

XIII. Investigation of a Positive Direct Antiglobulin Test and Immune Hemolysis

A. Significance of a Positive Direct Antiglobulin Test (DAT)

1. Introduction

- a. A positive DAT **does not** mean that an individual's RBCs have a shortened survival.
 - b. As many as 10% of hospital patients, and between 1 in 1000 to 1 in 9000 blood donors, will have a positive DAT without clinical manifestations of immune-mediated hemolysis.
2. *A positive DAT, with or without an associated shortened RBC survival, may be caused by the following phenomena in vivo:*
- a. **Autoantibodies** to intrinsic RBC antigens that coat RBCs with immunoglobulin or complement or both.
 - b. **Alloantibodies** present in the recipient of a recent transfusion that react with antigens on donor RBCs.
 - c. **Antibodies present in donor plasma, plasma products or blood fractions** that react with antigens on a transfusion **recipient's** cells.
 - d. **Maternal alloantibodies** that cross the placenta and coat fetal RBCs. These antibodies are often associated with HDN.
 - e. **Antibodies directed against certain drugs**, such as penicillin, that bind to RBC membranes.
 - f. **Red cell membrane modifications** resulting from therapy with certain drugs, notable those of the cephalosporin group, leading to nonspecific adsorption of proteins, including immunoglobulins, by RBCs.
 - g. **Drug/anti-drug complexes**, formed in response to the administration of drugs such as quinidine and phenacetin, that cause complement components and sometimes also IgG to be bound to RBCs.
 - h. **Heterophile antibodies** present in equine anti-human lymphocyte globulin that is used to reduce T-cell populations in organ and bone marrow transplant recipients.
 - i. **Non-antibody-mediated binding of immunoglobulins to RBCs in patients with hypergammaglobulinemia.** A positive DAT due to this phenomenon is also seen in patients treated with high-dose IV gammaglobulin.
3. The Pretransfusion DAT or Autocontrol
- a. Many workers used to routinely perform a DAT (some workers still do), in parallel with RBC typing or an autocontrol in parallel with serum tests for unexpected antibodies as part of pretransfusion testing.
 - b. Such an autocontrol serves essentially the same purpose as a DAT; to determine if RBCs are coated with globulins.
 - c. *For the most part, the DAT detects in vivo globulin coating, whereas the autocontrol may be positive due to in vivo **or** in vitro globulin coating.*

- d. The need to perform a DAT or autocontrol as part of routine pretransfusion testing has been the subject of considerable debate, particularly with the cost restraints currently imposed on the health-care industry.
- e. AABB Standards does not require that a DAT or autocontrol (AC) be performed as part of **routine** pretransfusion testing.
- f. *Reasons for the performance* of the DAT or AC are as follows:
 - 1) to screen for clinically unexpected autoimmune phenomena **and**
 - 2) to detect the early manifestation of an immune response to previous recent transfusions, particularly instances in which the newly formed alloantibody has been totally adsorbed by the transfused RBCs and cannot be detected in the serum.
- g. Independent studies have shown that there is minimal risk associated with eliminating the DAT/AC portion of routine pretransfusion testing.

4. Unwanted Positive DATs

- a. The results of DAT should reflect **in vivo** conditions, and should not be influenced by in vitro phenomena associated with the collection, storage or handling of blood samples.
- b. Before further studies are undertaken on a patient with a positive DAT, causes of **in vitro** coating should be excluded.
- c. *False positive* DAT results are most often associated with the use of refrigerated, clotted blood samples in which complement components coat RBCs in vitro.
- d. *Any positive DAT result obtained from a clotted blood sample should be confirmed using freshly collected, EDTA-anticoagulated specimen.*

B. Evaluation of a Positive DAT

- 1. Extent of testing.
 - a. **Clinical considerations should dictate the extent to which a positive DAT is evaluated.**
 - b. Dialogue with the attending physician is important before any additional serological tests are undertaken.
 - c. Interpreting the significance of serological findings requires knowledge of **the patient's diagnosis, drug therapy and recent transfusion history.**
 - d. The results of serological test **are not diagnostic**; their significance can **only** be assessed in relationship to the **patient's clinical condition, and laboratory data such as hematocrit, bilirubin, haptoglobin and reticulocyte count.**
 - e. If an anemic patient with a positive DAT manifest clinical signs and symptoms of hemolysis it is appropriate to determine if the hemolysis has an immune basis. On the other hand, **if there is no evidence of hemolysis, no further studies are necessary**, unless unexpected antibodies are present and transfusion is necessary.

