A review: antibodies with high-titer, low-avidity characteristics

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The term HTLA, standing for high-titer, low-avidity, has been used to name a category into which a group of IgG antibodies has been placed arbitrarily. Most examples of the antibodies in this group share several serological characteristics. First, the antibodies react weakly in the antiglobulin phase of hemagglutination tests. Second, they may not react to the same extent with the red cells of different antigen-positive donors: reaction strengths may vary from weakly macroscopic to microscopic. Third, the antibodies continue to produce the same weak reactions at high dilutions (1:64 or higher).

While these serological characteristics have been considered to be a hallmark of the antibodies in this group, it must be mentioned that some samples will be encountered that neither react to high dilutions nor produce weak or variable antiglobulin reactions. The variations in reactions seen with some HTLA antibodies and different red cell samples suggest that antigen density varies from the red cells of one donor to another. Some donor’s red cells may carry so little antigen and react so poorly in antiglobulin tests that they may be difficult to distinguish from antigen-negative red cells.

Antibodies grouped under the HTLA heading include anti-Csa (Cost-Stirling), -Csb (Ste), -Yka (York), -Kna (Knops-Helgeson), -Knb (Hall), the McCoy group -McCa through -McCf, -Slb (Swain-Langley), -Slb, -JMH (John Milton Hagen), -Ch (Chido) and -Rg (Rodgers). Some workers have also placed antibodies such as anti-Hy,21,22 -Gya,23-26 and anti-Yta27 under this heading, since they may also react weakly when the sera that contain them are tested undiluted and at high dilutions. The last antibody (anti-Yta) will not be discussed in this article since it belongs to a blood group system.

The antigens defined by HTLA antibodies are, with the exception of Csb, Knb, Slb, and McCb, of high incidence in the white population. Generally, more than 90 percent of random blood samples tested will be found to carry the determinants (Table 1). In contrast, the antigens defined by anti-Csb and anti-Knb are found on less than 5% of red cell samples. McCb and Slb are found on the red cells of 42% and 80% of blacks, respectively (Marilyn Moulds, personal communication, 1990). They are rarely found on the red cells of whites. Csb and Knb behave as if they are antithetical to Csa and Kna antigens, respectively. The expressions of the other antigens may be related one to another only at the phenotypic level. It has been shown that red cells lacking one of the antigens defined by these antibodies are more likely to lack or carry a

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<td>Gya</td>
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*The incidences of these antigens provided by Marilyn Moulds (personal communication, 1990)
greatly diminished expression of another of the antigens. Such phenotypic relationships make it difficult to identify the specificities of HTLA antibodies correctly.

**Phenotypic Relationships Between Cs\(a\), Yk\(a\), Kn\(a\), and McC\(a\)**

Studies with antibodies to the high-incidence antigens Cs\(a\) and Yk\(a\) were the first to show that a phenotypic relationship existed between antigens defined by different HTLA antibodies. Anti-Yk\(a\), described in 1969,\(^3\) was at first thought to be another example of anti-Cs\(a\) (an antibody whose description was reported in 1965), because it failed to react with Cs\(a\) red cells. Not until the red cells of the antibody former, Mrs. York, were tested and found to be Cs\(a\) positive, was it realized that an antibody to a new antigen had been found. Ninety-eight percent of red cell samples from white and black populations are Cs\(a\).\(^{1-4}\) Similarly, 98% of samples of blacks are also Yk\(a\), but the red cells of only 92% of whites carry the Yk\(a\) determinant. Molthan and Giles\(^4\) showed that samples of 12% Yk\(a\) whites and 15% of Yk\(a\) blacks also typed as Cs\(a\). If the Cs\(a\) and Yk\(a\) antigens were not associated with each other in some manner, the expected incidence of the Yk\(a\) red cells, Cs\(a\) red cells would be 0.16% and 0.04% in the white and black populations, respectively.

