EDITORIAL

To Be or Not To Be an Antibody: The "Agent" in Autoimmune Hemolytic Anemia

By W. Weiner

TO DOUBT a statement or theory is a right—nay a duty of every scientist—but this doubt should cease once reasonable evidence has been forthcoming.

Autoimmune hemolytic anemia is the name which has been given to a type of hemolytic disease clinically and serologically well defined. This name suggests that the cause of this anemia is an antibody directed against an antigen on the patient's cells and found in the serum and in the eluate produced from the cells of these patients. The name and the assumption connected with it have been accepted by the majority of workers in this field, but there are still a few who have expressed doubt about the immunologic cause of this anemia. They doubt the antibody nature of the "agent" found in the serum and on the cells of these patients. What evidence is there for the assumption that in the vast majority of cases the agent is a true antibody?

Of course, nobody can doubt that hemolytic anemia can be produced by an antibody. Thus was convincingly shown in the experiments of Dameshek and Schwartz in 1938 when they demonstrated that hemolytic anemia could be induced in the guinea pig by injection of anti-guinea pig red cell rabbit serum. Hemolytic disease of the newborn is an example of a hemolytic disease in man caused by an (iso-)antibody, and the immunologic nature of this disease is not questioned by anybody.

The trouble starts when an antibody is found which is directed against one of the patient's own antigens without this antibody having been transferred passively into the patient's circulation. Ehrlich's "horror autotoxicus" is then invoked and by Ehrlich's authority this concept is rejected. Most of these workers seem, however, to forget that long ago another great serologist, Landsteiner, in cooperation with Donath showed that true autoantibodies which are not passively transferred and are produced by the patients themselves do occur in man. Can there be anybody who doubts an autoantibody to be the cause of the attacks of paroxysmal cold hemoglobinuria? In spite of this precedence, quite a number of authors are horrified to contradict Ehrlich's authority and try in every way to avoid what seems to them the sacrilegious idea of an autoantibody. Most of them admit the similarity of the "agent" to an antibody but then still find various hairs which can be split to "prove" their difference.

Before going into the merits of the case, it will be well to try to define what is meant by an antibody. This is a notoriously difficult task, and no textbook on serology, hematology or pathology gives a completely satisfactory definition. However, for our own purpose, the following properties are important.

Submitted Oct., 1958; accepted for publication Apr. 27, 1959.
1. An antibody is a plasma globulin, usually of the gamma globulin type.
2. It combines specifically with its homologous antigen (but may occasionally cross react with related antigens).
3. The union of the antigen and antibody must be demonstrable.
4. The union takes place under specified conditions of temperature and in a specified medium and depends sometimes on the physical and chemical condition of the antigen.
5. With the use of appropriate procedures, the antibody-antigen complex can be separated; if cellular antigens are used, this process is called "elution of the antibody." In our own field, therefore, elutability should be added as a property of an antibody. This eluted antibody should be capable of reunion with the same antigen.
6. Some antibodies consume complement after their union with the antigen and some are hemolytic.

With these points to guide us, can we find reasonable evidence of the antibody nature of the "agent" in hemolytic anemia?

First, the "agent" found on the cells and often in the serum has been shown to be in most cases, a gamma globulin (Pirofsky, Fudenberg and Kunkel and many other authors). It is true that some coated red cells react better with an anti-beta-globulin serum than with anti-gamma globulin sera. This in all likelihood is due to the reaction of this Coombs serum with complement components which have been fixed by the antigen-antibody complex, the antibody again being in all likelihood a gamma globulin.

Secondly, it can certainly not be doubted that the "agent" combines with the red cells of the patient; this is where it is found, and if eluted from them, it will again combine with the same cells or cells of similar genetic make-up. Hundreds of cases must by now have been published or be known to the various workers in this field in which the "agent" in the serum (or eluate from the cells) had distinct and precise blood group specificity. This blood-group-specific antibody may be present on its own or be accompanied by an antibody showing panagglutinating properties. It is often only a question of technic to demonstrate the blood-group-specific antibody by absorption and possibly re-elution. On the other hand, one of the advocates of the nonimmune nature of hemolytic anemia says:

This concept [of the auto-immune character of this anemia] is based on the premise that the coating material represents an auto-antibody that develops as the sequela of active immunisation against an antigen in the patient's own cells. Because such antigens have not been demonstrated . . .

