Red cell transfusions in autoimmunized patients

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There are several different settings in which the transfusion of red cells to patients who have autoantibodies present in their serum may be necessary. Because the serological approach to the selection of blood varies in different clinical and immunohematologic situations, the various settings are described here.

Transfusion in "warm" antibody-induced (autoimmune) hemolytic anemia (AIHA)

Most investigators agree that transfusion is contraindicated for patients with this disease and elect to use alternative forms of therapy, until and unless transfusion becomes essential to prevent the patient's death from progressive anemia or its consequences.[1-6] There are several reasons for not giving these patients transfusions in other than life-threatening situations. First, AIHA is not simple anemia and is not corrected by the administration of red cells. In most instances, transfused red cells survive in vivo for no longer than the patient's own cells, meaning that the effects of transfusion are palliative and short-lived. Second, it is seldom possible to find compatible blood for transfusion to these patients, since most autoantibodies that cause AIHA have a specificity that results in their reacting with the red cells of almost all donor units.[4,7-12]

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Third, there is anecdotal evidence that transfusion in patients with AIHA can cause the disease to become more severe. Some workers have observed that patients with AIHA who require transfusions because of life-threatening anemia have a more severe long-term course, and that the disease is more difficult to control with steroid or other therapy than when remission can be induced by steroid therapy without supportive transfusions. It is possible that the introduction of additional red cells via transfusion, when those red cells are rapidly destroyed by the patient's autoantibody, acts as a stimulus to the patient's already malfunctioning immune system. The reason that the evidence is anecdotal is that a carefully controlled study cannot be performed. Those patients who must be given transfusions are self-selected based on the severity of the anemia.

Thus, it can be argued that patients who must be given transfusions are most severely affected and that the transfusions are necessary because of the severity of the disease and not because the disease is difficult to control after the patients have received transfusions. Despite this, many clinicians elect to give transfusions only when the patient has a life-threatening condition. Even if transfused red cells do not aggravate the condition, they do little to alleviate it for reasons discussed.

Although transfusions to patients with AIHA are contraindicated when not absolutely necessary (Pirofsky[5] has reported that the clinical condition of the patient and not the hematocrit value or the apprehensions of the physician must be treated), it is equally wrong to withhold transfusions because compatible blood is not available, once the decision to transfuse, based on clinical grounds, has been made. In such circumstances, blood incompatible with the autoantibody of the patient, but compatible with any alloantibodies present should be given. Such transfusions rarely cause overt transfusion reactions and, although the transfused cells seldom survive longer in vivo than those of the patient, they may keep the patient alive until such time as alternative therapies act to control the disease process.

Although AIHA patients should be given blood compatible with alloantibodies present in their sera, it is often technically difficult to ensure that donor units selected satisfy this criterion. The difficulty is that in 60% to 70% of such patients free autoantibody will be present in the serum of the patient.[1, 2, 4, 8] Most of the causative autoantibodies react with all red cells tested (*Table 1*).[13] Thus, when the autoantibody in the patient's serum reacts with all red cells, it becomes necessary to use a technique that will allow recognition of any alloantibodies simultaneously present. The difficulty in achieving this objective is demonstrated by the number of tests designed for this purpose (*Table 2*). The appropriate test selected is dictated by time and resources available, and by the serological expertise of the investigator.

Differential adsorption

With this technique, separate aliquots of the patient's serum are adsorbed with R_1R_1, R_2R_2, and rr red cells. The adsorbed aliquots (adsorbed X1, X2, or X3, dependent on the amount of free autoantibody in the serum) are then tested for antibody specificity. It is hoped that the autoantibodies will be removed and that any alloantibody present will be unadsorbed and demonstrable in one or more of the aliquots. Since Rh autoantibodies are so common in AIHA (*Table 1*), this technique is

Table 1. Specificities and reaction patterns of autoantibodies causative of AIHA

Test red cells	Group I* "Simple" specificity autoantibodies	Group 2† Anti-nl	Group 3‡ Anti-pdl	Group 4§ Anti-dl
Common Rh phenotype (e.g., R_1R_1, R_2R_2, rr)	As expected dependent on the specificity of the autoantibody present	+	+	+
D-deletion (e.g., D- -/D- -, Dc-/Dc-)		0	+	+
Rh_{null}		0	0	+

* Group 1 may be almost any specificity Rh antibody such as anti-c, -e, -C, -D, -E, -G, -rh_i is seldom present as the only autoantibody, and is frequently found in sera (or eluates) that contain anti-nl, -pdl, -dl. Some autoantibodies that initially appear to have simple specificity can be shown, by adsorption studies, to be more complex.[9, 13] Rarely, simple specificity autoantibodies directed against antigens such as M, N, Jk^a, Xg^a may be seen.