A similar phenotypic relationship has been reported for Kn\(a\) and McC\(a\).\(^7,9\) Kn\(a\) occurs with an incidence of 99% in the random population.\(^6\) McC\(a\) occurs in the white and black populations with an incidence of approximately 95%.\(^7,9\) Red cells that lack one of these antigens frequently lack the other. In a study conducted in the mid 1970s, Molthan and Moulds\(^7,9\) showed that 69% of McC\(a\) samples from whites and 34% from blacks were also Kn\(a\). In addition, seven of 94 McC\(a\), Kn\(a\) samples studied by these workers were also Yk\(a\), Cs\(a\). Thus, it appears that the Cs\(a\) and Yk\(a\) antigens are more frequently missing from or are greatly diminished in expression on McC\(a\) and/or Kn\(a\) red cells than on McC\(a\) or Kn\(a\) samples.

Kn\(a\), McC\(a\) red cells lack the low-incidence antigen Kn\(b\).\(^{11}\) Anti-Kn\(b\) reacts with the red cells of all Kn\(a\), McC\(a\) donors and the red cells of approximately 4% of Kn\(a\) people. The failure of the antibody to react with Kn\(a\), McC\(a\) samples suggests that such red cells represent a type of null phenotype with respect to the Knops blood groups. Data provided by Molthan suggest Kn\(a\), McC\(a\) red cells might also represent null red cells with respect to the McCoy groups. In two articles published in 1983,\(^8,12\) Molthan described a heterogeneous group of antibodies to relatively high incidence antigens called McC\(b\), McC\(c\), McC\(d\), McC\(e\), and McC\(f\). The common characteristic linking the sera was that they failed to react with Kn\(a\), McC\(a\) red cells.

**Heterogeneous Antibodies to McCoy Determinants**

Anti-McCc\(b\), an infrequent antibody, was reported to react with 45% of McC\(a\), Kn\(a\) samples from blacks. Anti-McCc\(c\), found predominantly in the sera of blacks, reacted with the red cells of 51% of blacks and 98% of whites. Anti-McCc\(d\) reacted with an antigen of high incidence carried on the red cells of both whites and blacks. Anti-McCc\(e\) (originally called anti-Zulu) and anti-McCc\(f\) were found by Molthan only in the sera of blacks. Anti-McCc\(e\) reacted with the McC\(a\) blood samples from some blacks (35%) and most whites (98%) and was nonreactive with McC\(a\) red cells of both groups. Anti-McCc\(f\) reacted with red cells of 91% of random blacks and 31% of random whites.

Red cells lacking one of the McCoy antigens are, according to Molthan, more likely to be missing another.\(^12\) For example, she found all McC\(a\) blacks to be McC\(c\) negative. Furthermore, both whites and blacks who were McC\(a\) were McC\(c\) negative. More McC\(c\) negatives blacks were McC\(c\) negative than those that were McC\(c\) positive. Molthan's conclusion, based on the analysis of data obtained from serological studies on the incidences of McC\(a\) through McC\(f\), was that some antigens were antithetical in blacks but not in whites, in particular McC\(a\) to McC\(b\) or McC\(c\) to McC\(d\). This association has not been readily acknowledged by other researchers, and doubts have been voiced by some serologists as to whether all McCoy antibodies described by Molthan exist as different yet related specificities. Serologists who suspect they have found sera such as anti-McCc\(b\), -McC\(d\), -McC\(e\), etc. cannot always have their findings confirmed by other workers using different or even the same red cell samples.

Lacey and her co-workers\(^13\) have described an antibody called anti-S\(a\) that may be the same as the anti-McCc of Molthan. S\(a\) antigen is missing from the Kn\(a\), McC\(a\) red cells of whites, but not all S\(a\) red cells are also McC\(a\). The incidence of S\(a\) (60% in blacks, 99% in whites) is similar to that reported for McC\(c\) by Molthan.\(^8\) Because extensive family studies that could establish the relationship between S\(a\) and
the Kn/McC groups were not possible, these workers chose to maintain the term S8 to avoid rigid association with Kn/McC determinants. Lacey et al. have also described three examples of what they call anti-S8, antibodies that react with S8 red cells and seemingly are directed against the product of the antithetical allele to S8.

