibodies.

It has also been suggested that warm-reactive autoantibodies may be divided into those that react preferentially with older cells and those that react with both older and reticulocyte-enriched populations. This theory has not been proven, but, if true, suggests that antibody to senescent red cell antigen may be involved.

### Table 18-4. DAT Results in AIHA Using Anti-IgG and Anti-C3 Reagents

<table>
<thead>
<tr>
<th></th>
<th>WAIHA</th>
<th>CAS</th>
<th>Mixed-Type AIHA</th>
<th>PCH</th>
<th>Drug-Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>355</td>
<td>275</td>
<td>61</td>
<td>17</td>
<td>157</td>
</tr>
<tr>
<td>IgG only†</td>
<td>67%</td>
<td>1%</td>
<td>18%</td>
<td>0</td>
<td>94%</td>
</tr>
<tr>
<td>IgG + C3†</td>
<td>24%</td>
<td>1%</td>
<td>71%</td>
<td>0</td>
<td>6%</td>
</tr>
<tr>
<td>C3 only†</td>
<td>7%</td>
<td>91%</td>
<td>9.8%</td>
<td>94%</td>
<td>0</td>
</tr>
<tr>
<td>Polyspecific-positive, monospecific-negative‡</td>
<td>1%</td>
<td>0.7%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DAT-negative</td>
<td>1%</td>
<td>6.3%</td>
<td>1.2%</td>
<td>6%</td>
<td>0</td>
</tr>
</tbody>
</table>

*Patients had both cold hemagglutinating autoantibodies reactive to 30 C or higher and warm-reactive autoantibodies.
†With or without IgM and/or IgA.
‡Polyspecific antihuman globulin gives a positive result but monospecific anti-IgG and anti-C3 reagents are negative.
Practical Significance. Tests against cells of rare phenotype and by special techniques have limited clinical application. In rare instances of WAIHA involving IgM agglutinins, determining autoantibody specificity may help differentiate such cases from typical CAS. It is rarely, if ever, necessary to ascertain autoantibody specificity in order to select antigen-negative blood for transfusion. If apparent specificity is directed to a high-incidence antigen (eg, anti-U), or when the autoantibody reacts with all red cells except those of a rare Rh phenotype (eg, –D–, Rhnull), compatible donor blood is unlikely to be available and there is little point in determining specificity. Such blood, if available, should be reserved for alloimmunized patients of that uncommon phenotype. In rare cases in which patients respond very poorly to infusion of “incompatible” red cells, it may be appropriate to consider using red cells lacking the Rh or high-incidence antigens.

Transfusion-Stimulated Autoantibodies. Transfusion itself may lead to the production of autoantibodies, which may persist and cause positive DATs for some time after transfusion yet not cause obvious red cell destruction. Such cell-bound autoantibodies sometimes display blood group specificity (eg, E, K, Jk*), but persist long after transfused red cells should have disappeared from the circulation, apparently adsorbed to the patient’s own antigen-negative red cells.

Transfusion of Patients with Warm-Reactive Autoantibodies

Inherent Risks. Patients with warm-reactive autoantibodies range from those with no apparent shortening of red cell life span to those with life-threatening anemia. Patients with little or no evidence of significant in-vivo red cell destruction often tolerate transfusion quite well, if required for reasons unrelated to the autoantibody. In general, however, transfusion of patients with WAIHA carries a greater-than-normal risk; even when autoantibody is not demonstrable in the serum, transfused blood is not truly compatible and effects of the transfusion are likely to be relatively short-lived.

When autoantibody is active in serum, it will be difficult to exclude the presence of alloantibodies, which increases the risk of adverse reaction. Transfusion may stimulate alloantibody production, complicating subsequent transfusions. Transfusion may intensify the autoantibody, inducing or increasing hemolysis and making serologic testing more difficult. Transfusion may depress compensatory erythropoiesis; destruction of transfused cells may increase hemoglobinemia and hemoglobinuria. In patients with active hemolysis, transfused red cells may be destroyed more rapidly than the patient’s own red cells. In rare cases this may promote hypercoagulability and disseminated intravascular coagulation. Transfusion reactions, if they occur, may be difficult to investigate.

Alternatives to Transfusion. Given the inherent risks of transfusion, alternative treatments should be considered. Corticosteroids, which inhibit antibody production and may also inhibit release of lysosomal enzymes, are generally considered a primary therapy. Marked in-vivo hemolysis may require intravenous administration of high doses of steroid. Intravenous immunoglobulin or immunosuppressive drugs such as cyclophosphamide may benefit some patients with autoantibody. Splenectomy is sometimes effective, but is usually considered only when other therapies are ineffective or contraindicated. In cases of severe hemolysis, plasma exchange or treatment with complement-inhibiting substances (eg, heparin) have been suggested, although without supportive
documentation. Filtration of the plasma through immunoadsorption columns (eg, containing protein A) may temporarily reduce the level of plasma IgG.

**Transfusion in WAIHA.** Transfusion is especially problematic for patients with rapid in-vivo hemolysis, who may present with a very low hemoglobin level and hypotension. Reticulocytopenia may accompany a rapidly falling hematocrit, and the patient may exhibit coronary insufficiency, congestive heart failure, cardiac decompensation, or neurologic impairment. Under these circumstances transfusion is usually required as a life saving measure. The transfused cells may support oxygen-carrying capacity until the acute hemolysis diminishes or other therapies can effect a more lasting benefit. These patients represent a significant challenge because serologic testing may be complex while clinical needs are acute.

Transfusion should not be withheld solely because of serologic incompatibility. The volume transfused should usually be the smallest amount required to maintain adequate oxygen delivery, not necessarily to reach an arbitrary hemoglobin level. Volumes of about 100 mL may be appropriate. The patient should be carefully monitored throughout the transfusion. The dangers of transfusion may be enhanced if the patient has mixed-type AIHA.

**Transfusion in Chronic WAIHA.** Most patients with WAIHA have a chronic stable anemia, often at relatively low hemoglobin levels. Those with hemoglobin levels above 8 g/dL rarely require transfusion, and many patients with levels of 5 g/dL (or even lower) can be managed with bed rest, without transfusion. Transfusion will be required if the anemia progresses or is accompanied by such symptoms as severe angina, cardiac decompensation, respiratory distress, and cerebral ischemia.

Most patients with chronic anemia due to WAIHA tolerate transfusion without overt reactions, even though the transfused cells may not survive any better than their own. Transfusion volume will be significantly greater than for the patient with acute hemolysis. Since transfusion may lead to circulatory overload or to increased red cell destruction, the decision to transfuse should be carefully considered. A few patients without acute hemolysis have had severe hemolytic reactions after transfusion, sometimes with no exceptional serologic evidence of autoantibody. This may be due to the sudden availability of large volumes of donor cells and the exponential curve of decay, by which the number of cells hemolyzed is proportional to the number of cells present. Reports of reactions in patients with HIV suggest these patients may be more susceptible to transfusion-induced DIC and hypercoagulability.

**Selecting Blood by Specificity.** If the decision is made to transfuse, selection of appropriate donor blood is essential. It is important to determine the patient’s ABO type and, if time permits, to detect potentially clinically significant alloantibodies. Adsorption and other special techniques described later in this chapter can greatly reduce the risk of undetected alloantibodies but may be time-consuming. If clinically significant alloantibodies are present, the transfused cells should lack the corresponding antigen(s).