† Examples of anti-nl may be, or contain, anti-Hr, anti-Hr_o and/or anti-Rh34.

‡ Examples of anti-pdl sometimes are, but more often contain, anti-Rh29, anti-LW and/or anti-U (anti-LW and anti-U can be identified when present in "pure" form in conventional antibody identification tests if the appropriate red cells are used).

§ Examples of anti-dl sometimes are, but more often contain anti-Wr^b, anti-En^a, anti-Kp^b, anti-K13, and/or anti-Vel (these antibodies can be identified when present in pure form in conventional antibody identification tests if the appropriate red cells are used).

Table 2. Methods for the isolation of alloantibodies in sera that contain warm reactive autoantibodies

Triple adsorption procedure
Partial elution/autoadsorption method
Antibody identification with diluted serum
Use of Rh phenotype-identical blood
Blind titration not recommended

coupled with determination of the patient's Rh phenotype. Such tests are performed with saline-active anti-Rh antisera to avoid false positive reactions due to the patient's antibody-coated red cells. As an example, a patient's serum may contain auto-anti-pdl (*Table 1*) that reacts with all except Rh_{null} red cells. In the serum aliquot adsorbed with R_1R_1 red cells, all anti-pdl may have been removed and anti-c might be identified. In the aliquot adsorbed with rr red cells, the anti-pdl and anti-c may have been adsorbed, and anti-D left unadsorbed. If the patient's red cells have been shown to be D−, C−, E−, c+, e+, it will be clear that the anti-pdl and anti-c are autoantibodies, whereas the anti-D is an alloantibody. In other words, the patient could be given rr blood, which would be incompatible with the auto-anti-pdl and auto-anti-c, but must not be given Rh+ blood, which would be incompatible with the allo-anti-D.

Since antibodies in other than the Rh system may be present, and since their presence may also be masked by autoantibody in the serum, the R_1R_1, R_2R_2 and rr cells used should be selected with care. They should lack other antigens, i.e., one Jk (a−), one JK (b−), one Fy (a−), one Fy (b−), so that non-Rh system alloantibodies remain unadsorbed in one or more of the adsorbed aliquots. Although this triple adsorption procedure involves much work, and is time-consuming, it is one of the best methods for the detection and identification of alloantibodies present in sera that contain autoantibodies.

Partial elution and autoadsorption

The ideal red cells to adsorb autoantibodies from a serum and leave al-

loantibodies unadsorbed are those of the patient. However, on most occasions when there is autoantibody in the serum of a patient with AIHA, the red cells of the patient are maximally coated with autoantibody. Attempts to autoadsorb the serum with such cells are seldom successful, because there are too few unbound antigen sites to effect adsorption of appreciable amounts of serum autoantibody. This difficulty can be obviated by eluting in vivo bound autoantibody while leaving the antigen sites of the cells unaffected, so that they will then adsorb autoantibody from the serum. In practice, many elution methods are unsuitable since in the process of removing autoantibody the red cells are destroyed. A compromise involves the heat elution method, which, if the time of elution is reduced, results in partial antibody dissociation without red cell destruction.[14] The washed red cells of the patient are heated at 56 C for 5 or 10 minutes, then washed again to remove the dissociated antibody. The red cells, which still have some antibody bound, but now have more sites available for autoantibody adsorption, are used to adsorb the patient's serum. This method occasionally works well. Presumably, the difference represents the binding affinity of the autoantibody (much or little removed during the 56 C step). The method has also been modified so that after the 56 C elution, the patient's red cells are premodified with papain or ficin. This modification is effective if the autoantibody to be adsorbed reacts with protease-treated red cells. However, it must be remembered that some autoantibodies (such as the anti-Pr series and certain specificities of anti-En^a) complex with antigens that are denatured when red cells are protease-treated.

Although this method employs the ideal red cells, its shortcoming is that sometimes too little in vivo-bound autoantibody is eluted to permit adsorption of serum autoantibody.

Antibody identification tests with diluted serum

Another technique to identify alloantibodies in sera containing autoantibodies seeks to dilute the autoantibody to a point of reduced reactivity so that alloantibody activity can be seen.[4]

The patient's serum is titrated against pooled red cells. An attempt is made to determine the titer of the autoantibody that will usually react with all cells by ignoring mixed field reactions of higher titer that may represent an alloantibody reacting with the cells of one or more of the donors. For example, if the autoantibody gives a 1+ reaction at a dilution of 1 in 8, the serum is diluted 1 in 8 and a panel study is performed. It is hoped that alloantibodies present will be identifiable from their strong reactions with certain of the panel cells. As an example, if all Fy (a+) red cells gave 3+ reactions, while all Fy (a−) samples gave the 1+ reaction expected of the autoantibody, evidence of the presence of allo-anti-Fy^a would have been obtained. This method will detect only alloantibodies that react to higher titer than the autoantibody present. However, this fact is not a serious disadvantage since free serum autoantibodies in patients with AIHA seldom have high titers.