Many researchers who encounter sera that fail to react with Kn(a−), McC(a−) red cells opt to call the antibodies anti-"Kn/McC" even though the antibodies may react with some examples of Kn(a+), McC(a−) or Kn(a−), McC(a+) red cells. The term is thus used to denote the failure of the antibodies to react with the Kn/McC null red cells and does not identify the specific antigen to which the antibodies are directed. [It must be remembered that antibodies failing to react with Kn(a−), McC(a−) red cells include not only anti-Kna, -Knb, -McCa through anti-McCc but may also include antibodies such as anti-Cs or -Yka if the Kn(a−), McC(a−) red cells selected for testing lack one or both of these antigens as well.] In a similar manner, some workers use the term anti-"Cs/Yk" to denote antibodies that have failed to react with known Cs(a−), Yka red cells. Further work to establish the absolute identities of antibodies nonreactive with Kn/McC or Yk/CS null red cells is not warranted. No matter which specificity would be identified by further testing (anti-Kna, -McCa, -McCc, -Cs, -Yka, etc.), it would be considered of no clinical significance and would be ignored for transfusion purposes.27–28

**Heterogeneity of Antibodies to JMH, Cs, Yk**

Anti-JMH (also called "The Boys" or "The Over 60s") is an antibody of high-titer and low-avidity defining an antigen with an incidence of 99% or more.14-16 The JMH− phenotype has been shown to arise through two distinct mechanisms. One mechanism represents a transient state during which the antigen of JMH+ persons is depressed to the point that their red cells may behave as if they are JMH− with moderately or weakly reactive typing reagents. The transient condition has been reported to occur in persons over 50 years of age or in certain disease states. Such people can be recognized in serological tests because their sera contain anti-JMH and their red cells are weakly positive in direct antiglobulin tests (DATs). The second mechanism is due to the inheritance of an autosomal dominant gene. People of this type have negative DATs and have not been found to produce anti-JMH.

Antibodies identified as anti-JMH can behave as if they have slightly different specificities. Two patients (VG and RM) studied by Moulds et al. produced red cells with near normal, rather than reduced, expressions of JMH, yet made alloantibodies that failed to react with the JMH− red cells of other donors. The anti-JMH-like antibody of VG did not react with autologous red cells, the red cells of RM, or other JMH− samples. In contrast, the antibody of RM did not react with RM red cells or other JMH− red cells, but did react with the red cells produced by VG. The antigen status of VG and RM appeared to be inherited as a dominant trait. A monoclonal antibody (H8) studied by Daniels and Knowles appears to behave similarly in serological typing tests to the anti-JMH-like antibody of RM. However, H8 can block the binding of the VG antibody or other anti-JMH antibodies to JMH+ red cells.

Antibodies to the Cs and Yk antigens may also react as a heterogeneous group. Giles and Molthan and Giles have reported that different examples of anti-Yka appear to vary in their abilities to react with red cells carrying weak expressions of the antigen. In a case reported by Giles,2 the red cells of one patient who had produced a weakly reactive anti-Yka were found to react with the more potent anti-Yka produced by a second patient. The red cells of the first patient might be thought of as carrying part but not all of the epitopes comprising Yka. Thus, patient No. 1 produced antibody to epitopes (portions of the Yka antigen) missing from her own red cells. Her red cells were able to react with anti-Yka produced by patient No. 2 because they contained antibodies to the epitopes remaining on the red cells of patient No. 1. The serological findings seen with patient No. 1 can also be explained by an alternative hypothesis. The red cells of patient No. 1, and perhaps those of other antibody formers as well, carry some, albeit minute, quantities of antigen. The presence of the antigen can be demonstrated only when potent or more avid typing sera are used.