- f. Approximately 3% of patients receiving IV penicillin and 15-20% of patients receiving alpha-methyl dopa (Aldomet) will develop a positive DAT, but less than 1% of those patients will have hemolytic anemia. the attending physician should be alerted so that appropriate surveillance for hemolysis can be maintained, *but if there is no evidence of hemolysis, no additional **blood bank** testing need be done.*
 - g. *Transfusion of non-ABO group specific plasma to A and/or B antigens present on the recipient's cells may give rise to a positive DAT and occasionally may result in accelerated destruction of those RBCs. If signs of hemolysis occur investigation of the DAT can be limited to demonstrating the presence of anti-A and/or -B on the recipient's RBCs.*
 - h. Patients receiving anti-lymphocyte globulin (ALG) or anti-thymocyte globulin (ATG) produced in horses develop a positive DAT within a few days after such therapy is initiated. *This appears to be related to high-titer heterophile antibodies in these products.* The problem can be avoided using AHG which has been partially neutralized with horse serum.
2. Collection of Blood Samples
- a. To verify that a positive DAT is not the result of **in vitro uptake of complement components by clotted specimens, an EDTA-anticoagulated sample should be obtained.**
 - b. This is used for the DAT and also provides a source of RBCs for elution if necessary.
 - c. A freshly collected clotted blood sample is needed for serum studies.
 - d. If a cold hemagglutinin is suspected, the clotted specimen **should be maintained at 37C until the serum has been separated.**
3. Initial Serological Studies
- a. Tests with anti-IgG and anti-C3d AHG reagents to *determine the types of proteins coating the RBCs.*
 - b. *Serum* tests to detect and identify unexpected antibodies. Such studies should be undertaken at RT, 37C and IAT (antibody screen and panel if necessary).
 - c. Test with an eluate prepared from the coated RBCs to characterize the coating antibody. The eluate should be tested by IAT against panel cells. The panel cells should include R1, R2, r RBCs and include one K+, one Jk(a+b=) and one Jk(a=b+) sample. If warm reactive auto-antibodies are suspected, the designated Rh phenotypes afford maximal sensitivity for detecting auto-antibodies with Rh related activity (eg, auto-anti-e). If the patient has been recently transfused, the suggested phenotypes serve to provide optimal sensitivity for detecting alloantibodies to Rh, Kell and Kidd system antigens. Such antibodies are frequently associated with an immune response to recently transfused RBCs.
4. Further Studies.
- a. **WHEN NO UNEXPECTED ANTIBODIES ARE PRESENT IN THE SERUM, ONLY AUTOANTIBODY IS PRESENT IN THE ELUATE AND THE PATIENT HAS NOT BEEN RECENTLY TRANSFUSED, NO FURTHER SEROLOGICAL TESTING IS NECESSARY.**
 - b. If alloantibody appears to be present in serum or eluate, or both, additional studies may be required to confirm specificity.

- c. If both serum and eluate are nonreactive at all test phases, and if the patient is known to have received high-dose IV penicillin, then tests with penicillin-coated RBCs should be considered.
- d. If the patient has been transfused with ABO-incompatible blood components, such as group O platelets administered to a group A recipient, tests with A and B RBCs may be done to ascertain whether passively acquired anti-A or anti-B is responsible for the positive DAT.
- e. Allo-immune hemolysis, resulting in HDN or associated with an immune response to recently transfused RBCs has been discussed already.