This titration method is much superior to the blind titration method. In that method, the patient's serum is serially diluted and tested against the red cells of random donors. Those units reacting to the lowest titer are selected for transfusion as "least incompatible." The danger with such a procedure is that it is performed without knowledge of the specificity of any autoantibodies or al-

loantibodies present. If a patient has auto-anti-e with a titer of 8, and an allo-anti-c with a titer of 4, an R_2R_2 unit (c+, e−) will appear more compatible than an R_1R_1 unit (c−, e+) and will be selected as least incompatible. Whereas the patient would probably tolerate transfusion of an R_1R_1 unit (incompatible with autoantibody), a transfusion reaction is likely to occur if he is given an R_2R_2 unit (incompatible with alloantibody). The reason that an alloantibody of lower titer than free autoantibody is more likely than the autoantibody to cause a frank transfusion reaction is that the autoantibody is already clearing incompatible red cells (the patient's own) while the alloantibody is inactive in vivo until incompatible red cells are transfused.

Transfusion of Rh phenotype identical blood

In an emergency, when a patient with AIHA and severe anemia requires a transfusion, there may be too little time for any of the above procedures. In such instances blood of the same Rh phenotype as the patient's can be given. Determining the Rh phenotype of the patient and potential donors takes only minutes. Such blood will be compatible with allo-anti-Rh antibodies present in the patient's serum. However, such a procedure does not safeguard against antibodies such as anti-K, anti-Fy^a, anti-Jk^a, and should be used only in extreme emergencies.

Comparison of the methods described

In *Table 1* the methods listed in order for selecting blood for patients with autoantibodies in their sera become less time-consuming but also less efficient. The method used will depend, at least in part, on the urgency. No method for identifying alloantibodies in sera that contain autoantibodies is foolproof. Even after units have been selected with maximum care, the blood should be transfused slowly and the patient carefully watched for signs of any adverse reaction.[6]

Some investigators believe that the triple adsorption procedure is no more time-consuming than the partial elution, autoadsorption method, because in the former the fully phenotyped R_1R_1, R_2R_2, and rr red cells can be available in any laboratory that handles such cases. In contrast, in the latter procedure, the red cells of each patient must be treated. However, one advantage of the partial elution, autoadsorption procedure is that following the autoadsorption, the serum can be used for compatibility tests instead of for antibody identification studies. Any unadsorbed antibodies present will be alloimmune (unless adsorption of the autoantibodies is incomplete). In the triple adsorption procedure this is not possible, since it is not known which of the unadsorbed antibodies are autoantibodies, and which are alloantibodies, until identification studies are completed and reviewed in light of the patient's red cell phenotype.

Transfusion in AIHA when the patient's serum contains no free autoantibody

When the patient's serum contains no free autoantibody, it is easy to determine whether alloantibodies are present. If none are detected, or if blood lacking the antigen(s) to which any present is (are) directed is selected, it will appear from in vitro tests that the blood selected is compatible. This is not the case. The patient's autoantibody, bound to the patient's cells at the time the serum was collected, does not remain bound. More autoantibody is made and

some autoantibody molecules elute from the patient's red cells. Thus, when the blood is transfused it will be incompatible in vivo if it carries the antigen against which the autoantibody is directed. For this reason, an AIHA patient with no free autoantibody in the serum is no more suitable for transfusion than one with serum autoantibody. The contraindications for transfusion discussed earlier apply in both patient groups.

Transfusion in AIHA when no alloantibodies are present

When it has been shown with a reasonable degree of certainty that the AIHA patient to receive a transfusion does not have any alloantibodies, a different consideration applies. If blood lacking the antigen against which the autoantibody is directed is available, such red cells may survive longer in vivo than the patient's own cells. For example, in an R_1r patient with auto-anti-e, R_2R_2 cells will survive longer than R_1R_1 or R_1r cells.[3] Since transfusion in AIHA is contraindicated, enhanced in vivo survival of transfused red cells is advantageous because the number of transfusions is reduced. However, opportunities to transfuse cells more compatible than the patient's own arise infrequently. In our experience,[2, 8, 9] only 2% to 3% of patients with AIHA have just "simple" specificity Rh autoantibodies, e.g., anti-c, -e, -C, -D, -Ce, -G, present. In most instances, when an antibody such as auto-anti-e is present, it is accompanied by other autoantibodies of "broader" specificity, e.g., anti-nl, anti-pdl (*Table 2*). In an R_1r patient in whom auto-anti-e can be demonstrated after auto-anti-pdl has been removed by adsorption, there is no advantage in transfusing R_2R_2 blood, since such blood will be incompatible with, and will be destroyed by, the anti-pdl.