**Heterogeneous Antibodies to Ch and Rg**

Unlike the other antibodies described so far, anti-Ch and anti-Rg are directed against antigens adsorbed to, rather than antigens produced by, red cells. The Ch and Rg determinants have been reviewed recently in this journal, so no more than a brief description of the antigens and antibodies will appear here. Anti-Ch and anti-Rg react with the red cells of 98% and 97% of
random adults, respectively.\textsuperscript{17-19} Most normal donors are Ch+ and Rg+, 3% are Ch+Rg−, and 2% Ch−Rg+. Production of the antigens defined by these antibodies is controlled by alleles at two closely linked loci called \( C4A \) and \( C4B \) that encode proteins of the complement component C4.\textsuperscript{20,34} These loci are closely linked to the HLA genes on chromosome number 6.\textsuperscript{35,36} Ch and Rg appear to be located on the alpha chains of their respective two-chain C4 proteins and behave serologically as if they are part of the C4d component.\textsuperscript{37,38} O’Neill et al.\textsuperscript{20} found the Rg determinant was associated with \( C4A \)-encoded proteins while Ch component.\textsuperscript{37,38} These workers found all Rg+ and Ch− people are C4-deficient and lack both proteins.

Anti-Ch and anti-Rg are produced by immunized Ch− and Rg− people, respectively. The antibodies can be neutralized by the soluble substances occurring in the plasmas of Ch+, Rg+ donors. Inhibition is seen in two forms, complete and partial, a finding that indicates subtle differences exist between antibodies of the same specificity.\textsuperscript{20,41-44} Through electrophoretic or adsorption/elution studies, Nordhagen et al.\textsuperscript{43,44} and Giles et al.\textsuperscript{45-49} have all been able to show that two Rg (Rg1 and Rg2) and six Ch (Ch1 through Ch6) determinants exist. The plasmas of most Rg+ donors contain Rg1 and Rg2, thus their phenotypes are written as Rg1,2. The plasmas of approximately 3% of random people demonstrate Rg1 but not Rg2 (Rg1,−2) and behave as partial inhibitors of anti-Rg. Neither Rg1 nor Rg2 are found in the plasma of people who are Rg− (Rg:−1,−2). All examples of anti-Rg studied by Giles\textsuperscript{46,47} have contained antibodies to Rg1 and Rg2. Most Ch+ donors are thought to express the six Ch determinants in their plasma and phenotypically are Ch1, 2, 3, 4, 5, 6. Some people, however, have been found who fail to produce one or more of the Ch determinants. Their plasmas behave as partial inhibitors of anti-Ch. These people can produce an antibody to the determinant they lack and thus they appear, in serological tests, to be Ch+ with anti-Ch.\textsuperscript{49} Giles\textsuperscript{46,47} reports most anti-Ch contain antibodies to Ch1. Anti-Ch2 is found as a component in 25% and anti-Ch5 in 16% of anti-Ch sera.

At the level of transfusion practice, it is impractical to identify the different anti-Ch specificities that might be present in the serum of a Ch− person, particularly because lengthy adsorption and elution studies must be done. From a clinical point of view, Ch− people with anti-Ch (or Rg− people with anti-Rg) are sufficiently served if their sera are handled as if they contain only one antibody. Anti-Ch and anti-Rg are considered benign in vivo, in terms of premature red cell destruction.

**Phenotypic Relationship Between Gy\textsuperscript{a} and Hy**

The red cells of blacks that lack the low-incidence antigen Hy react poorly with anti-Gy\textsuperscript{a} and thus are Hya−, Gya+w.\textsuperscript{23} The red cells of whites who are Gya− react in typing tests as if they are Hy−.\textsuperscript{23,50,51} Of four Hy−, Gya− people studied by Reid et al.,\textsuperscript{51} one patient produced red cells that would adsorb, and yield on elution, anti-Hy, indicating they carried some Hy antigen. Red cells of the other three patients failed to adsorb or elute anti-Hy; the red cells of all four failed to adsorb and elute anti-Gya. Hy−, Gya− people have all been of European extraction. The Hy−, Gya+w phenotype derived in typing tests is, so far, associated with blacks.