If the autoantibody has apparent and relatively clear-cut specificity for a single antigen (eg, anti-e) and there is active ongoing hemolysis, blood lacking that antigen may be selected. There is evidence that in some patients such red cells survive better than the patient’s own red cells. In the absence of hemolysis, autoantibody specificity can usually be ignored, although donor units negative for the antigen may be chosen because this is a simple way to circumvent the autoantibody and detect
potential alloantibodies. If the autoantibody shows broader reactivity, reacting with all cells but showing some relative specificity (eg, reacts preferentially with e-positive red cells), the use of blood lacking the corresponding antigen is debatable. It may be undesirable to expose the patient to Rh antigens absent from autologous cells, especially D and especially in women who may later bear children, simply to improve serologic compatibility testing with the autoantibody (eg, when a D-negative patient has autoanti-e).

**Selecting Blood by Random Testing.** In many cases of WAIHA no autoantibody specificity is apparent. The patient's serum reacts with all red-cell samples to the same degree, or reacts with red cells from different donors to varying degrees for reasons seemingly unrelated to Rh phenotypes. Even if specificity is identified, the exotic cells used for such identification are not available for transfusion. Some workers test a large number of donor blood samples and select those units that give the weakest reactions in vitro, although no data exist to indicate better in-vivo survival of these least-incompatible red cells. Other workers recommend ignoring any specificity of the autoantibody and, if the presence of alloantibody has been excluded, advocate giving blood of the patient's Rh phenotype to avoid subsequent development of Rh alloantibodies. However, in cases of severe and progressive anemia, it may be essential to transfuse blood that does not react with the patient's autoantibody.

**Suitable Components.** Red blood cells are preferable to whole blood if transfusion is undertaken. Use of leukocyte-reduced components can lessen the chance of febrile reactions and make the interpretation of such reactions, should they occur, more readily attributable to red cell antibodies.

**Frequency of Testing.** If the patient has not been transfused or pregnant since a previous investigation, most workers consider repeat testing to be unnecessary. Each transfusion makes pretransfusion testing for subsequent transfusions more complicated. Extensive adsorption tests are impractical if repeated transfusion is required within a short period of time. Although not all immunologists agree, Judd has suggested that repeated extensive serologic testing is not needed within a week of the previous investigation, unless results of routine tests for unexpected antibodies are significantly different from those of previous tests. Repeat testing may sometimes be streamlined by testing adsorbed serum against cells from recently transfused donor units, because these are the ones likely to have stimulated any current alloantibody production. This approach cannot be used if the patient has received multiple transfusions over a number of days.

**Cold Agglutinin Syndrome**

Cold agglutinin syndrome (also called cold hemagglutinin disease, CHD) is the hemolytic anemia most commonly associated with cold-reactive autoantibodies and accounts for approximately 16-32% of all cases of immune hemolysis. It occurs as an acute or chronic condition. The acute form is often secondary to lymphoproliferative disorders (eg, lymphoma) or *Mycoplasma pneumoniae* infection. The chronic form, which causes mild to moderate hemolysis, is often seen in elderly patients, sometimes associated with lymphoma, chronic lymphocytic leukemia, or Waldenstrom's macroglobulinemia; Raynaud's phenomenon and hemoglobinuria may occur in cold weather. CAS is often characterized by rapid sedimentation, at room temperature, of red cells in an EDTA specimen; clumping of red cells may be obvious in such a sample, sometimes so strong that
the cells appear to be clotted. Problems with ABO and Rh typing and other tests are not uncommon.

DAT

Complement is the only protein detected on the red cells in almost all cases. The cold-reactive autoagglutinin is usually IgM, which binds to red cells in the comparatively low temperature of the peripheral circulation, and causes complement components (C3 and C4 in particular) to attach to the red cells. As the red cells circulate to warmer areas, the IgM dissociates, but the complement remains. Regulatory proteins convert the bound C3 and C4 to C3d and C4d, and it is the anti-C3d (-C4d) component of polyspecific antiglobulin reagents that accounts for the positive DAT. The presence of C3d, per se, does not shorten red cell survival.

In rare cases the cold-reactive autoantibody is IgG, which may or may not bind complement. The presence of this IgG may be missed by the DAT unless all phases of testing are done in the cold, using cold saline and antiglobulin reagent.

Serum

IgM cold-reactive autoagglutinins associated with immune hemolysis usually have a titer above 1000 when tested at 4 C; in vitro, they rarely react with saline-suspended red cells above 32 C. If 30% bovine albumin is included in the reaction medium, 70% of clinically significant examples will react at 37 C. Hemolytic activity against untreated red cells can sometimes be demonstrated at 20-25 C and, except in rare cases with Pr specificity, enzyme-treated red cells are hemolyzed in the presence of adequate complement.

Determination of the true thermal amplitude or titer of the cold autoagglutinin requires that the specimen be collected and maintained strictly at 37 C until the serum and cells are separated, to avoid in-vitro autoadsorption. Alternatively, plasma can be used from an EDTA-anticoagulated specimen that has been warmed for at least 15 minutes at 37 C (with repeated mixing) and then separated from the cells, ideally at 37 C. This should release autoadsorbed antibody back into the plasma.

In chronic CAS the IgM autoagglutinin is usually a monoclonal protein with kappa light chains. In the acute form, the autoreactive antibody is polyclonal IgM, with normal kappa and lambda light-chain distribution. Rare examples of IgA and IgG cold-reactive autoagglutinins have also been described.

Eluate

Elution is seldom necessary in obvious cases of CAS. If the red cells have been collected properly and washed at 37 C, there will be no immunoglobulin on the cells and no reactivity will be found in the eluate. Because the serum antibody level is often very high, it is essential to test the last saline wash before elution to ensure that unbound autoagglutinins have been removed.

Specificity of Autoantibody

The autoantibody specificity in CAS is usually of only academic interest. CAS is most often associated with antibodies with I specificity; less commonly, i specificity is found, usually associated with infectious mononucleosis. On rare occasions, cold-reactive autoagglutinins with Pr or other specificities, are seen (see Method 6.3). Dilution of the serum may be necessary to demonstrate specificity of very high-titer antibodies.

Autoantibody specificity is not diagnostic for CAS. Autoanti-I may be seen in healthy subjects as well as patients.
with CAS. The nonpathologic forms of autoanti-I, however, rarely react to titers above 64 at 4 °C, and are usually nonreactive with I-negative (i<sub>cord</sub> and i<sub>adult</sub>) red cells at room temperature. In contrast, the autoanti-I of CAS may react quite strongly with I-negative red cells in tests at room temperature, while equal or even stronger reactions are observed with I-positive red cells. Auto-anti-i antibodies react in the opposite manner; they give much stronger reactions with I-negative red cells than with red cells that are I-positive. The cold-reactive autoantibody found in mixed-type AIHA may not show specificity, may react only weakly at 4 °C, but usually reacts at up to 30 °C. Procedures to determine the specificities of cold-reactive autoantibodies are given in Methods 6.2 and 6.3.

### Transfusion in CAS

Patients suffering from CAS can often be managed without transfusion. Acute cell destruction may be reduced by keeping the patient in a warm room (about 40 °C), to prevent complement binding by the cold agglutinin. Steroid therapy is usually not successful; however, if the antibody is low-titered but reacts at a high thermal range, steroids may be helpful. Plasma exchange has been helpful in some cases, but the effects are usually not lasting. Transient hemolysis associated with infectious diseases requires treatment of the infectious disease.