Some investigators disagree with the policy of transfusing blood compatible with simple specificity auto-anti-Rh antibodies present on the grounds that it involves the introduction of foreign Rh antigens. For example, an R_1R_1 patient with auto-anti-e who is given R_2R_2 blood is exposed to the antigens c and E that are lacking in his red cells. Thus, although the R_2R_2 red cells may survive better than would R_1R_1 cells, their introduction can in theory at least stimulate the production of allo-anti-c and allo-anti-E. In our experience[7] such a risk is so small as to be of no practical consideration. We have found the de novo production of alloantibodies by patients with AIHA to be a rare event. Those patients making alloantibodies at the time of diagnosis of the AIHA continue to make them despite the immunosuppressive forms of treatment used. Those who do not have alloantibodies present when AIHA is diagnosed seldom seem to begin a new immune response even when they receive a transfusion. One exception, as would be expected, involves the antigen D. Rh-negative patients with AIHA who receive transfusions with Rh-positive blood are almost as likely as other Rh-negative patients given Rh-positive blood to make anti-D. For this reason, when we encounter, for example, an rr patient who must be given a transfusion and who has auto-anti-e in the serum, we use rr blood to avoid stimulating production of anti-D, which would be likely if R_2R_2 blood were used. It can be argued that R_2R_2 blood can be used for the first series of transfusions and then rr blood can be used if the patient makes anti-D, and if further transfusions become necessary. However, our concern is that if the patient is admitted to a different hospital where the transfusion records are not available, the potential

for a serious transfusion reaction exists. The patient's red cells may be mistyped as D+ (a relatively easy mistake to make with red cells heavily coated with antibody, DAT 4+, and the serum allo-anti-D may be overlooked if much free autoantibody, e.g., anti-nl, is present. Thus, it may appear that the patient is D+ with an autoantibody incompatible with all cells present in the serum. Rh-positive blood may be used and may cause a serious transfusion reaction because of the hidden allo-anti-D.

On some occasions it is possible theoretically to avoid transfusing blood incompatible with autoantibodies by the use of ultrarare units.

Autoantibodies directed against the antigens Hr, Hr_0, Rh29, LW, U, Wr^b, En^a, Kp^b, K13 have been incriminated as causative of AIHA[13] (*Table 2*). When such an antibody is involved, only rare donor units would be compatible and would be expected to survive for a longer period in vivo than the patient's own cells. However, most workers including this author consider units such as those from D--/D--, U-negative, Kp(b−) donors too rare to be used in cases of AIHA, and instead reserve them for patients alloimmunized against one of the high-incidence antigens. It must be remembered that in AIHA the patient's own cells are incompatible and that blood incompatible with the autoantibody will usually survive as long as the patient's own cells. Such survival may be long enough to get the AIHA patient through an acute hemolytic episode until such time as treatment takes effect. In cases of alloimmunization this consideration does not apply and most persons managing a patient whose serum contains allo-anti-U, or allo-anti-Kp^b or similar antigens would not use incompatible blood unless there was no alternative.

Washed red cells and AIHA

It is not unusual to be asked to supply washed red cells for transfusion to patients with AIHA. However, the condition itself is not one in which washed cells have any significant advantage over packed red cells. Sometimes the request is made "in order that washing remove those incompatible antigens." The autoantibodies causative of AIHA are directed against antigens that are integral parts of the red cell membrane that are not altered when red cells are washed. Thus, requests for washed red cells for patients with AIHA should be refused unless there are reasons, such as presence of leukoagglutinins or a history of febrile transfusion reactions, that would indicate the need for washed red cells in a non-AIHA patient.