Active immunization against an antigen on the patient's own cells would be very difficult to observe. What can be and is being observed is the presence of an "agent" reacting with an antigen on the patient's cells; in other words, the mechanism of autoimmunization has yet to be elucidated, but the antibody is there for all to see. Among workers in this field, it has become a routine investigation to search for the antigen which is defined by the "agent" in the serum or on the cells—but more on this point later.
As to the third point, the union of the antigen with the “agent” is indeed easily demonstrable by the methods commonly used in blood grouping (agglutination and Coombs tests). The “agent” can also equally easily be absorbed by cells carrying the appropriate antigen and eluted from them.

The fourth point made above shows the great similarity between the “agent” of hemolytic disease and the common antibodies used in everyday blood grouping. We all know that some of these “agents” act preferentially at 37°C., others at 22°C. or 4°C. The 37°C. “agents” act on albumin-suspended cells, trypsinized, papainized or ficinized cells and, of course, give a positive Coombs test, a behavior shared by the common immune antibodies (e.g. anti-Rhesus). The “cold agents” act very similarly to “cold” antibodies. They act on saline-suspended cells somewhat better than on albumin-suspended cells. Occasionally their action is enhanced by enzyme treatment of the cells and their Coombs tests are usually much more weakly positive than those obtained with the “warm” variety. Naturally, quite a number of other substances will react in similar ways, but it is important to stress that their action is “similar,” not identical. Lectins, for example, have many properties in common with antibodies, but they have not been found in the plasma and are, of course, not gamma globulins. Silica will mimic the action of antibodies in some respects, but nobody will mistake silica for antibodies. Jandl and Simmons have shown that metallic cations can “sensitize” (and agglutinate) red cells, but nobody would mistake the “agent” on these cells for an antibody, as it falls far short of the other criteria characteristic for an antibody mentioned above. Positive direct Coombs tests have been reported in other conditions of definitely nonimmunologic nature like phenylhydrazine poisoning or lead poisoning. Though Muirhead et al. apparently produced in the serum of their dogs an “agent” which gave an “indirect Coombs test” and stated that they could elute the “agent” off the cells, their protocols do not seem to bear out these statements. Their experiments certainly need confirmation, and more than a ± reaction would be expected to make the results acceptable throughout. There is no doubt whatsoever that various poisons—and lead is one of them—will alter the surface of the red cell drastically. This altered surface can no doubt absorb serum proteins nonspecifically and these may well react with the antibody in the Coombs serum. The same can be observed in vitro when tanned red cells are allowed to absorb plasma proteins. It is even possible that a surface change of the red cell may expose groupings otherwise below the surface which will react with the antibody in the Coombs serum. It is thus common experience that some cases of pernicious anemia in deep relapse show weakly positive direct Coombs tests. Not even the most efficient of elution technics will, however, produce an eluate active on other cells. In other words, the findings of a positive direct Coombs test must not be taken to be identical with the diagnosis of an autohemolytic anemia until other causes for this reaction have been eliminated. The latter are so rare that most workers in this field will probably not even bother to think of lead poisoning, etc. As far as is known, lead poisoning has not been described in
man as the cause of a direct positive Coomb test, but Harris\textsuperscript{12} described a case of fuadin sensitivity in which he found the cells of the patient to give a positive Coombs test due to an antibody which combined with the cells in the presence of fuadin. Here again, however, the “agent” was an antibody which though not blood-group-specific obviously combined with an antigen or antigens on the red cell which may possibly have been changed into a hapten by the fuadin.

However, anybody who has tried has been able to elute the “agent” off coated cells in a true hemolytic disease. The eluted “agent” will behave and act exactly like an antibody and if an efficient elution method is used, the potency and avidity of the “agent” can be maintained over several passages. Obviously the specificity remains the same. The “agents” in the “cold” type are known to consume complement and have been shown in many instances to be hemolytic.