If transfusion is undertaken, up to 50% of the cells transfused initially may be destroyed, until cells acquire some resistance to complement destruction by binding of C3d. If transfusion is required, use of a blood warmer is often recommended, but the need for this is controversial.

### Pretransfusion Testing

Antibody detection tests should be performed in ways that minimize cold-reactive autoantibody activity yet still permit detection of clinically significant alloantibodies. The use of albumin and other potentiators increases the reactivity of the autoantibodies and should be avoided. To avoid the detection of bound complement, some workers use an IgG-specific reagent, rather than a polyclonal antoglobulin serum. Some workers perform compatibility tests strictly at 37 °C, but this may miss some potentially significant alloantibodies.

### Adsorption Procedures

When cold-reactive autoantibody reactivity persists, autoadsorption studies (see Method 6.1) can be performed if the patient has not been transfused within the past 3 months. This should make it possible to detect alloantibodies masked by the cold-reactive autoantibody. If the patient has been recently transfused, rabbit red cells may be used to remove autoanti-I and -IH from sera, but this may remove clinically significant alloantibodies, notably anti-B, -D, -E, and others. A preparation of rabbit red cell stroma is commercially available. Alternatively, allogeneic adsorption studies can be performed as for WAIHA (see below).

### Mixed-Type AIHA

Although some patients with WAIHA may also have IgM antibodies that react to high titer at low temperature, a separate mixed-type AIHA accounts for approximately 7-8% of all AIHAs. These patients have “cold” agglutinins that have low titers at 4 °C but high thermal amplitudes, reacting at 30 °C or above. Mixed-type AIHA often presents as an extremely acute condition characterized by very low hemoglobin concentrations and complex serum reactivity present in all phases of testing. Transfusion should be carefully considered, especially since prompt corticosteroid therapy is frequently successful. However, even with steroid therapy, this type
of AIHA may be persistent, with intermittent periods of hemolysis. Mixed-type AIHA can be idiopathic or secondary, often associated with systemic lupus erythematosus. Typical serologic findings are described below.

**DAT**

Both IgG and C3d are usually detectable on patient’s red cells. Presumably, the IgG is due to the warm autoantibody, while the C3d is bound by the effects of IgM autoantibody.

**Serum**

Both warm-reactive IgG autoantibodies and cold-reactive, hemagglutinating IgM autoantibodies are present in the serum. These usually result in reactivity at all phases of testing, with virtually all cells tested. The IgM hemagglutinating autoantibody(ies), unlike those in CAS, usually have titers less than 64 at 4°C but react at 30°C or above. If the presence of cold hemagglutinin leads to misclassification as CAS, the option of corticosteroid therapy may be dismissed, but steroids are often effective in mixed-type AIHA. Mixed-type AIHA should also be distinguished from WAIHA in a patient with a strongly reactive, but normal, cold agglutinin. Care should be taken to avoid loss of antibody reactivity by autoadsorption of the cold agglutinin component.

Albumin and other potentiators of agglutination increase the reactivity of the cold autoagglutinin; anti-IgG, rather than a polyspecific antihuman globulin reagent, should be used for the IAT. If adsorption studies are done to detect alloantibodies, it may be necessary to perform adsorptions at both warm and cold temperatures. The cold (IgM) agglutinin may also be circumvented by treating the serum with sulfhydryl reagents, leaving intact the warm-reactive (IgG) autoagglutinin and most clinically significant alloantibodies.

**Eluate**

A suitably prepared eluate will contain a warm-reactive IgG autoantibody.

**Specificity of Autoantibodies**

The unusual cold-reactive IgM hemagglutinating autoantibody can have specificities typical of CAS (ie, I or i) but often has no apparent specificity. The warm-reactive IgG autoantibody often appears serologically indistinguishable from specificities encountered in typical WAIHA, but may have atypical features.

**Transfusion in Mixed-Type AIHA**

Patients with mixed-type AIHA often present with severe anemia and marked intravascular hemolysis. Transfusion in such cases may result in increased hemolysis, which may be life-threatening. Fortunately, these patients usually show a dramatic initial response to corticosteroid therapy and often do not need to be transfused. Transfusion may be less problematic if hemolysis is less acute.

If blood transfusions are necessary, the considerations in the selection of blood for transfusion are identical to those described for patients with acute hemolysis due to warm antibody type AIHA (see above). Considerations about blood warmers are the same as for CAS.

**Paroxysmal Cold Hemoglobinuria**

The rarest form of DAT-positive AIHA is PCH. In the past, it was characteristically associated with syphilis, but this association is now unusual. More commonly, PCH presents as an acute transient condition secondary to viral infections, particularly in young children. It can also occur as an idiopathic
chronic disease in older people. One large study found that none of 531 adults having well-defined immune hemolytic anemias had PCH, while 22 of 68 (32%) children were shown to have PCH.

**DAT**

The autoantibody in PCH is IgG, but as with IgM cold-reactive autoagglutinins, it reacts with red cells in colder areas of the body (usually the extremities), causes C3 and C4 to bind irreversibly to red cells, and then dissociates from the red cells at warmer temperatures. Red cells washed in a routine manner for the DAT are coated only with complement components, but IgG may be detectable on cells that have been washed with cold saline and tested with cold anti-IgG reagent. Keeping the system nearer its optimal binding temperature allows the cold-reactive IgG autoantibody to remain attached to its antigen.

**Serum**

The IgG autoantibody in PCH is classically described as a biphasic hemolysin, since binding to red cells occurs at low temperatures but hemolysis does not occur until the coated red cells are warmed to 37°C. This is the basis of the diagnostic test for the disease, the Donath-Landsteiner test (see Method 6.7). The autoantibody may agglutinate normal red cells at 4°C, but rarely to titers greater than 64. Because the antibody rarely reacts above 4°C, the serum is usually compatible with random donor cells by routine crossmatch procedures and pretransfusion antibody detection tests are usually nonreactive.

**Eluate**

Because complement components are usually the only globulins present on circulating red cells, eluates prepared from red cells of patients with PCH are almost invariably nonreactive.

**Specificity of Autoantibody**

The autoantibody of PCH has most frequently been shown to have P specificity, reacting with all red cells (including the patient’s own red cells) except those of the very rare p or Pk phenotypes. Exceptional examples with other specificities have been described.

**Transfusion in PCH**

Transfusion is rarely necessary for adult patients with PCH, unless hemolysis is severe. In children, especially under age 6, the thermal amplitude of the antibody tends to be much wider than in adults and hemolysis more brisk, and transfusion may be required as a lifesaving measure. Normal bank blood can usually be used. While there is some evidence that p red cells survive better than P-positive (P1-positive or P1-negative) red cells, the prevalence of p blood is approximately 1 in 200,000 and the urgent need for transfusion usually precludes attempts to obtain this rare blood. Transfusion of random donor blood should not be withheld from PCH patients whose need is urgent. Red cells negative for the P antigen should be considered only for those patients who do not respond adequately to random donor blood.

**DAT-Negative AIHA**

Clinical evidence of hemolytic anemia is present in some patients whose DAT is nonreactive. Frequently, autoantibody cannot be detected in either eluate or serum. There may be several reasons why the DAT is negative. Antibodies with low binding affinity may dissociate from the red cells during saline washing of the cells for the DAT. Washing with low ionic strength saline or saline at 37°C or 4°C
may help retain antibody on the cells. There may be too few antibody molecules on the cell for detection by routine methods, but may be demonstrable by methods such as flow cytometry, enzyme-linked antiglobulin tests, solid phase, or direct Polybrene.