AIHA and transfusion; final considerations

Having dealt at length with resolution of difficulties encountered in the transfusion of patients with AIHA, it should again be stressed that transfusion is best avoided. Not all investigators agree about the potential danger of transfusion (subsequent increase in the amount of autoantibody made), but there can be no disagreement that the effects of transfusion are palliative and short-lived and are not curative of the disorder. Thus, when the AIHA patient has a hemoglobin of 10 g/dl, transfusion is virtually never necessary. When the hemoglobin is 6 g/dl, it is likely that the patient will be reasonably well compensated, having reached that hemoglobin level gradually. When the hemoglobin is 3 g/dl, transfusion may not be essential. With such a hemoglobin level, it may be possible to treat the patient with bed rest and supportive oxygen if necessary until the hemoglobin starts to

rise, as steroid therapy decreases the rate of in vivo red cell destruction and of autoantibody production. A physician with an anemic patient in whom further red cell destruction is occurring will often want to add red cells to the patient's circulation. Although it is not the responsibility of the blood bank to decide when transfusion is essential, it is the responsibility of the blood bank to point out that transfusions are not curative and, if administered too soon, may have an adverse long-term effect on the patient.

Just as it is wrong to transfuse too early in AIHA, it is wrong to withhold blood when transfusion becomes essential on clinical grounds because no compatible blood is available. Once the physician notices signs of cardiac distress, regardless of the hemoglobin or hematocrit levels, and reports that there is a danger that the patient may die of anemia, blood must be issued. Depending on the time available, the blood must be selected by that procedure in *Table 2* for which time is available and, if necessary, blood grossly incompatible with the free serum autoantibody, but compatible with any alloantibodies present, must be used.

Transfusion in the presence of drug-induced antibodies

In some instances, antibodies whose production has been stimulated by certain drugs cause no problems in the selection of blood. For example, the sera of patients who have made antibodies directed against penicillin-coated red cells will not react with normal red cells.[15] Similarly, in those drug-induced situations in which immune complexes of antibody and drug form in the patient's plasma, or those in which the patient's red cell membranes are altered and bind plasma proteins nonspecifically, there will be no antibody against normal red cells present in the patient's serum unless blood group alloantibodies are simultaneously present.[7, 15]

However, some drugs, methyldopa (Aldomet), levodopa, and mefenamic acid, stimulate the production of autoantibodies that react with normal red cells. Since the autoantibodies are similar to those in patients with AIHA,[7, 15] many of the same considerations as those mentioned earlier about transfusion in AIHA apply. First, any blood transfused must be compatible with alloantibodies present in the patient's serum. The procedures in *Table 2* described in detail earlier may be necessary in order that any alloantibodies present be detected and identified. The need for transfusion in this group of patients is seldom as acute as in the most severe form of AIHA, so that there is usually time for the triple adsorption procedure to be employed.

The contraindications for transfusion in AIHA do not generally apply in this group of patients. First, most patients in whom a positive DAT develops (and have free serum autoantibody) in this setting are those being treated with methyldopa. Second, although autoantibody production is not particularly rare, it occurs in about 20% of all patients treated; the higher the dose of methyldopa the greater the chance of autoantibody production. Antibody-induced in vivo red cell destruction occurs in less than 1% of all treated patients.[4, 7, 15] Third, in those patients in whom a hemolytic episode develops, the episode is transient and can be reversed by withdrawal of the drug.

There are three considerations regarding transfusion in these patients. The first involves the detection and

identification of any alloantibodies present. Such antibodies must be avoided in transfusion.

Second, if the patient is experiencing in vivo destruction of his own red cells, it is likely that the transfused cells will have the same shortened in vivo survival. This can be avoided if blood compatible with the autoantibody can be provided. However, most of the autoantibodies encountered are of broad specificity, and the opportunities of providing blood more compatible than the patient's own cells are few.[7, 8, 15]

The danger of providing more immunogen and stimulating an increase in the level of autoantibody being made does not apply. Once drug-induced antibody-mediated in vivo hemolysis is detected, the drug in question is withheld. Drug-induced autoantibody production is halted by withdrawal of the drug so that there is no danger of worsening the patient's long-term course. Indeed, in the most severe cases of methyldopa-induced in vivo hemolysis, transfusion may be beneficial (after or at the same time as cessation of methyldopa therapy) in maintaining the patient's red cell level until the in vivo hemolytic episode stops.

Third, in patients in whom autoantibody production occurs without an in vivo hemolytic episode, the transfused cells can be expected to survive normally if alloantibodies are successfully avoided, like the patient's own.[4] It is convenient to withdraw the drug and wait until autoantibody production stops before undertaking compatibility tests to provide blood, but this is not necessary. If surgery must be performed while the patient's DAT is positive and autoantibody is present in the serum, personnel in the blood bank must be prepared to undertake necessary tests to identify any alloantibodies present (*Table 2*), then issue blood that may appear grossly incompatible in in vitro tests, but which can be expected to survive normally in vivo. There is no excuse for delaying necessary surgery when the drug-induced autoantibodies are not causing in vivo red cell destruction.