Two other antigens of high incidence, Jc\textsuperscript{a} and Jo\textsuperscript{a}, have been reported to have a phenotypic relationship with Gy\textsuperscript{a} and Hy. Laird-Fryer et al.\textsuperscript{52} found antibodies to Jc\textsuperscript{a} in the sera of five black women. Each serum reacted with all red cells except those that were either Hy−, Gya− or Hy−, Gya+w or from other anti-Jc\textsuperscript{a} antibody formers. Weaver et al.\textsuperscript{53} used two examples of anti-Hy and four of anti-Jo\textsuperscript{a} in tests with the red cells of seven Hy−, Gya+w, one Hy−, Gya−, and 20 Jo(a−) people. These workers found all Hy− and/or Gya− red cells were also Jo(a−). Four of the 20 Jo(a−) were also Hy−. The phenotypic association has been challenged by Brown.\textsuperscript{54} This researcher studied the red cells of a pregnant, black woman. The phenotype of the patient was Jo(a−), Hy+, Gya+. Her antibody reacted with all of four Hy−, Gya+w and two Hy−, Gya− red cell samples.

**Significance of HTLA Antibodies**

The majority of reports that appeared in the late 1970s and early 1980s indicate that HTLA antibodies, with the possible exception of anti-Hy, have little effect on antigen-positive red cells in vivo.\textsuperscript{14, 21-26,55-69} Tilley
and co-workers showed that $^{51}$Cr-labeled Yk(a+) red cells survived normally in a patient who had produced anti-Yk, reacting to a titer of 1000. Shore and Steane successfully transfused 11 Cs(a+) units to a patient with anti-Cs during a bleeding episode requiring surgery. Postoperatively, a $^{51}$Cr-labeled red cell survival study was performed using strongly reactive Cs(a+) red cells. These researchers reported that essentially normal survival was observed. Red cell survival studies using serologically incompatible red cells have also shown that antibodies in the Knops-McCoy groups behave normally in patients with the antibodies. Valko et al. detected normal survival of $^{51}$Cr-labeled McC(a+) red cells in a patient with anti-McC. Viggiano and Ballas showed that $^{51}$Cr-labeled Kn(a+), McC(a+) red cells survived normally in a patient with anti-Kn/McC. This case is of particular significance since the patient may have been of the Kn/McC null phenotype. Twenty-nine units of antigen-positive red cells were transfused to the patient without evidence of accelerated red cell destruction. Both Harpool and Wells et al. have reported that the transfusion of Sl(a+) units to patients with anti-Sla in the serum did not cause transfusion reactions or apparent premature destruction of transfused red cells.

Sabo et al. have described the normal survival of labeled JMH+ red cells in three different patients with anti-JMH. Tregellas et al. described uneventful transfusions to nine patients with the antibody. Of these patients, one received more than 20 units of JMH+ blood over a 10-month period. An 80-year-old patient studied by Whitsett et al. had anti-JMH in the serum. His red cells typed as JMH- even though an eluate made from his DAT+ red cells yielded anti-JMH. As expected, $^{51}$Cr-labeled JMH+ red cells survived normally in this patient. Several studies have shown that the transfusion of Ch+ or Rg+ units to patients with the corresponding antibodies failed to produce any evidence of accelerated destruction of red cells.65-67,69

There are conflicting data concerning the effect of anti-Gy on incompatible red cells. Moulds et al. reported that two Gy(-) women, who each made anti-Gy during pregnancy, showed symptoms of a reaction (fever, chills) following the administration of incompatible, uncharacterized, but presumably Gy(a+) units. Ellisor et al. found that small volumes of $^{51}$Cr-labeled Gy(a+) red cells survived normally in a previously transfused Gy(a-) 71-year-old man who produced anti-Gy only for a brief period. Fischer et al. successfully transfused two Gy(a+) units to an 81-year-old Gy(a-) woman with gastrointestinal bleeding. The serum of this patient contained a potent anti-Gy reacting to 1:512 in hemagglutination tests. There was an increase in the patient's hemoglobin following transfusion, and no changes in bilirubin or liver enzyme levels were noted, even though the posttransfusion red cells became positive in the DAT.