C. Immune Hemolysis

1. *Immune hemolysis is defined as* **shortened RBC survival resulting from an immune reaction.**
2. If bone marrow compensation is adequate, hemolysis may not result in anemia.
3. Hemolysis is only one cause of anemia, and there are many causes of hemolysis unrelated to immune reactions. The serological investigations carried out in the blood bank **do not** determine whether a patient has hemolytic anemia.
4. The *diagnosis of hemolytic anemia* rests on **clinical findings and laboratory data such as hemoglobin or hematocrit values, reticulocyte count, RBC morphology, bilirubin, haptoglobin and LDH levels.** Sometimes, RBC survival studies are informative.
5. The serological 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.**
6. Immune hemolytic anemia may be classified in various ways.
7. In one series involving 300 patients with immune hemolytic anemias, **48-70% had immune hemolysis associated with warm-reactive antibodies, 16% had cold agglutinin syndrome, 7-8% had mixed type AIHA, 2% had paroxysmal cold hemoglobinuria and 12-18% had drug-induced hemolysis.**

D. Warm Autoimmune Hemolytic Anemia (WAIHA)

1. **The most common type of autoimmune hemolytic anemia is associated with warm-reactive (37C) antibodies.** WAIHA patients present **the most difficult serological problems you will encounter in the clinical blood bank.** They may present with all of the following: will not type correctly, antibody screen strongly positive, crossmatches strongly incompatible, DAT strongly positive, eluate reactive with all cells tested and life threatening anemia.
2. Direct Antiglobulin Test (DAT)
 - a. The DAT should be performed with a variety of antiglobulin reagents to classify the coating protein.
 - b. When monospecific anti-IgG and anti-C3d reagents are used, one of three patterns of reactivity may be found:
 - 1) **67% of the cases, RBCs are coated with both IgG and complement.**
 - 2) 20% of the cases, RBCs are coated with IgG alone.
 - 3) 13% of the cases, RBCs are coated with complement alone.

3. Serum

- a. The patient's serum may contain very little free autoantibody if the autoantibody has been primarily adsorbed by the patient's RBCs *in vivo*.
- b. *Autoantibody usually appears in the serum when all the specific antigen sites on the RBCs have been occupied, and no more antibody can be bound in vivo.* In this case the DAT is usually **strongly positive**.
- c. Approximately 50% of patients with AIHA will have sera containing *autoantibody reactive with all cells tested*, reagent as well as donor. The autoantibody is usually IgG and is best demonstrated by IAT.
- d. **The serum may contain alloantibodies in addition to auto-antibodies.** It is very important to identify underlying alloantibodies.

4. Eluate

- a. The presence of the IgG autoantibody on the RBCs can be confirmed by elution, using the digitonin acid technique or with organic solvents such as ether or xylene.
- b. IgG may be present at such low levels that no detectable antibody is recovered by elution.
- c. If only complement is found coated to the RBCs, the eluate will have no serological activity.

5. Specificity of the Autoantibody

- a. The specificity of auto-antibodies associated with WAIHA **is very complex**.
- b. **Specificity is often initially directed against the Rh antigen complex.**
- c. Apart from Rh specificity, there have been other reports of warm auto-antibodies with other specificities (refer to textbook).
- d. It is rarely, if ever, necessary to perform additional testing to ascertain autoantibody specificity in order to select antigen-negative blood for transfusion. In some patients, simple specificity will be readily apparent (eg, anti-e). When there is evidence of immune hemolysis and the simple autoantibody specificity is clear cut - not relative- there may be some benefit in providing antigen negative blood.
- e. It is important **not to expose the patients to Rh antigens that their own red cells lack, especially D.**
- f. If specificity is directed at a high incidence antigen compatible donor blood is unlikely to be found. If such blood is available, it should be reserved for alloimmunized patients of that uncommon phenotype.
- g. The specificity of warm-reactive auto-antibodies is usually only of academic interest, unless the patient is actively hemolyzing and requires blood transfusion.