Transfusion in "cold" antibody-induced autoimmune hemolytic anemia, cold hemagglutinin disease (CHD)

Unlike AIHA, there are no contraindications for giving transfusions to patients with CHD. In this form of hemolytic anemia, the patient's serum contains an autoantibody (most often IgM) that complexes with the patient's red cells when the body temperature drops. Such complexing frequently takes place in the body extremities when the patient is exposed to cold. As the body temperature returns to normal, or as blood from the cold-exposed extremities flows back to the body where the temperature is normal, the performed antigen-antibody complexes activate complement. The patient then experiences a hemolytic episode involving complement-mediated intravascular hemolysis.[1–4, 16]

In vivo hemolysis in CHD is not ongoing as it is in AIHA, and patients with CHD can be given transfusions in a controlled environment maintained at a temperature that will prevent the in vivo uptake of antibody by red cells. Such transfusions do not result in transfusion reactions or in hemolytic episodes.[16] Further, unlike AIHA, in which autoantibodies are often polyclonal and introduction of donor red cells may stimulate an increase in autoantibody production, those autoantibodies causative of CHD are monoclonal. There is no evidence that transfusion of red cells

to patients with CHD causes any increase in amount, broadening of specificity, or widening of thermal range of the monoclonal cold autoantibody. For these reasons red cell transfusions in CHD can be of benefit to the patient. However, in most patients with CHD, the disease is clinically mild and transfusion is seldom necessary. Many patients with the disease can avoid hemolytic episodes and resultant anemia by avoiding exposure to cold. In those few patients with severe forms of this disorder, suppression of autoantibody production by alkylating agents such as chlorambucil or in vivo denaturation of the IgM autoantibody molecules by sulphydryl compounds such as mercaptopyridoxine are the treatment methods of choice, and can be supplemented by supportive red cell transfusions when necessary.

Since transfusion is not contraindicated, the only major consideration is to ensure that blood compatible with any alloantibodies present is used. This task, as in AIHA, is often complicated by the reactions of free autoantibody, this time optimally reactive at low temperatures in the patient's serum. As discussed below, the use of blood compatible with the patient's autoantibody is seldom possible, but is even less often necessary. *Table 3* lists a number of methods used to avoid the actions of cold-reactive autoagglutinins and permits the recognition of clinically significant alloantibodies.

Table 3. Methods for the isolation of alloantibodies in sera that contain cold reactive autoantibodies

Autoadsorption procedure
Prewarmed tests
IATS read with anti-IgG
Blind titration not recommended

Autoadsorption procedure

In this method, the cold-reactive autoagglutinin is removed by adsorption with the patient's red cells. To obtain cells not already agglutinated by the autoantibody, blood samples are drawn into ethylenediaminetetraacetic acid (EDTA) or other anticoagulant and placed in a portable water bath such as a wide-necked thermos vessel containing water heated to 37 to 40 C. The samples are returned to the blood bank and the red cells are washed with saline prewarmed to 37 C. In this way red cells of the patient that do not have any or very much autoantibody bound to them are obtained. These red cells are used to autoadsorb the patient's serum at 4 C. If it is known that the autoantibody present reacts with protease-treated red cells (anti-I, anti-IH, and anti-i react strongly with such cells, the anti-Pr antibodies do not react at all) the autoadsorption procedure can be made more efficient by use of ficin or papain-treated patient red cells.[2, 4, 7, 17, 18]

When the patient's autoantibody is of moderate titer this method works well. All autoantibody is removed in one to three autoadsorptions. However, in cases of severe CHD in which the autoantibody is of high titer, six or more autoadsorptions may fail to remove all autoantibody.[4] In such cases prewarmed tests and/or IATS read with anti-IgG may become necessary to recognize alloantibodies.

Prewarmed tests

In this method the in vitro tests are performed at a temperature above that at which the cold autoagglutinin is active. The patient's serum and the test red cells are prewarmed to 37 C in separate tubes. The serum is added to the cells with a prewarmed pipette and

the tests are incubated and read at 37 C. For indirect antiglobulin tests, the test red cells are washed with saline prewarmed at 37 C and in a heated centrifuge (a serofuge inside a 37 C incubator works well). Once washed, the red cells are no longer in contact with the patient's serum so that there is no need to warm the antiglobulin serum. Care must be taken to ensure that all steps are performed in a manner that prevents any drop in temperature of the tests. Many autoantibodies causative of CHD are extremely avid and will agglutinate red cells in seconds if the temperature of the serum-cell mixture falls appreciably below 37 C.