Nonroutine Reagents

The causative antibody may be IgM or IgA not detected by routine antiglobulin reagents. Anti-IgG, anti-C3d, and the combined anti-C3b,-C3d reagents are the only licensed products available for use with human red cells. Antiglobulin reagents that react with IgA, IgM, or C4 are available commercially but have been prepared for use with endpoints other than agglutination. These must be used cautiously and their hemagglutinating reactivity carefully standardized by the user. Quality control must be rigorous. Because agglutination with antiglobulin reagents is more sensitive than precipitation, a serum that appears to be monospecific by precipitation tests may react with several different proteins when used in agglutination tests.

Antigen Dispersion

The patient with autoantibodies with specificity in the Kell system may have depressed red cell expression of the Kell antigens. When this occurs, antibody may be detected in the serum and eluate, but the DAT may be negative or very weakly positive. This may provide in-vivo protection of autologous cells. Donor cells of common Kell type may be destroyed, but cells lacking the corresponding antigen (usually high-incidence) may survive well. When the autoantibody subsides, autologous cells again express normal amounts of antigen.

Serologic Problems with Autoantibodies

In pretransfusion tests on patients with autoantibodies, the following problems may arise:

1. Cold-reactive autoantibodies can cause autoagglutination, resulting in erroneous determinations of ABO and Rh type.

2. Red cells strongly coated with globulins may undergo spontaneous agglutination with high-protein anti-Rh blood grouping reagents, and occasionally even with low-protein reagents.

3. The presence of free autoantibody in the serum may make antibody detection and crossmatching tests difficult to interpret. If time permits, the presence or absence of alloantibody should be determined (see Methods 6.4-6.6) before blood is transfused.

Although resolving these serologic problems is important, delaying transfusion in the hope of finding serologically compatible blood may, in some cases, cause greater danger to the patient. Only clinical judgment can resolve this dilemma. Dialogue with the patient's physician is important.

Resolution of ABO Problems

There are several approaches to the resolution of ABO typing problems associated with cold-reactive autoagglutinins. Often, it is only necessary to maintain the blood sample at 37 C immediately after collection and to wash the red cells with warm (37-45 C) saline before testing. It is helpful to perform a parallel control test, using 6% bovine albumin in saline, to determine if autoagglutination persists. If the control test is nonreactive, the results obtained with anti-A and anti-B are usually valid. If autoagglutination still occurs, interpretation of the
results can be difficult, but comparing the strength of the observed reactions may be informative. If the blood sample has been kept at room temperature, or if cold-reactive autoagglutinins are particularly potent, it may be necessary to treat the red cells with sulfhydryl reagents.

**Dispersing Autoagglutination**

Because cold-reactive autoagglutinins are almost always IgM, and sulfhydryl reagents denature IgM molecules, reagents such as 2-mercaptoethanol (2-ME) or dithiothreitol (DTT) can be used to abolish autoagglutination (see Method 2.11). Other means of dispersing autoagglutination use the ZZAP reagent or glycine-HCl/EDTA (see Methods 2.14 and 6.4). Some reagent sera should not be used with chemically modified red cells, eg, monoclonal ABO grouping reagents with enzyme-treated red cells. Appropriate controls are essential for all tests.

An alternative but less effective means of dispersing autoagglutination due to cold-reactive autoantibodies is to incubate the red cells at 45°C for 10 minutes and wash them several times in saline at that temperature.

**Problems with ABO Serum Tests**

When the serum agglutinates group O reagent red cells, the results of serum tests may be unreliable. Repeating the tests using prewarmed serum and A, B, and O red cells at 37°C will often resolve any discrepancy, but anti-A and/or -B in some patients' sera do not react at 37°C. Alternatively, adsorbed serum (either autoadsorbed or adsorbed with allogeneic group O red cells) can be used. Because rabbit red cells express a B-like antigen, sera adsorbed with rabbit red cells or stroma may not contain anti-B, and sera adsorbed in this manner should not be used for ABO serum tests.

**Resolution of Rh Problems**

Spontaneous agglutination of red cells by cold- or warm-reactive autoantibodies may also cause discrepant Rh typing. The same procedures described for the resolution of ABO problems may be useful, but most Rh typing discrepancies occur when IgG-coated red cells undergo spontaneous aggregation in the high-protein reagents designated for use by slide or rapid tube tests (sometimes referred to as modified-tube anti-Rh). The use of low-protein reagents, such as monoclonal/polyclonal blends, may be necessary to avoid erroneous results (see Chapter 13). IgG antibody can be dissociated from the cells by treatment with chloroquine diphosphate (Method 2.13), by glycine-HCl/EDTA (Method 2.14), or by other elution methods that leave red cells intact for subsequent typing. IgM-coated cells can be treated with sulfhydryl reagents (such as 2-ME or DTT, Method 2.11) to circumvent spontaneous agglutination.

**Detection of Alloantibodies in the Presence of Warm-Reactive Autoantibodies**

If the patient who has warm-reactive autoantibodies in the serum needs transfusion, it is important to evaluate the possible simultaneous presence of alloantibodies to red cell antigens. Some alloantibodies may make their presence known by reacting more strongly or at different phases than the autoantibody, but initial studies may not suggest the existence of masked alloantibodies. It is helpful to know which of the common red cell antigens are lacking on the patient's red cells, to predict which clinically significant alloantibodies the patient might produce. Antigens absent
from autologous cells could well be the target of present or future alloantibodies. When the red cells are coated with IgG, antiglobulin-reactive reagents, such as most anti-Fy\textsuperscript{a} or anti-Jk\textsuperscript{a}, cannot be used to test IgG-coated cells unless the IgG is first removed (see Method 2.14). Monoclonal reagents or other “saline-reactive” antisera that do not require an antiglobulin test may be helpful in typing the DAT-positive red cells. Cell separation procedures (see Methods 2.15 and 2.16) may be necessary if the patient has been transfused recently.

Methods to detect alloantibodies in the presence of warm-reactive autoantibodies attempt to remove, reduce, or circumvent the autoantibody. Methods that use PEG, LISS, or enzymes generally enhance autoantibodies; avoiding these reagents may allow detection of most significant alloantibodies. Other procedures involve adsorption, the principles of which are discussed in Chapter 17. Three widely used approaches are discussed below.

**Autologous Adsorption**

In a patient who has not been recently transfused, autologous adsorption (see Method 6.4) is the best way to detect alloantibodies in the presence of warm-reactive autoantibodies. The adsorbed serum can be used in the routine antibody detection procedure.

Autoadsorption generally requires some initial preparation of the patient’s red cells. At 37°C, in-vivo adsorption will have occurred and all antigen sites on the patient’s own red cells may be blocked. It may be necessary, therefore, to remove autoantibody from the red cells to make the sites available. Treatment of the autologous red cells with proteolytic enzymes also increases their capacity to adsorb autoantibody. ZZAP, a mixture of papain and dithiothreitol, is sometimes used to treat the cells (see Method 6.5); the sulfhydryl component makes the IgG molecules more susceptible to the protease and dissociates the antibody molecule from the cell. Multiple, sequential autoadsorptions may be necessary if the serum contains high levels of autoantibody. Once autoantibody has been removed, the adsorbed serum is examined for alloantibody activity.