6. Detection of Alloantibodies in the Presence of Warm-Reactive Auto-antibodies.
 - a. If warm-reactive auto-antibodies are present in the serum, it is important to establish that alloantibodies are not also present when the patient concerned requires blood transfusion.s
 - b. Alloantibodies may not be readily apparent from the results of initial studies; their presence may be masked by auto-antibodies.
 - c. It is helpful to know the blood group phenotype of the patient's cells to aid in predicting what alloantibodies they may have now or may make in the future.
 - d. When the RBCs are coated with IgG, AHG reactive reagents such as anti-Fy^a or anti-Jk^a cannot be used unless the IgG is removed from the RBCs prior to testing. A procedure for this, involving **chloroquine diphosphate** is found in the Technical Manual.
 - e. **Autologous adsorption (autoadsorption) is the best way to detect alloantibodies in the presence of warm-reactive auto-antibodies. (MEMORIZE THIS PROCEDURE)**
 - 1) At 37C, in vivo adsorption will have occurred and all antigen sites on the patient's own RBCs may be blocked. It is necessary, therefore, to elute autoantibody from the RBCs to make the sites available for in-vitro autoadsorption. Gentle heat elution works very well.
 - 2) Treatment of the autologous RBCs with proteolytic enzymes increases their capacity to adsorb autoantibody.
 - 3) Patient serum is added to an equivalent volume of patient enzyme treated cells and incubated at 1 hour at 37C. During this incubation period antibody in the patient serum will be adsorbed onto the antigens on the patient cells.
 - 4) The serum/cell mixture is spun down, the "autoadsorbed serum" is removed and tested against reagent screen cells. Interpretation of results is as follows:
 - a) all RBCs tested give negative results, auto-antibody only
 - b) all RBCs tested still positive, autoabsorption unsuccessful, repeat up to 3 times
 - c) pattern of pos and neg reactions, allo-antibody present, perform a panel with autoadsorbed serum.
 - 5) **This procedure cannot be performed on recently transfused patients.** The patient may have an underlying alloantibody, the transfused cells may possess the antigen causing autoantibody **and alloantibody to be adsorbed out.**
7. Transfusion Considerations
 - a. **It is best to avoid transfusing patient who have AIHA.**
 - b. If transfusion is **essential, the smallest volume of RBCs necessary to maintain adequate oxygen transportation should be given.**
 - c. Many times serologically compatible blood may not be obtainable, and there is substantial risk that concomitant alloantibodies could cause a severe hemolytic transfusion reaction.

- d. The clinical need **must justify** the risk of transfusion, but needed transfusions should not be withheld because serologically compatible blood cannot be found. The doctor must sign a release form whenever incompatible blood must be given.
- e. If alloantibodies have been identified, blood lacking the corresponding antigen(s) should be selected for transfusion.
- f. In many cases of WAIHA, no autoantibody specificity is apparent; the patient's serum reacts with all RBC samples tested to the same degree, or reacts with RBCs from different donors to varying degrees.
 - 1) In such cases, some workers test a large number of donor blood samples (eg, 12-20) with patient serum, or if autoadsorption was successful, with autoadsorbed serum, and select those units that give the weakest reactions in vitro.
 - 2) There are no data to indicate better in vivo survival of these "**least incompatible**" units, but some workers feel more comfortable issuing the least incompatible units.
 - 3) It is best to transfuse blood of the same Rh phenotype of the patient if possible.
- g. Although RBC survival of transfused cells may not be normal, transfusion with incompatible blood may provide sufficient oxygen-carrying capacity until other therapy can effect a more lasting benefit.
- h. In summary, transfusion of patient with WAIHA should be undertaken **only if absolutely essential**, with the realization that the blood is **not truly compatible** and its effects are likely to be brief.
 - 1) The volume transfused should be the **least amount to maintain adequate oxygen transportation**.
 - 2) An attempt should be made to **differentiate autoantibody from alloantibody** in the serum. If specificity is obvious, **the appropriate donor blood should, if feasible, be selected**.
 - 3) **Blood should never be withheld from a patient with severe life-threatening anemia because of incompatibility due to auto-antibodies.**