If the autoantibody in the patient's serum neither agglutinates red cells nor causes complement activation at 37 C, this method is suitable.

Although the test will detect only those alloantibodies active at 37 C, and not those such as anti-P_1, anti-Le^b, anti-A_1, active at lower temperatures, this is no disadvantage. It is now accepted[3] that alloantibodies active only at temperatures below 37 C are of no clinical importance and can be ignored in the selection of blood for transfusion.

A disadvantage of this method is that some cold autoantibodies, although unable to agglutinate red cells in tests performed at 37 C, can bind to the red cells and cause complement activation. If the IATS are then read with a broad spectrum antiglobulin reagent, they may be weakly or moderately positive due to membrane-bound complement. Again, these reactions may interfere with the recognition of alloantibodies present and in such cases it may be necessary to read the IATS with an anti-IgG reagent.

IATS read with anti-IgG

The use of anti-IgG to read prewarmed IATS permits detection and recognition of IgG alloantibodies without interference from membrane-bound complement activated by the autoantibody. It is theoretically possible that such a procedure would result in nondetection of a complement-binding alloantibody. However, the chance of one patient's serum containing a cold autoantibody capable of complement activation at 37 C and an alloantibody detectable only via the C3-anti-C3 portion of the indirect antiglobulin test must be small.

Blind titrations

When it is necesary to select blood for a patient with CHD, some workers use the patient's serum in titrations against the red cells of potential donors. The units reacting to the lowest titer are then issued as "least incompatible." There is no evidence that this method is of benefit to the patient and the method has the disadvantage that any alloantibodies present in the serum may be overlooked if they are of lower titer than the autoantibody. This consideration is real, for unlike AIHA in which serum autoantibodies are usually of low titer, autoantibodies in the sera of patients with CHD may be of high titer (1000 or greater).

Comparison of methods described

Unlike AIHA, where the method to be used is dictated largely by the amount of time available, the method for selecting blood for a patient with CHD is dictated by the in vitro behavior of the cold autoantibody. Although some autoantibodies can be removed from the patient's serum by autoadsorption, when this is not possible prewarmed tests become necessary. With an autoantibody of wide thermal range that fixes complement at 37 C, it may be necessary to read IATS with anti-

IgG. With the sera of patients with CHD, the investigator must use that method or combination of methods that most completely avoids the reactions of the cold autoantibody and most efficiently demonstrates alloantibodies.

Use of washed red cells in transfusions for patients with CHD

Because the major mechanism of in vivo red cell destruction in CHD is complement-dependent intravascular hemolysis, it is occasionally necessary to give such patients washed red cells. In some patients, complement utilization is such that the limiting factor in in vivo hemolysis is depletion of one of the complement components. When this occurs, an additional supply of complement via the transfusion of plasma must be avoided. Although complement activation does not occur in in vitro tests when plasma is used, it must be remembered that all of the complement components are present in unaltered form in plasma. They do not act in vitro because of the chelation of calcium ions by most anticoagulants. However, in vivo, free calcium ions are available so that if plasma is transfused the complement components it contains can be utilized.

Washed red cells for transfusion to patients with CHD are rarely needed and the limiting factor of in vivo hemolysis is not complement depletion. When asked to supply washed red cells for a patient with CHD, blood bank personnel should ask whether complement depletion has actually been documented by assays of the CHD patient's serum.

Transfusion of incompatible blood in CHD

In vivo complexing of a patient's cold autoantibody and his own or transfused red cells can usually be prevented by ensuring that the patient is not exposed to cold. For this reason, it is usually possible to transfuse red cells incompatible with the patient's autoantibody and still obtain a good response. This is fortunate because autoantibodies causative of CHD are such that in many instances compatible blood does not exist (*Table 4*). All i adult donors have some I antigen on their red cells, all I+ donors have red cells that carry some i, and no human Pr-negative red cells with the possible exception of those of the M^k homozygotes are known.

Of the antibodies shown in *Table 4*, anti-I and anti-IH are the most common

Table 4. Specificities of autoantibodies causative of CHD

System	Antibody
I system*	Anti-I, anti-IH (both common) Anti-i (rare)
Pr system†	Anti-Pr_{1h}, anti-pPr_{1d}, anti-Pr_2, Anti-Pr_3, Anti-Pr_a
P system	Anti-P (PCH only)‡
Others	Anti-Gd, anti-H_T, anti-Sd^x
	Some cold autoagglutinins whose specificity has not yet been determined
	Anti-T (in T-activated polyagglutination)§

* These antibodies react more strongly with protease-treated (ficin, papain, bromelin), than with untreated red cells.

† These antibodies do not react with protease-treated red cells.