One more point about the specificity of these “agents.” Pirofsky,\textsuperscript{3} though admitting that blood-group-specific antibodies have been described, tries to get out of the dilemma by stating that “most auto-antibodies [sic!] associated with secondary hemolytic anemia seem to act as a non-specific pan-agglutinin.” Specificity of an antibody can only be demonstrated if the antibody fails to react with one particular cell population. This is often difficult enough to find in ordinary blood grouping if the antibody is directed against one of the “public antigens,”\textsuperscript{13} and it is little wonder that antibodies found in autoimmune hemolytic anemia provide a nice “headache” to even experienced serologists. The first antibody serologically defined\textsuperscript{14} had anti-e specificity (a “near-public” antigen). Since then, it has been shown\textsuperscript{15} that those antibodies which are encountered least frequently in ordinary blood grouping work are most frequently encountered in the different serologic climate of hemolytic anemia. In other words, it is conceivable—and there is some experimental evidence for this assumption—that there are present in the rhesus system, and possibly in other systems as well, antigens which are of extreme frequency. These antigens cannot by their nature as “very public antigens” be expected to provide the antigenic stimulus for the production of antibodies unless a transfusion is given to a recipient who, as a great exception, lacks this very frequent antigen. There is, however, nothing to stop them from producing an antibody in acquired hemolytic anemia. They would still be specific for these very frequent antigens in spite of the fact that they react with even the largest cell panels. (But does not anti-Tj\textsuperscript{*} do the same thing?) The assumption of “nonspecific” antibodies is positively lindingering and does not help further knowledge, as it may stop workers from investigating these “agents” further. The antibody in the “cold” hemolytic anemias, for instance, has up to a short time ago been regarded as the nonspecific antibody par excellence. It has been claimed that it is not even species-specific. At least in one case of a “cold” hemolytic anemia, however, it was shown by Wiener et al.\textsuperscript{16} that this antibody too may have blood group specificity and define a very frequent blood group antigen (I). Apart from their theoretical importance, these findings have an immediate practical
application, as, with their recognition, it would be possible to find compatible donors for these sometimes very ill patients. "Nonspecific" is therefore a word which might well disappear from the serologic dictionary. An antibody by definition must combine with an antigen, and a nonspecific antibody is a *contradictio in adjecto*. This is not to deny that positive Coombs tests produced by toxic "agents" are nonspecific. No antibody-antigen union is involved in these cases. One must insist, however, that the assumption of an antibody makes it essential to assume specificity, and if specificity has been shown to be a property of an "agent," the "agent" can easiest be explained as being an antibody.

The "agent" of hemolytic disease has, therefore, the following properties: It is a gamma globulin and combines specifically with one or more antigens on the patient's own red cells (and possibly on other tissues as well). This combination can easily be demonstrated. The union between the antigen and the "agent" is governed by well defined conditions of temperature, medium and state of the antigen. The "agent" can be eluted from the cells, and, lastly, it occasionally consumes complement and lyses the cells. This would seem to be reasonable evidence of the antibody nature of the "agent" found in hemolytic disease and can surely be accepted as such by even the most fastidious workers. This statement does not, of course, deny the possibility of a very occasional case presenting a direct Coombs test not due to the coating of the red cells by an antibody, but it would be important that the protagonists of the "nontibody nature" of autoimmune hemolytic anemia publish appropriate cases and thus produce unequivocal evidence of their existence in man.

In the meantime, however, I would like to make a plea to all workers in this field to have the courage to call an antibody an *antibody* and to accept the possibility of an autoantibody produced by the patient under the exceptional circumstances of hemolytic disease. It does not help our understanding or further work if recourse is taken to expressions like "agent," "E.C.M.," etc. It would appear that no matter what names it receives, "an antibody by any other name will still combine with its specific antigen."

REFERENCES

7. Boyd, W. C., and Reguera, R. M.:


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