E. Cold Agglutinin Syndrome (CAS), formerly called Cold Hemagglutinin Disease (CHD)

1. Introduction

- a. CAS is the **most common type of hemolytic anemia associated with cold-reactive auto-antibodies**, and accounts for approximately 16% of all cases of immune hemolysis.
- b. It occurs as an acute or chronic form.
 - 1) The acute form is often **secondary to lymphoproliferative disorders (eg, lymphoma) or Mycoplasma pneumoniae infection**.
 - 2) The chronic form is often seen in elderly patients and results in a mild to moderate degree of hemolysis. **Raynaud's phenomenon and hemoglobinuria may occur in cold weather.**

2. Direct Antiglobulin Test

- a. **Complement is the only globulin detected on the RBCs.**

- b. **The cold-reactive auto-agglutinin is IgM that binds to RBCs in the peripheral circulation where the temperature may fall to 32C. IgM binds complement components to the RBCs at this temperature. As the RBCs return to warmer parts of the circulation, the IgM dissociates, leaving RBCs coated only with complement.**
3. Serum
- a. IgM cold-reactive auto-agglutinins associated with immune hemolysis are **usually present at titers greater than 1000 when tested at 4C.**
 - b. They rarely react in vitro above 32C in tests with saline suspended RBCs. However, if 30% bovine albumin is included in the reaction medium, 70% of clinically significant examples will react at 37C (show high thermal amplitude).
 - c. Antibody screening tests may be performed strictly at 37C (pre-warmed). The patients serum, screen cells and saline to be used for washing are allowed to warm to 37C. Serum is then added to the cells and incubated for 1 hour. After incubation the cells are **immediately** washed with the warm saline and taken to Coombs. If a pattern of reactivity is observed a prewarmed panel is done in the same manner.
4. Eluate
- a. If the RBCs have been collected and washed at 37C, *no antibody reactivity will be found in the eluate*, as only complement components are present on the RBCs in vivo at 37C.
 - b. Eluates are usually only done when the DAT is positive with anti-IgG.
5. Specificity of the Autoantibody
- a. **The most common specificity associated with CAS is anti-I.**
 - b. Less commonly, anti-i is found, usually associated with **infectious mononucleosis.**
6. Detection of Alloantibodies in the Presence of Cold-Reactive Auto-antibodies.
- a. Potent cold-reactive autoagglutinins, like warm-reactive auto-antibodies, may mask the presence in serum of clinically significant alloantibodies.
 - b. If the autoagglutinins are reactive at 32C, or if they bind complement to RBCs in the IAT, it is advisable to perform a **cold autoadsorption. (MEMORIZE THIS PROCEDURE)**
 - 1) Enzyme treat patient cells to enhance adsorption of autoantibody onto the patient cells.
 - 2) Allow the serum specimen to clot at 4C for 1 hour.
 - 3) Add serum to equivalent volume of patient's own cells.
 - 4) Incubate at 4C for 1 hour.
 - 5) Harvest autoadsorbed serum and test against screen cells. Negative reactions with all screen cells is indicative of no underlying alloantibody. Positive reactions with all three screen cells indicates the need for an additional autoadsorption with new aliquot of cells (maximum of 3 autoadsorptions can be done). If 1 or 2 of the three screen cells is positive perform a panel and identify antibody specificity.
 - 6) Autoadsorption **cannot be performed on recently transfused patients.**

- c. *Rabbit Erythrocyte Stroma Test (REST)*
 - 1) It was discovered that rabbits possess the I antigen on their RBCs. Rabbits are bled and their RBCs hemolyzed. The cells are washed free of hemoglobin and put into a preservative solution.
 - 2) Patient serum is added to the cell stroma and incubated at 4C to adsorb out the anti-I.
 - 3) *This method can be utilized if the patient has been recently transfused.*
 - 4) More than 1 adsorption may be necessary.