Beattie and Castillo found decreased survival of Hy+ red cells given to a patient, L. Rivers, who had produced anti-Hy. Two units of incompatible blood were administered to correct the manifestations of acute blood loss. The patient's hemoglobin increased from 2.9 to 7.0 g/dL immediately following transfusion. Two days later, the patient's hemoglobin decreased to 4 g/dL, without further blood loss. Posttransfusion red cells had a positive DAT and there was an increase in the patient's plasma hemoglobin, with a concomitant decrease in haptoglobin. The significance of another example of anti-Hy was studied by Hsu et al. using $^{51}$Cr-labeled red cells. When administered to his patient in small doses, $^{51}$Cr-labeled Hy+ red cells showed a shortened survival curve. This finding suggests that this patient's antibody could cause abnormal red cell survival. Nevertheless, the antibody behaved as if it were clinically benign in a mononuclear macrophage assay. In contrast, the anti-Hy of L. Rivers behaved as if it were clinically significant in the same macrophage assay. A third anti-Hy sample produced results in the macrophage assay indicative of an insignificant antibody.

**Resolving Serological Problems Due to HTLA Antibodies**

Anti-Yk, -Kn, -McC, -Ch, -Rg, and anti-McCc (or anti-Sla) are encountered often in serological testing. In contrast, the other antibodies, such as anti-Cs, -McC are uncommon. All of the HTLA antibodies are IgG in nature and with the exception of the anti-Gya described by Clark et al. do not appear to bind complement. As mentioned previously, examples of the antibodies have been noted to react variably in hemagglutination tests with the red cells of different adults. They may react more weakly with cord red cells, as it appears the antigens they define are often poorly developed on these cells. (This can explain why HTLA antibodies have not been reported to cause hemolytic disease of the newborn.) The difference in reactions between cord red cells and those of adults may not be readily apparent in tests with undiluted strongly reactive
antibodies; they may become apparent only when sera are tested in titration.

For the last 15 months, our laboratory has been testing samples submitted for antibody identification by both solid phase red cell adherence (SPRCA) and routine tube hemagglutination procedures. During this period, over 40 examples of HTLA antibodies of defined specificities (anti-Cs, -Yk, -Kn, -McC, -Gy, -Hy, -JM, -Ch, -Rg) or unidentified HTLA antibodies have been tested. In SPRCA tests, many of the antibodies produced moderate adherence reactions of similar strengths with the immobilized red cells of different donors (Rolih, Fisher F, Robichaux M, unpublished observations). However, we cannot conclude at this point that most HTLA antibodies behave in a nonvariable manner in SPRCA assays because the antibodies under evaluation, for the most part, reacted with little donor-to-donor variation in parallel tube hemagglutination tests.

One difficulty of working with sera that contain antibodies of the HTLA group is that they often are present with other antibodies of clinical significance. Thus, they create problems for those responsible for supplying suitable red cells for transfusion. In particular, reactions of HTLA antibodies may mask those of more clinically significant antibodies in hemagglutination or solid-phase tests. Titration studies can be employed to determine the probability that weak, variable reactions are due to antibodies with HTLA characteristics. If the antibody produces nonvariable reactions at several consecutive dilutions of serum, then the presence of an HTLA antibody may be suspected. Unfortunately, titration studies do not differentiate HTLA antibodies from antibodies of clinical significance, such as anti-Yt, -Lu, or anti-Ge, that might also react to high titers, yet show low-avidity characteristics. Titration studies also do not readily identify those sera that contain more than one antibody.