**Recent Transfusion.** Autologous adsorption is most applicable to patients who have not been recently transfused, so will not have an admixture of transfused red cells that might adsorb alloantibody. Recent, in this context, usually means within the normal life span of red cells, or 3-4 months. Autologous adsorption studies may be informative even after transfusion as recent as one week, especially if there is evidence of markedly shortened red cell survival. Failure to detect alloantibody in “autoadsorbed” serum from a recently transfused patient should never be considered conclusive proof that no alloantibody exists.

**Sources of Autologous Cells.** The patient’s own red cells, a portion of which will be younger and less dense than transfused red cells, can sometimes be separated by simple (Method 2.15) or gradient centrifugation for use in autoadsorption experiments. However, these techniques are highly variable and may require expensive equipment and/or considerable expertise in interpretation. If the patient is to be transfused, it can be advantageous to collect and save additional aliquots of pretransfusion cells, to be used for later adsorptions.

**Allogeneic Adsorption**

The use of allogeneic red cells for adsorption may be helpful when the patient has been recently transfused or when insufficient autologous red cells are available. The goal is to remove autoantibody and leave the alloantibody...
in the adsorbed serum. The adsorbing cells must be negative for the antigens against which the alloantibodies react. Because alloantibody specificity is unknown, cells of several different phenotypes will usually be used to adsorb several aliquots.

Given the number of potential alloantibodies, the task of selecting cells may appear formidable. In actuality, however, the selected cells need only demonstrate those few alloantibodies of clinical significance likely to be present. These include the common Rh antigens (D, C, E, c, and e), K, Fya and Fy\(^b\), Jk\(^a\) and Jk\(^b\), and S and s. Cell selection is made easier by the fact that cells can be rendered negative for some of these antigens by appropriate treatment prior to adsorption. Antibodies to other antigens are rare, detect low-incidence antigens, or have little clinical significance even if present. Antibodies to high-incidence antigens cannot be excluded by allogeneic adsorptions, because the adsorbing cells will almost inevitably express the antigen and adsorb the alloantibody along with autoantibody.

**Patient’s Phenotype Unknown.** When the patient’s Rh phenotype is not known, group O red cell samples of three different Rh phenotypes (R\(_1\), R\(_2\), and r) should be selected. One should lack Jk\(^a\) and another Jk\(^b\). If treated with ZZAP, these cells would lack all antigens of the Kell system and M, N, S, s, Fya\(^a\), and Fy\(^b\).

Each aliquot may need to be adsorbed two or three times. The fully adsorbed aliquots are tested against reagent red cells known either to lack or to carry common antigens of the Rh, MNS, Kidd, Kell, and Duffy blood group systems. If an adsorbed aliquot is reactive, that aliquot (or an additional specimen similarly adsorbed) should be tested to identify the antibody. Adsorbing several aliquots with different red cell samples provides a battery of potentially informative specimens. For example, if the aliquot adsorbed with Jk(a–) red cells subsequently reacts only with Jk(a+) red cells, the presence of alloanti-Jk\(^a\) can confidently be inferred.

The adsorbed serum can also be used for crossmatching. If the quantity of adsorbed serum is limited, crossmatching may be a more advantageous use than antibody identification; if necessary, adsorbed aliquots may be pooled for compatibility tests.

If ZZAP is not available, cells treated only with proteolytic enzyme can be used, but they must be K-negative because Kell system antigens will not be destroyed. Untreated cells may be used, but antibody will be more difficult to remove and the adsorbing cells must, at a minimum, include at least one negative for the S, s, Fya\(^a\), Fy\(^b\), and K antigens, in addition to the Rh and Kidd requirements above.

**Patient’s Phenotype Known.** The number of different red cell phenotypes required may be reduced if the patient’s phenotype is known, or can be determined by separation of autologous from transfused red cells (see Method 2.15). If the patient’s Rh and Kidd phenotypes are known or can be determined, adsorption can be performed with a single sample of allogeneic ZZAP-treated red cells of the same Rh and Kidd phenotypes as the patient.

**Problems Encountered.** Occasionally autoantibody will not be removed by three sequential adsorptions. Further adsorptions can be done, but multiple adsorptions are likely to dilute the serum. If the adsorbing cells do not appear to remove the antibody, the autoantibody may have an unusual specificity that does not react with the cells used for adsorption. For example, autoantibodies with Kell specificity would not be removed by ZZAP-treated cells. Some anti-En\(^a\), -Wr\(^b\), or other antibodies may not react with cells treated with ZZAP or proteolytic enzymes.
Autoantibodies Mimicking Alloantibodies

Autoantibodies sometimes have patterns of reactivity that are easily mistaken for alloantibody. For example, the serum of a D-negative patient may have apparent anti-C and -e reactivity. The anti-C reactivity may reflect warm-reactive autoantibody, even if the patient's cells lack C. The autoantibody nature of the reactivity can be demonstrated by autologous and allogeneic adsorption studies. The apparent alloanti-C would, in this case, be adsorbed by C-negative red cells, both autologous and allogeneic. This is quite unlike the behavior of a true alloanti-C, which would be adsorbed only by C-positive red cells.

Detection of Alloantibodies in the Presence of Cold-Reactive Autoantibodies

Cold-reactive autoagglutinins rarely mask clinically significant alloantibodies if serum tests are conducted at 37 C and if IgG-specific reagents are used for the antiglobulin phase. In rare instances it may be necessary to perform autoadsorption at 4 C (see Method 6.1). Achieving complete removal of potent cold-reactive autoagglutinins is very time-consuming, and may be facilitated by treating the patient's cells with ZZAP or enzymes before adsorption.

If autologous adsorption studies are inappropriate because the patient has been recently transfused, or if complete removal of the autoantibody is not possible, tests for alloantibody activity may be carried out strictly at 37 C. Both the patient's serum and the reagent red cells should be warmed to 37 C before mixing. The tests should be centrifuged at 37 C. If centrifugation at 37 C is impossible, and if time permits, tests can be incubated at 37 C for 1-2 hours to allow the red cells to settle, and they can then be examined for agglutination without centrifugation. Because bovine albumin and other enhancement media increase the reactivity of cold-reactive autoantibodies, their use should be avoided. The red cells should be washed in saline at 37 C and then tested with anti-IgG to avoid the effects of in-vitro complement binding by the cold-reactive autoantibody. EDTA-anticoagulated plasma, or serum to which EDTA has been added (2 mL serum plus 0.25 mL 4.45% K$_2$EDTA), can also be used to avoid interference by complement-binding autoantibodies.

Drug-Induced Immune Hemolytic Anemias

Drugs sometimes induce the formation of antibodies, either against the drug itself or against intrinsic red cell antigens that may result in a positive DAT, immune red cell destruction, or both. Some of the antibodies produced appear to be dependent on the presence of the drug for their detection or destructive capability while others do not. In some instances, a reactive DAT may result from nonimmunologic effects of the drugs.

The patient's medications may include over-the-counter medications and others not prescribed by a physician. Environmental chemicals can cause similar serologic and clinical effects. These include chlorinated hydrocarbons (found in insecticides) and chemicals used in dyes and manufacturing processes. The bite of the brown recluse spider may be associated with severe intravascular hemolysis, generally with a negative DAT, although examples with positive DATs have been reported.