7. Selection of Blood for Patients with CAS

- a. Patients suffering from CAS rarely require transfusion.
- b. If the need arises, compatibility tests should be performed in ways that minimize cold-reactive autoantibody activity yet still permit detection of clinically significant alloantibodies.
- c. Some workers perform compatibility tests strictly at 37C. The donor RBCs and patient's serum are warmed to 37C prior to mixing, and RBCs are washed with 37C saline for the AHG test (**prewarmed technique**).
- d. The use of albumin and other potentiators of agglutination should be avoided, since these enhance the complement-binding properties of the autoantibody.
- e. IgG AHG is used for the AHG test.
- f. If the prewarmed technique does not work perform an autoabsorption.
- g. Blood for transfusion *should be warmed* during infusion into the patient.

F. Mixed Type AIHA

1. Introduction

- a. These patients have cold agglutinins that have low titers at 4 C but high thermal amplitude.
- b. Can be idiopathic or secondary, often associated with systemic lupus erythematosus.
- c. Often presents as an extremely acute condition characterized by very low hemoglobin and complex serum reactivity present in all phases of testing.

2. Direct Antiglobulin Test

- a. *Both IgG and C3d* are detectable on patient's RBCs.
- b. IgG is due to warm autoantibody and C3d I bound by the effects of the IgM autoantibody.

3. Serum

- a. Both warm reactive IgG and cold reactive IgM auto-antibodies are present *resulting in reactivity at all phases of serum testing.*
- b. Cold agglutinin titer is **less than 64** at 4 C.
- c. *Both warm and cold auto-absorptions may be necessary* to determine the presence of alloantibodies.

4. Eluate - will be reactive due to the presence of IgG autoantibody.

5. Specificity of Autoantibody

- a. Cold autoantibody may have typical specificity, anti-I or -i, but often has no apparent specificity.

- b. The IgG warm autoantibody is serologically indistinguishable from specificities encountered in WAIHA.

6. Selection of Blood

- a. Transfusion should be considered carefully, especially since prompt corticosteroid therapy is frequently successful.
- b. *Transfusion may result in increased hemolysis*, which may be life threatening.
- c. Considerations for blood selection is identical to that for WAIHA and CAS.

G. Paroxysmal Cold Hemoglobinuria (PCH)

1. Introduction

- a. This is the **rarest** form of autoimmune hemolytic anemia.
- b. PCH presents as an acute transient condition secondary to viral infections, particularly in young children or as an idiopathic chronic disease in older people.
- c. It is marked by episodes of hemoglobinemia and hemoglobinuria *after exposure to the cold*.

2. Direct Antiglobulin Test

- a. The autoantibody in PCH is an **IgG** protein. Like IgM cold-reactive autoagglutinins, **the IgG autoantibody in PCH reacts with RBCs in colder parts of the body, causes complement components to be bound irreversibly to RBCs, and the IgG elutes off at warmer temperatures in the body.**
- b. Consequently, complement only is detected on the RBCs.

3. Serum

- a. The IgG autoantibody in PCH is classically described as a **biphasic hemolysin**, since it binds to RBCs at low temperatures and then causes hemolysis when the coated RBCs are warmed to 37C. This is the basis of the diagnostic test for the disease, **the Donath-Landsteiner test**.
- b. The autoantibody often agglutinates normal RBCs at 4C, **but rarely to titers greater than 64**.

4. Eluate

- a. As in CHD, only complement components are present on RBCs in vivo at 37C.
- b. Consequently, eluates prepared from RBCs of patients with PCH are *invariably nonreactive*.

5. Specificity of the Autoantibody

- a. The autoantibody of PCH has most frequently been shown to have **anti-P** specificity.
- b. The *antibody reacts with all RBCs* (including the patients) except those of the very rare p or Pk phenotypes.

6. Detection of alloantibodies which may be masked by auto-antibodies in PCH is the same as that described for CHD except only cold auto-adsorption may be done. The REST procedure only removes anti-I. So if a patient has been recently transfused, autoabsorption cannot be done.
7. Selection of blood for patients with PCH.
 - a. Sera from patients with PCH will be compatible with random donor RBCs when tested by routine crossmatch procedures.
 - b. The causative antibody rarely reacts as an agglutinin above 4C.
 - c. While there is some evidence that p RBCs survive better than RBCs of a common P-system phenotype, the incidence of p blood is approximately *1 in 200,000*.
 - d. PCH patients often require transfusion before rare blood can be obtained, and the transfusion of random donor blood should not be withheld for patients requiring urgent transfusions.
 - e. Rare p blood should be considered **only** for those patients who do not respond to random donor blood.