‡ This autoantibody has the same specificity as the allo-anti-p made by persons with P^k. It differs markedly from anti-P_1 and anti-$P+P_1+pk$(anti-Tj^a).

§ In vivo cell destruction due to anti-T in T-activated polyagglutination is rare. In most cases of transient red cell T-activation, anti-T is not demonstrable in the patient's serum while the red cells are T-activated.

in CHD. Despite this, I-positive blood is usually suitable for transfusion. In 30 years of work in immunohematology I have seen only one patient who needed a transfusion of i adult blood. The patient was undergoing continuous intravascular hemolysis, and transfused I-postive red cells were rapidly eliminated even though the patient was in a heated room and covered with electic blankets. Chlorambucil therapy had been started and during the period between initiation of therapy and the first effects of the drug he was maintained with transfusions of i adult blood. Within 48 hours of the initiation of drug therapy, the activity of cold autoagglutinin had diminished sufficiently so that his own and transfused I-positive red cells began to survive. Prolonged chlorambucil therapy prevented further in vivo red cell destruction and the patient recovered. There seems no doubt that the i adult blood kept this patient alive during the most acute phase of the hemolytic episode. Such cases are extremely rare.

Transfusion of warm blood in CHD

Since hemolytic episodes in CHD are initiated by a reduction in the patient's body temperature, it is obvious that blood that does not cause such a reduction must be used for transfusions. This can usually be accomplished by allowing the unit of blood to warm to room temperature before infusion is started. In some cases it may be necessary to infuse blood through a warming coil.

Transfusion in the presence of benign autoantibodies

In addition to those autoantibodies already described that cause reduced in vivo survival of the patient's own red cells, there are a number that react strongly in in vitro tests, yet they are benign in vivo.

Although most investigators are aware of harmless cold autoagglutinins, it is less widely appreciated that about 1 in 1000 hematologically normal individuals has warm-reactive autoantibodies.[19] These persons have a positive DAT. Autoantibodies of every specificity have been eluted from their red cells, and as many as 30% of them have autoantibody in the serum[8] (*Table 1*). Although it is somewhat frightening that these patients must be given transfusions, e.g., at elective surgery or following accidents, there is no evidence that the transfusions cause harm. I am not aware of a single case in which transfusion of incompatible (with the autoantibody) blood caused a benign autoantibody to become pathologic. Further, all of the evidence suggests that the transfused red cells have a normal in vivo survival time. In the selection of blood for these patients, the prime consideration becomes the recognition of any alloantibodies present whose reactions may be masked by autoantibodies present. Obviously, the methods listed in *Table 2* and described earlier should be used to detect alloantibodies. Blood should not be withheld from these patients, nor should necessary surgery be postponed, because donor units are apparently incompatible. As long as blood compatible with any alloantibodies in the patients' sera is provided, normal in vivo survival of the transfused red cells can be expected.

An exactly parallel situation exists when cold-reactive, benign autoantibodies, which are very common, are encountered. Since these autoantibodies are usually of low titer and of restricted thermal range, blood for transfusion can usually be readily selected by use of the

autoadsorption or prewarmed test methods (*Table 3*).

Summary

Warm antibody-induced hemolytic anemia

1. Avoid transfusion except in life-threatening situations.
2. When necessary, transfuse blood incompatible with autoantibodies, but compatible with any alloantibodies present.
3. When no alloantibodies are present, it is occasionally possible to transfuse blood compatible with the autoantibodies.
4. Washed red cells are seldom indicated.
5. 1 is better than 2 or 3, but
6. Never withhold blood because no compatible units are available, when clinical findings indicate that transfusion is essential.

Drug-induced autoantibodies

1. Antibodies directed against drug-coated red cells cause no problems in the selection of blood.
2. Antibodies to the drug (immune complex mechanism), or drugs that modify the red cell membrane cause no problems in the selection of blood.
3. Antibodies induced by methyldopa, levodopa, and mefenamic acid therapy must be treated like those in warm antibody hemolytic anemia. However, in these patients transfusion need not be avoided.

Cold antibody-induced hemolytic anemia

1. Transfusion is not contraindicated.
2. Any warm-reactive alloantibodies present in sera containing cold autoagglutinins must be detected, identified, and avoided in transfusion.
3. Blood compatible with the autoantibody is seldom necessary or available.
4. Warmed or washed red cells or both are sometimes indicated.

Benign warm and cold autoantibodies

1. Transfusion is not contraindicated and should not be withheld because of in vitro incompatibility.
2. Recognition and avoidance of alloantibodies present in sera containing these autoantibodies is essential.

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