Theories of the Immune Response and Drug-Dependent Antibodies

Numerous theories have been suggested to explain how drugs induce immune
responses and what relation such responses may have to the positive DAT and immune-mediated cell destruction observed in some patients. For many years, drug-associated positive DATs were classified into four mechanisms: drug adsorption, immune complex formation, non-specific adsorption, and autoantibody production. Such classification has been useful, but many aspects lacked definitive proof or detailed mechanisms. These theories were further complicated by evidence that some drugs created immune problems involving aspects of more than one mechanism. More recent theories, still unproven, tend toward a more comprehensive approach applicable to all drugs, allowing for differing manifestations.

Most drugs have a molecular weight substantially below the 5-kDa level usually considered the threshold for effective immunogenicity. Drugs may act as haptens, eliciting antibody only after they bind firmly to or become part of a protein carrier. Most drugs associated with an immune response react very weakly with cellular proteins, but drug-induced immune responses often show extraordinary specificity to particular cellular targets, such as red cells or platelets. This exquisite specificity renders less plausible the classical explanation of induction by the hapten carrier.

Current theories propose that drugs elicit antibodies based on their ability to interact with specific cell membrane components, thus altering the normal components so that they are no longer recognized as self. A new configuration or “neoantigen” is produced, consisting of both drug and cell component, and the host’s immune system perceives this neoantigen as foreign. The antibody is produced directly against the neoantigen, not against a haptenic constituent.

This theory assumes that drugs bind with variable affinity to particular membrane components present on red cells, granulocytes, or platelets. The degree of association would be expected to reflect such factors as the chemical structure of the drug or its metabolite(s), the concentration of the drug, the structure of cellular membrane proteins, and the capacity of the host proteins to associate with the drug (possibly dependent on pH, temperature, ionic strength and, most probably, host genetic factors). Because conditions must be just right to create a sufficiently high affinity to provoke an immune response, instances of drug-induced immunity are relatively rare.

The polyclonal immune response provoked by such a configuration consists of antibodies that can recognize at least three categories of epitopes. Some react essentially with the drug portion itself, some with combinations of the drug and cellular component, and others with essentially cellular membrane components (see Fig 18-1). In some cases red cells or other specific cell types are the sole or primary targets, while at other times a variety of cell types may be affected. Why certain drugs repeatedly target single or multiple cell lines is not always clear.

**Serologic and Clinical Classification**

Drug-induced antibodies can be classified into three groups according to their clinical and serologic characteristics. In one group the drug binds firmly to the cell membrane and antibody is apparently largely directed against the drug itself. This is known as the drug adsorption mechanism, and antibodies to penicillin are the best described of this group.

The second group of drug-dependent antibodies reacts with drugs that do not bind well to the cell membrane. The reactive mechanism of these antibodies was previously described as immune complex formation, but this appears not to be quite the case. Antibodies in this group cause acute intravascular hemolysis,
but they may be difficult to demonstrate serologically.

Antibodies of the third group have serologic reactivity independent of the drug, despite the fact that it was the drug that originally induced the immune response. Serologically, they behave as autoantibodies.

Some drugs are associated with a positive DAT due to nonimmune mechanisms. These drugs, especially first-generation cephalosporins, alter the red cell membrane in a way that induces nonspecific adsorption of proteins, including but not limited to immunoglobulins. Hemolytic anemia is not associated with this mechanism.

**Drug-Dependent Antibodies Reactive with Cell-Bound Drug: Penicillin-Type Antibodies**

Drugs with a high binding affinity may induce a seemingly drug-specific immune response. The high degree of association with the cellular component(s) may cause the drug to act as a true hapten. These drugs with very high binding affinity may be more immunogenic than drugs that bind to cell membrane components with lower affinity. Because the antibodies produced are directed predominantly to the drug (hapten) itself, they can be expected to be inhibitable by a pure form of the drug (ie, hapten inhibition), and may require that the drug be bound to a solid matrix detection system (ie, red cells) for in-vitro detection.

Such drugs have previously been classified as reacting by the drug adsorption mechanism, with penicillin antibodies the primary example (see Fig 18-2). Hemolytic examples of anti-penicillin are not always completely inhibited with pure penicillin. This may simply reflect the need of these very high titers of antibody for a very high hapten concentration to cause complete inhibition. Alternatively, this may indicate that at least a portion of the polyclonal anti-

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**Figure 18-1. Proposed unifying theory of drug-induced antibody reactions (based on a cartoon by Habibi as cited by Garratty 24).** The thicker, darker lines represent antigen-binding sites on the F(ab) region of the drug-induced antibody. Drugs (haptens) bind loosely, or firmly, to cell membranes and antibodies may be made to: a) the drug (producing in-vitro reactions typical of a drug adsorption (penicillin-type) reaction); b) membrane components, or mainly membrane components (producing in vitro reactions typical of autoantibody); or c) part-drug, part-membrane components (producing an in-vitro reaction typical of the so-called immune complex mechanism). [24(p55)]
penicillin response recognizes some shared component of the membrane-drug neoantigen. If this occurs, the in-vitro serologic reactions resemble both the drug adsorption and “immune complex” mechanisms.

**General Observations.** The clinical and laboratory features of drug-induced immune hemolytic anemia operating through this mechanism are:

1. The DAT is strongly positive due to IgG coating. Rarely, complement coating may also be present, but weakly.
2. Tests for unexpected serum antibodies are nonreactive unless the patient also has alloantibodies to red cell antigens.
3. Antibody eluted from the red cells reacts with drug-coated red cells but not with uncoated red cells.
4. The serum contains a high-titer IgG antibody, at least when the target is penicillin.

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**Figure 18-2.** The drug-adsorption mechanism. The drug binds tightly to the red cell membrane proteins. If a patient develops a potent anti-drug antibody, it will react with the cell-bound drug. Such rbc's will yield a positive result in the DAT using anti-IgG reagents. Complement is usually not activated and lysis is primarily extravascular in nature. Penicillin-G is the prototype drug. (Reprinted with permission from Petz and Branch.)
5. Hemolysis occurs only in patients receiving very large intravenous doses of drug; for penicillin, the hemolysis-inducing dose is millions of units daily for a week or more.

6. Hemolysis develops gradually, but may be life-threatening if the etiology is unrecognized and drug administration is continued.

7. Discontinuation of the drug is usually followed by increased cell survival, although hemolysis of decreasing severity may persist for several weeks.

**Penicillin Antibodies.** Approximately 3% of patients receiving large doses of penicillin intravenously (ie, millions of units per day) will develop a positive DAT, but less than 5% of these will develop hemolytic anemia. Intravascular hemolysis is rare. A possible mechanism for the positive DAT is given in Fig 18-2. The penicillin becomes covalently linked to the red cells in vivo, and if the patient has antibodies to penicillin, they bind to the penicillin bound to the red cells. The result is that the penicillin-coated red cells become coated with IgG. Complement is not usually involved. If cell destruction occurs, it takes place extravascularly, probably in the same way that red cells coated with IgG alloantibodies are destroyed.

Drugs such as penicillin have several distinct haptenic groups, of which the most important is the benzyl-penicilloyl (BPO) group. With sufficiently sensitive techniques, most adult human sera can be shown to contain BPO antibodies. These are usually IgM and of low titer; however, some sera contain an IgG component. The high prevalence of penicillin antibodies in the normal population probably reflects the widespread exposure to this drug. Circulating IgM and IgG antibodies to penicillin are not involved in allergic reactions to the drug, which are due to IgE antibodies.