H. Drug Induced In-Vivo RBC Sensitization

1. Introduction

- a. Drugs sometimes induce the formation of antibodies, either against the drug itself or against intrinsic RBC antigens.
- b. Most drugs have a molecular weight substantially below the 5000 dalton level usually considered to be the threshold for effective immunogenicity.
- c. *Drugs act as haptens*, eliciting antibody only after they have been firmly bound to a protein carrier.
- d. Once formed, the antibody can react with the small hapten independent of any protein attachment.
- e. Drugs may cause a positive DAT, which may or may not be associated with immune hemolysis, by one of four mechanisms.

2. Drug Adsorption (DA)

- a. Approximately 3% of patients receiving large doses of penicillin IV (eg, millions of units per day) and cephalosporins will develop a positive DAT, but less than 5% of these will develop hemolytic anemia.
- b. Intravascular hemolysis is **rare**.
- c. *The mechanism of the positive DAT is clear.*
 - 1) The penicillin is adsorbed to the RBCs in vivo.
 - 2) If the patient has formed antibodies to penicillin, the anti-penicillin will react with the penicillin bound to the RBCs.
 - 3) This results in penicillin-coated RBCs becoming coated with IgG.
 - 4) Complement is not usually involved. If hemolysis occurs, RBCs are destroyed extra vascularily, probably in the same way that RBCs coated with IgG alloantibodies are destroyed.

- d. The clinical and laboratory features of penicillin-induced hemolysis are:
- 1) The *DAT is strongly positive* due to IgG coating.
 - 2) Unless alloantibodies are present coincidentally, screening tests for unexpected serum antibodies will be nonreactive.
 - 3) Antibody eluted from the RBCs will *react with penicillin-coated RBCs but not with uncoated RBCs*.
 - 4) A high-titer IgG antibody to penicillin is always present in the serum.
 - 5) Hemolysis typically develops only in patients receiving very large doses of IV penicillin.
 - 6) Hemolysis is subacute in onset, but may be life-threatening if the etiology is unrecognized and penicillin administration is continued.
 - 7) Discontinuation of penicillin therapy is usually followed by cessation of hemolysis. However, hemolysis of decreasing severity may persist for weeks.

3. Immune Complex (IC) Adsorption

- a. Some drugs do not bind directly to RBCs, but have a high affinity for their specific antibodies and form *antigen-antibody complexes that circulate in the plasma*.
- b. These immune complexes *attach nonspecifically to RBCs* and initiate *complement activation* of the RBC surface which may lead to **intravascular hemolysis**.
- c. RBCs that are not hemolyzed have a positive DAT because of complement coating, but immunoglobulin may also be bound.
- d. The immune complexes may dissociate after activating complement and go to react with other RBCs. This may explain why small amounts of the drug can induce acute hemolytic episodes.
- e. This mechanism is the one **least often encountered** when investigating drug-induced hemolysis.
- f. The characteristic findings are:
 - 1) Acute intravascular hemolysis with hemoglobinemia and hemoglobinuria is the usual presentation. Renal failure occurs in approximately 50% of the cases.
 - 2) Once antibody has been formed, the patient may experience severe hemolytic episodes after taking only small quantities of the drug.
 - 3) The antibody can be either IgM or IgG.
 - 4) The RBCs are often coated only with complement.
 - 5) In-vitro reactions such as agglutination, hemolysis and reactive IATs can only be demonstrated when the patient's serum and reagent RBCs are incubated in the presence of the drug.