Serologists can take several approaches to resolving problems with samples containing HTLA antibodies. One is to identify the specificity of the HTLA antibody. Another is to determine the group of antigens toward which the antibody is most likely directed without determining the actual specificity. Yet another approach is to employ procedures designed to circumvent the interfering reactions of such antibodies, and thus ignore the HTLA antibody present.

In most cases, time will not be well spent if it is devoted to the identification of the HTLA antibody. First, identification procedures require the use of large numbers of correctly characterized, antigen-negative red cells. Most routine blood banks and many reference laboratories do not possess such red cells in the numbers needed to identify the HTLA antibody correctly. If too few antigen-negative red cells are used in identification tests, the chance that an HTLA antibody will be incorrectly identified is great. Second, once identified, the HTLA antibody will be ignored because of its dubious clinical significance. Red cells selected for transfusion will carry the corresponding antigen, since searches for antigen-negative units are not warranted and cause unnecessary delays in issuing blood for transfusion.

Workers in some reference laboratories attempt to determine at least the group of related antigens toward which an HTLA antibody is directed by testing the sera against standardized selected red cell panels employing three or four examples of null phenotypes such as Kn(a-), McC(a-), and Cs(a-) red cells. Tests with null red cells can help differentiate HTLA antibodies from clinically significant antibodies capable of reacting with low avidity, yet to high titers. For example, an antibody that fails to react with most, or all, Kn(a-), McC(a-) red cells of the selected panel is more likely directed at a determinant missing from the Kn(a-), McC(a-) red cells, than against a high-incidence antigen such as Yt, Lu, or Ge. No further testing is done to identify the antibody failing to react with the null-type red cells, since such specificities (anti-Kn, -Kn, -McC, etc.) have all been shown to be of dubious importance with respect to transfusion. In terms of clinical reports, antibodies whose phenotypic preferences have been established in tests with null red cells can be listed in terms such as “antibody to Knops/McCoy group” or anti-“Kn/McC.” As mentioned previously, such terms are used to denote the failure of the antibodies to react with the Kn/McC null red cells and do not imply the antibodies are directed against any specific Knops or McCoy antigen.

Tests to establish phenotypic preferences, like full identification procedures, may not be warranted, since the antibodies are ignored for transfusion purposes. A more useful expenditure of time would be to employ procedures that eliminate the reactions of the nuisance HTLA antibodies so samples could more easily be evaluated for the presence of significant specificities. The antigens Ch, Rg, and JM are destroyed by the proteolytic enzymes papain and ficin. Therefore, anti-Ch, -Rg, and -JM fail to react in tests employing
enzyme-treated red cells. The reactions of antibodies to Csa, Yka, McCa, Knα, Gyα etc. may be unchanged, slightly enhanced, or slightly reduced when enzyme-treated red cells are substituted for untreated red cells in hemagglutination or SPRCA tests.

The antigens defined by most HTLA antibodies are denatured to some extent by solutions of the chemicals 2-aminoethylisothiouronium bromide (AET) or dithiothreitol (DTT). A 6% solution of AET decreases the expression of Yka, McCa, Knα, JMH on red cells to such an extent that they fail to react with their respective antibodies, or do so only poorly. The Hy and Gyα antigens also appear to be denatured by AET. A 0.2 M solution of DTT is similar in its effects to AET. If modification is to be performed in test tubes, one volume of packed cells prepared from 8 to 10 drops of a 5% suspension of red cells is incubated at 37°C for 30 minutes with 4 volumes of 0.2 M DTT.74,75 The red cells are then washed in saline before they are used in testing. Laboratories performing solid phase red cell adherence assays can accomplish the same modification by treating immobilized reagent red cell monolayers with 50 µL 0.2 M DTT for 30 minutes at 37°C.77 Results obtained with enzyme-, AET-, or DTT-treated red cells should be interpreted with caution since antigens other than those defined by HTLA antibodies may be denatured. For example, antigens such as Fya, Fyb, S, s, and Xgα are destroyed by enzymes. The Kell system, paraKell, and YRα antigens are destroyed by AET.71 Similarly, Kell, paraKell, Cartwright, LW and Dombrock antigens are destroyed by DTT.76,78,79 When negative results are obtained with enzyme- or DTT-treated red cells, steps must be taken to ensure that the reactions obtained with untreated red cells were not due to a clinically significant antibody.