**Cephalosporins.** Cephalosporin drugs, which are related to penicillins, may behave in a similar manner. The drugs bind firmly to red cells, which then interact with the specific cephalosporin antibody. Anti-cephalosporins may cross-react with penicillin-treated red cells, but cells prepared with the specific cephalosporin may be necessary to demonstrate reactivity.

The cephalosporins are generally classified by “generations,” based on their effectiveness against gram-negative organisms (see Table 18-5). Approximately 4% of patients receiving first- or second-generation cephalosporins develop a positive DAT, although some series have reported a higher prevalence, possibly due to drug dosages or the different antiglobulin reagents used. Most positive DAT results, especially those seen with first-generation cephalosporins, reflect nonimmunologic adsorption of protein (see below), a mechanism not associated with reduced red cell survival. There have been occasional reports of red cell destruction resulting from therapy with first- and second-generation cephalosporins, probably resulting from effects of specific anti-cephalosporin antibodies. Even more dramatically reduced red cell survival has been associated with newer second- and third-generation cephalosporin, and in some cases a drug-independent autoantibody mechanism may also be involved. The prevalence and severity of cephalosporin-induced immune red cell destruction appears to be increasing.

**Other Drug-Dependent Antibodies: “Immune Complex” Mechanism**

This mechanism is the least frequent finding in drug-induced immune-mediated red cell destruction. Quinidine and phenacetin are prototype drugs. The following observations are characteristic.

1. Acute intravascular hemolysis with hemoglobinemia and hemoglo-
binuria is the usual presentation. Renal failure occurs in approximately 50% of cases.

2. Once antibody has been formed, severe hemolytic episodes may recur after exposure to very small quantities of the drug.

3. The antibody can be either IgM or IgG.

4. Complement is usually the only globulin found on the red cells.

5. Drug must be present in vitro for demonstration of the antibody in the patient's serum.

Many drugs have been implicated as causing immune hemolysis, yet most are represented by only one or a few examples. The immune hemolysis was previously attributed to the formation of drug/anti-drug immune complexes that interacted loosely and nonspecifically with the cell, resulting in the binding of complement and lysis of the cell.

The neoantigen concept proposes that these drugs do, in fact, associate with the cell membrane, though not with high affinity. The neoantigen, composed partly of drug, partly of membrane components, constitutes the stimulus to antibody formation and the target of antibodies once formed (see Fig 18-1).

The distinction between these “immune complex” drug-dependent reactions and those of the “drug-adsorption” mechanism may be more apparent than real. Classification depends in large part on the ability to bind the implicated drug to red cells in vitro for testing.

### Drug-Independent Antibodies: Autoantibody Production

Some drugs induce autoantibodies that appear serologically indistinguishable from those of WAIHA: red cells are coated with IgG, and the eluate as well as the serum reacts with virtually all cells tested, in the absence of the drug. At times blood group specificity has been demonstrated, similar to that seen in AIHA. The antibody has no in-vitro activity with the drug, directly, or indirectly.

The best studied of such cases are those induced by α-methylldopa. A closely related drug, L-dopa, has also been implicated, as have several drugs unrelated to α-methylldopa, including

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### Table 18-5. Some Cephalosporins

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Trade Name*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First Generation</strong></td>
<td></td>
</tr>
<tr>
<td>Cefadroxil</td>
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</tr>
<tr>
<td>Cefazolin</td>
<td>Ancef, Kefzol</td>
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<td>Keflex</td>
</tr>
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<td>Keflin</td>
</tr>
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<tr>
<td>Cephradine</td>
<td>Anspor</td>
</tr>
<tr>
<td><strong>Second Generation</strong></td>
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<td>Ceclor</td>
</tr>
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<td>Mandol</td>
</tr>
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<td>Zefazone</td>
</tr>
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<td>Monocid</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>Cefotan</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>Mefoxin</td>
</tr>
<tr>
<td>Cefprozil</td>
<td>Cefzil</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>Zinacef, Kefurox</td>
</tr>
<tr>
<td>Cefuroxime axetil</td>
<td>Ceftin</td>
</tr>
<tr>
<td><strong>Intermediate</strong></td>
<td></td>
</tr>
<tr>
<td>Cefixime</td>
<td>Suprax</td>
</tr>
<tr>
<td><strong>Third Generation</strong></td>
<td></td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>Cefobid</td>
</tr>
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<td>Cefotaxime</td>
<td>Claforan</td>
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<tr>
<td>Ceftazidime</td>
<td>Fortaz, Ceptaz, Pentacef, Tazicef, Tazidine</td>
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<td>Cefizox</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>Rocephin</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>Moxam</td>
</tr>
</tbody>
</table>

*Several forms are also marketed under other trade names. This list is intended to be informational, not inclusive.
procainamide and nonsteroidal anti-inflammatory drugs (eg, mefenamic acid and sulindac). (See Table 18-6.) In some cases drug-dependent antibodies are also present.

Proof that a drug causes autoantibody production is difficult to obtain. Sufficient evidence would include: demonstration that autoantibody production began after drug administration; resolution of the immune process after withdrawal of the drug; and recurrence of hemolytic anemia or autoantibodies if the drug is readministered. The last requirement is crucial and the most difficult to demonstrate.

**Possible Mechanisms.** Previous theories to explain autoantibody formation include:

1. The drug alters an intrinsic red cell antigen so that it is no longer recognized as self by the immune system.
2. The drug interferes with suppressor T-cell function, allowing overproduction of autoantibody by B cells. Studies attempting to confirm this theory have given mixed results.
3. The hemolytic autoantibody is qualitatively different, or recognizes a slightly different epitope, from the nonhemolytic autoantibody found in the majority of DAT-positive patients receiving α-methyldopa.

Current theories suggest that the drugs bind to the cell membrane and/or subtly alter membrane structures, forming a neoantigen. The autoantibodies produced are those directed against membrane components of the neoantigen, which are crossreactive with the drug and do not require it for subsequent reactivity. Blood group specificity may occur if antigens present on the cell are involved with the neoantigen.

With some drugs, drug-dependent antibodies have been shown in addition to drug-independent autoantibodies. Such drug-dependent antibodies have not been demonstrated with α-methyldopa, procainamide, or mefenamic acid, suggesting that possibly at least two different mechanisms may be involved.

**α-Methyldopa.** The prototype drug responsible for this mechanism, α-methyldopa, is now rarely prescribed, as more effective antihypertensive drugs with fewer side effects have become available. The clinical and laboratory features associated with α-methyldopa-induced autoantibodies are as follows:

1. Approximately 15% of patients receiving α-methyldopa develop a positive DAT. Only 0.5-1.0% of patients taking α-methyldopa develop hemolytic anemia.

| Table 18-6. Some Drugs That May Induce Drug-Independent Antibodies (Autoantibodies) |
|---------------------------------|------------------|------------------|
| α-Methyldopa                    | Cianidanol*      | Levodopa         |
| Azapropazone*                   | Chlorpromazine   | Mefenamic Acid   |
| Carbimazole*                    | Cyclofenil       | Nomifensine*     |
| Catergen                        | Diclofenac*      | Phenacetin*      |
| Cefotetan*                      | Fenfluramine     | Procaínamide     |
| Cefoxitin*                      | Glafeine*        | Streptomycin*    |
| Chaparral                       | Ibuprofen        | Teniposide*      |
| Chlorinated Hydrocarbons*       | Latamoxef*       | Tolmetin*        |

*These drugs may also induce drug-dependent antibodies.
2. The DAT becomes positive only after 3-6 months of α-methyldopa therapy.
3. Red cells are usually coated only with IgG; weak complement coating has been reported occasionally.
4. Development of a positive DAT is dose dependent; approximately 36% of patients taking 3 g of the drug daily develop a positive DAT, compared with 11% of patients receiving 1 g per day.
5. Antibodies in the serum and on the red cells are indistinguishable from those found in WAIHA.
6. The strength of the positive DAT becomes progressively weaker once α-methyldopa therapy is discontinued. It may take from 1 month to 2 years for the DAT to revert, but in patients with clinical hemolysis, hematologic values usually improve within a week or so after the drug therapy is discontinued.