4. Membrane Modification

- a. *Drugs of the cephalosporins are the only drugs that are thought to alter RBC membranes in such a way that the RBCs adsorb all proteins in a nonspecific manner. When this occurs in vivo it can cause a positive DAT.*
- b. In addition to this non-immune adsorption of proteins, the cephalosporins can also *induce a positive DAT by the mechanism described for penicillin.*
- c. The drug binds firmly to RBCs, which then interact with the specific anticephalosporin antibody.
- d. Approximately 4% of patients receiving cephalosporins develop a positive DAT.
- e. There have been *occasional reports of hemolysis* resulting from cephalosporin therapy; these are thought to result from the effect of specific anticephalosporin antibodies, rather than from nonimmunologic adsorption of proteins following membrane modification.

5. Induction of Autoimmunity

- a. The first cases of WAIHA resulting from ***alpha methyldopa*** (Aldomet, Aldoclor, Aldoril) therapy were described in 1966.
- b. Following alpha methyldopa therapy, auto-antibodies are formed that react with intrinsic RBC antigens. They do not react with the drug in vitro, either directly or indirectly.
- c. The serological findings are **indistinguishable** from those associated with WAIHA; often, the autoantibody can be found to have an Rh-related specificity.
- d. It has been suggested that the drug interferes with suppressor T-cell function allowing over-production of autoantibody by B cells.
- e. **Therapy with alpha methyldopa accounts for more cases of drug-induced positive DATS and immune hemolysis than all of the other drugs.**
- f. The clinical and laboratory features are as follows:
 - 1) Positive DATs occur in approximately 15% of patients receiving alpha methyldopa. Only 0.5 - 1.0% of patients taking alpha-methyldopa develop hemolytic anemia.
 - 2) RBCs are usually coated only with IgG, but occasionally weak complement coating is also present.
 - 3) The DAT usually becomes positive only after 3-6 months of therapy.
 - 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 RBCs are indistinguishable from those found in idiopathic WAIHA.
 - 6) The strength of the positive DAT becomes progressively weaker once alpha-methyldopa therapy is discontinued. This may take from 1 month to 2 years. In patients with hemolytic anemia due to alpha-methyldopa therapy, hematologic values usually improve within the first week or so after the drug therapy is discontinued.

I. Laboratory Investigation of Drug-Induced Hemolysis

1. Introduction

- a. The drug-related problems most commonly encountered in the blood bank are those associated with a positive DAT, and alpha-methyldopa is by far the most common cause of a drug-induced positive DAT.
- b. Drugs of the alpha-methyldopa group may cause reactive IAT in serum/red cell mixtures without added drug.

2. The serological evaluation of drug induced hemolysis is carried out in essentially the same manner as that to investigate AIHA.

- a. Monospecific antiglobulin sera are useful for the DAT.
 - 1) Drugs that cause a positive DAT by the immune complex mechanism primarily bind complement to RBCs.
 - 2) Penicillin or alpha-methyldopa cause IgG to be bound to the RBCs.
 - 3) Red cells that have adsorbed proteins nonimmunologically in association with drugs of the cephalosporin group react with some or all antiglobulin sera.
- b. The patient's serum should be tested for unexpected antibodies by the routine procedures used in pretransfusion testing.
 - 1) If the serum does not react with untreated RBCs, the tests should be repeated against ABO-compatible RBCs in the presence of any drug that the patient has been receiving.
 - 2) Techniques for testing may be those described in published cases, if the drug is one already reported as being immunogenic.
- c. If these tests are not informative, an attempt should be made to coat normal RBCs with the drug, and test the patient's serum and an eluate from the patient's RBCs against the drug coated cells.
 - 1) This is the method of choice when penicillin or the cephalosporins are the suspected cause of the positive DAT.
 - 2) The definitive test result for a penicillin-induced positive DAT is a positive IAT with the eluate and penicillin-coated RBCs but not with the eluate and untreated RBCs.
 - 3) Drugs that induce a positive DAT by the immune complex mechanism often bind only complement to RBCs, so that the eluate may be nonreactive, even when the drug is added to the test system.

Exam 6 Online ***Discuss Laboratory Practical***