When the detection or identification of weakly reactive examples of antibodies showing low-avidity characteristics needs to be facilitated, it can be accomplished by doubling or tripling the amount of serum used in testing (hemagglutination tests) or by extending the incubation period at 37°C to 60 minutes (hemagglutination, SPRCA). Such changes in testing protocols may enhance the reaction of the antibodies to such an extent that identification of the antibody, if it is absolutely needed to resolve crossmatching difficulties, will be easier. As mentioned previously, rarely does the use of enzyme-treated red cells expedite identification, unless antibodies to the enzyme-sensitive antigens Ch, Rg, or JMH are present. Anti-Ch and -Rg produce weak, variable reactions in tests because only small amounts of the respective antigens are adsorbed to the red cell surfaces. Several researchers have reported that the identification of anti-Ch and -Rg can be facilitated if red cells coated with higher than normal levels of C4 are used.80-82 Such red cells can be prepared by incubating them briefly at 37°C in the presence of fresh serum and isotonic sucrose solutions of low ionic strength. During incubation, large amounts of complement components, including C4, are deposited on the red cell membrane. If serum used in this procedure has Ch and Rg, then large amounts of both antigens will be bound. Anti-Ch and -Rg will react strongly with C4-coated red cells prepared in this manner, even though such sera would produce only weak reactions with normal red cell samples. In fact, some examples normally reactive at the antiglobulin phase will react with the C4-coated red cells at the room temperature phase of testing.

In(Lu) and Antigens Defined by HTLA Antibodies

The dominant In(Lu) gene is responsible for the more common form of the Lu(a−b−) phenotype. The gene is independent of the Lutheran locus, yet causes suppression of Lutheran and para-Lutheran antigens. In(Lu) also suppresses P1, Auα, i, and An/Wj antigens. Recently, Daniels et al.83 have shown that several antigens defined by antibodies with HTLA characteristics may also be affected by this gene. In a study of members of 12 families of Lu(a−b−) propositi due to In(Lu), these workers found that the Knα, Knα-like, McCa, and Slα antigens were consistently more weakly expressed on the red cells of Lu(a−b−) members than on the red cells of Lu(a+) or Lu(b+) members. Suppression of Knα, McCa, and Slα were not as dramatic as that described for P1, Auα, or i. Csα and Yka determinants were depressed on the red cells of Lu(a−b−) propositi in some, but not all, families studied. Ch and Rg determinants did not appear to be affected by In(Lu) in any family studied.

HTLA Antibodies Concluded

In conclusion, it can be said that the HTLA group of antibodies cause difficulties in serological testing because of the weak reactions they produce in the indirect antiglobulin test. The antibodies are frequently encountered and most specificities are directed against antigens of high incidence in both white and black populations. Those described here, with the exception...
of some examples of anti-Hy and perhaps some anti-Gy, have not been shown to cause significant destruction of antigen-positive red cells. The antibodies are considered to be nuisance antibodies because the reactions they produce interfere with the identification of reactions due to other, clinically significant antibodies. The interfering reactions of the antibodies can be eliminated by treating test red cells with reagents such as DTT and AET.

References
High-titer, low-avidity antibodies


71. Eckrich RJ. Inactivation of Holley (Hy) and Gregory (Yy) antigens by dithiothreitol (DTT). Immunohematology 1988;4:12-3.


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IMMUNOHEMATOLOGY, VOLUME 6, NUMBER 3, 1990