Nonimmunologic Protein Adsorption

The positive DAT associated with some drugs is due to a mechanism independent of antibody production. Hemolytic anemia associated with this mechanism occurs extremely rarely, if at all.

Cephalosporins (primarily cephalothin) are the drugs with which this was originally associated. Red cells coated with cephalothin (Keflin) and incubated with normal plasma will adsorb albumin, IgA, IgG, IgM, and β and α (ie, complement) globulins in a nonimmunologic manner. If this occurs, a positive DAT will be seen with antihuman reagents to many serum proteins, not just anti-IgG or anti-C3.

Although it was once thought that these drugs altered the red cell membrane in some way, making the cells adsorb proteins nonspecifically, a different mechanism has been proposed. Cephalothin can bind firmly, in vitro, to red cells at acid pH by a mechanism unlike that of other β-lactam antibiotics. This alternative binding method leaves exposed a β-lactam moiety of the molecule, to which several proteins can then become bound.

In addition to this nonimmune adsorption of proteins, the cephalosporins can also induce a positive DAT by the drug adsorption mechanism described for penicillin by the so-called immune complex mechanism, and even by the production of drug-independent autoantibodies (see above). Newer generations of cephalosporins (see Table 18-5) have been associated with severe immune hemolysis.

Other drugs that may cause nonimmunologic adsorption of proteins and a positive DAT include diglycoaldehyde, suramin, and cisplatin.

Laboratory Investigation of Drug-Induced Antibodies

The drug-related problems most commonly encountered in the blood bank are those associated with a positive DAT. IgG- and complement-specific antiglobulin reagents are useful in classifying cases of drug-related hemolysis. Typical DAT results are shown in Table 18-3. Drug-dependent antibodies characteristically bind only complement to the cells. Drugs, such as penicillin, that bind firmly to the cells and induce IgG antibodies are usually associated with IgG-only DAT results. Drug-independent antibodies induce serologic phenomena similar to those in AIHA of other etiologies. For investigation of suspected hemolysis due to drug-dependent antibodies, special methods are often required.

Adding Drug to the Test System

The patient’s serum should be tested for unexpected antibodies by routine proce-
dures. If the serum does not react with untreated red cells, the tests should be repeated against ABO-compatible red cells in the presence of the drug(s) suspected of causing the problem.

If the drug is one already reported as being immunogenic, testing methods may be available in the case reports. If such information is not available, an initial screening test can be performed with a solution of the drug at a concentration of approximately 1 mg/mL in phosphate-buffered saline at a pH optimal for solubility of the drug. Techniques are given in Methods 6.9 and 6.10. For some drugs, a solvent other than saline may be required. The physical properties of drugs, such as solubility and stability, may be found in several drug reference books, through consultation with the hospital pharmacist, or by contacting the pharmaceutical company. Detection of some drug-related antibodies can be enhanced if the implicated drug is added to wash solutions. The gel test has also been shown to be very sensitive in detecting drug-dependent antibodies.

If these tests are not informative, attempts can be made to coat normal red cells with the drug, and the patient's serum and an eluate from the patient's red cells tested against the drug-coated red cells. This is the method of choice when penicillin or cephalosporins are thought to be implicated (see Method 6.8). Results definitive for penicillin-induced positive DAT are reactivity of the eluate against penicillin-coated red cells and absence of reaction between the eluate and uncoated red cells. If the positive DAT is due to complement binding, the eluate is likely to be nonreactive, even when the drug is added to the test system.

Other Observations

The actual molecule that induces the immune response may be a metabolite of a drug, rather than the drug itself. It may be helpful to test drug metabolites for reactivity by one of the above methods, having obtained the metabolites by collecting serum or urine from other individuals taking the implicated drugs.

Drug-induced immune hemolysis has been associated with apparent blood group specificity. In some reported cases the drug apparently bound to cells in association with specific antigen receptors (eg, Jka, e, I). Drugs involved have included chlorpropamide, glafenine, rifampicin, and nomifensine. Most other examples of immune hemolysis associated with these same drugs have not shown blood group specificity.

Drugs that have been reported to cause a positive DAT and hemolytic anemia are listed in Appendix 18-1.

References


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**Suggested Reading**


### Appendix 18-1. Some Drugs Associated with Immune Hemolysis and/or Positive DATs Due to Drug-Induced Antibodies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic Action</th>
<th>Possible Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Analgesic, antipyretic</td>
<td>DD-IC</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>Analgesic, antipyretic</td>
<td>DD-IC</td>
</tr>
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<td>Amphotericin B</td>
<td>Antifungal, antibiotic</td>
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</tr>
<tr>
<td>Ampicillin</td>
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<td>DD-IC</td>
</tr>
<tr>
<td>Antazoline</td>
<td>Antihistamine</td>
<td>DD-IC</td>
</tr>
<tr>
<td>Apazone (azapropazone)</td>
<td>Anti-inflammatory, analgesic</td>
<td>DI, DD-DA</td>
</tr>
<tr>
<td>Buthiazide (butazide)</td>
<td>Diuretic, antihypertensive</td>
<td>DD-IC</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>Antibacterial</td>
<td>DD-DA</td>
</tr>
<tr>
<td>Carbimazole</td>
<td>Thyroid inhibitor</td>
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</tr>
<tr>
<td>Catergen</td>
<td>Diarrheal astringent, treatment of hepatic disease</td>
<td>DI</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Antibacterials</td>
<td>NIA</td>
</tr>
<tr>
<td>First generation</td>
<td></td>
<td>DD-IC, DD-DA, DI</td>
</tr>
<tr>
<td>Second generation</td>
<td></td>
<td>DD-IC, DD-DA, DI</td>
</tr>
<tr>
<td>Third generation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chaparral</td>
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<td>Gonad-stimulating principle</td>
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<td>Diclofenac</td>
<td>Anti-inflammatory</td>
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</tr>
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<td>Diglycoalteddehyde</td>
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<td>Anti-inflammatory, analgesic</td>
<td>DI, DD-IC</td>
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<td>Fluorescein</td>
<td>Injectable dye</td>
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<td>Anti-inflammatory</td>
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<td>Melphalan</td>
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<td>Methotrexate</td>
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(continued)
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<td>Analgesic, anti-inflammatory</td>
<td>DD-DA, DD-IC, DI</td>
</tr>
</tbody>
</table>

Mechanisms listed are based on descriptions in the literature.\(^{39-43,50}\)

DAT = Direct antiglobulin test.


DD-DA = Drug-dependent. Drug adsorbed onto red cells, antibody reacts with drug on cells.

DD-IC = Drug-dependent. “Immune complex mechanism.” Requires drug, serum, and red cells for serologic demonstration. For most of these drugs there are only single or very few case reports.

NIA = Nonimmunologic adsorption of proteins.

? = Mechanism unclear or unknown.