Journal of the Minnesota Academy of Science

Volume 29 | Number 1 Article 37

1961

Progress Report on the Isolation of Human Antibodies from Cattle Anti-Human Sera

Charles W. Anderson
Central High School

PROGRESS REPORT ON THE ISOLATION OF HUMAN ANTIBODIES FROM CATTLE ANTI-HUMAN SERA[1]

CHARLES W. ANDERSON
Central High School, Norwood, Minnesota

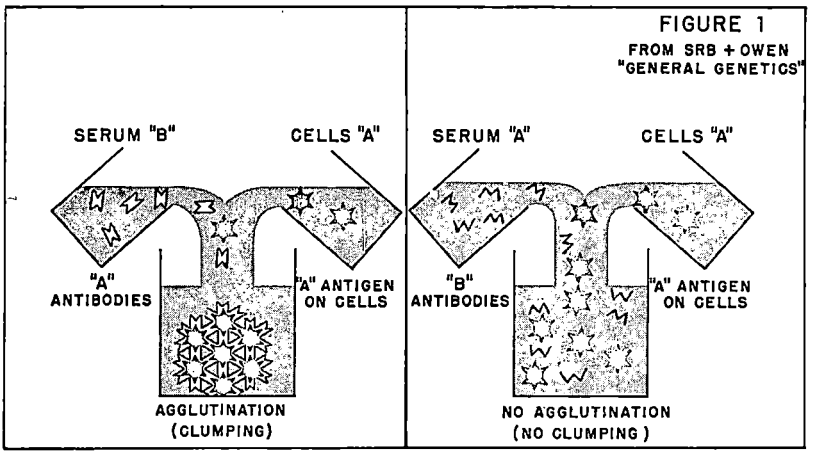
This progress report is concerned with the isolation, detection, and preparation of human antibodies from cattle anti-human sera and can be classified into the field of immunology which deals generally with the mechanisms by which living tissue reacts to foreign living or non-living biological materials.

It would be rather difficult to describe the work which has been done without prefacing the remarks with a few words and diagrams of explanation. The terms "agglutination," "antigen," "antibodies," "absorption," etc., may be familiar to a few but meaningless to the majority; it is to the latter group that this introductory explanation is directed.

It has been known since about 1875 that when blood cells of one species of animal are mixed with the blood serum of another species of animal, clumping of the cells or agglutination, as it is called, will occur. This clumping or agglutination is due to the reaction of an antigen, located on the surface of the red blood cells, with its corresponding antibody found in the serum. This same phenomenon will occur often in the mixing of human blood from different individuals, and it is through the techniques of blood grouping, developed since 1900, that safe transfusions of blood have been made possible.

An example of agglutination due to an antigen-antibody reaction is shown on the left of Figure 1. Here it can be seen that antibodies in a serum unite specifically with their corresponding antigen on the cells to tie the cells together in clumps. If the antigens and the antibodies present do not correspond, then no clumping or agglutination will occur. This is shown on the right of Figure 1. Antibodies are generally found in the serum and antigens are located on the surface of the red blood cells. When they come together, the cells (with their antigens) and the antibodies tie together and form this clumping of cells. If the antigens and the antibodies do not correspond, then the

[1] Acknowledgments are made to:
Dr. W. H. Stone, Dr. Margery Gray, Dr. W. J. Tyler, Mr. Earl Edwards, Badger Regional Blood Center, the University of Wisconsin Genetics Department, the National Science Foundation, the Future Scientists of America Foundation and School District #108, Norwood, Minnesota, for aid and assistance in making this study possible.



cells will not agglutinate. The "key" (antigen) must "fit" the "lock" (antibody) before agglutination can occur, so to speak. In other words, antigens and antibodies show specificities in their reactivity.

At the present time, a great deal of research is being done on human blood groups. There are several reasons for this. One has to do with the use of human blood for transfusion and the prevention of transfusion reactions. A blood transfusion may be considered a homograft from the standpoint that it is a tissue (even though it is nonproliferative) and may be transplanted from one member of the species to another member of the same species, provided that cross-matching has been done. Reactions to transfusions are seemingly rare these days with methods and techniques employed by efficient technicians in cross-matching patient's and donor's bloods. Reactions do take place occasionally and are usually due to an antigen on the donor's erythrocytes which reacts with the corresponding antibody in the patient's blood or stimulates the formation of antibodies which will react with blood used in subsequent transfusions or vice versa.

Another of the research areas deals with the appearance of hemolytic disease of the newborn which occurs primarily as a result of cross circulation of the fetal blood between the developing infant and the mother. Through the use of blood typing sera and other typing tests, most of these cases can be discovered prior to parturition and proper preparations can be made to treat and insure recovery of the infant after it is born. Another aspect of blood group research lies in the study of population genetics. Differences have been found with respect to the frequencies of erythrocytic antigens in different population groups, e.g., about 99% of the Chinese are Rh_0 positives while the Basques, on the other hand, have a higher incidence of Rh_0 negatives than other indigenous groups. Similar studies have been done and are now in progress relative to other blood group antigens and population groups, and corresponding differences in the frequencies of these antigens have been established.

In all fields of human blood group research, blood typing is a necessity and there is a continuing need for sources of typing sera which will detect specificities on the human erythrocytes and other tissues which show blood group antigenicity. It would seem desirable to find a method for producing some of these antisera in larger quantities. There are, according to Race and Sanger (1958; 1959), 59 known human erythrocytic specificities and of this time there are probably more which have been discovered. A. E. Mourant in a recent publication (1959) says: ". . . If sufficient quantities of the more recently discovered diagnostic sera can be made available and suitable placed workers trained to use them, this alone will greatly increase our knowledge. *Much depends upon the possibility, at present barely investigated at all, of making antisera in animals rather than waiting their appearance in human beings.*" There is a limit to the amount of antisera than can be obtained from man. Rabbits, even though they are prolific, yield only small quantities of blood, and smaller quantities of serum. Therefore, cattle would be desirable animals from which to obtain antisera, provided that they develop antibodies in response to an injection or series of injections of human red blood cells, since one can remove from two to four liters of blood at one time from them without difficulty.

The major sources of commercial blood typing antisera are limited to:

1. People who have been immunized through blood transfusions,
2. Women who have been immunized through a pregnancy,
3. Those who knowingly allow themselves to be immunized,
4. Some animals, rabbits in particular, can be immunized to certain of the human red cell factors, particularly M and N, and
5. Plants provide agglutinins which are specific for human red cell factors and research is continuing along these lines in hopes of being able to produce cheaper blood typing reagents.

The purpose of this project has been to find specific agglutinins in cattle sera, from cows which have been purposely immunized by injections with human erythrocytes. Booster injections last summer produced the development of antibodies specific to human erythrocytes in titers up to 32,000 (1 part antisera to 31,999 parts of saline). Thus far the studies have been fruitful in that anti-M or an agglutinin specific for those human red blood cells containing the M antigen or factor has been isolated. This has been accomplished by means of absorbing the cattle sera with the human erythrocytes which contain all (it is hoped) of the factors of the blood which was originally injected with the exception of M. In this process, these antigens remove species specific agglutinins in the cattle sera, in a process which can be likened to a blotter soaking up ink. Everything is removed except

for the anti-M which is suspended in the remaining sera. Thus far, over 100 different human bloods have been typed with this anti-sera in the blood laboratory and no false positive or negative reactions have been observed. Concurrent testing has been done using commercially available anti-sera as a control. The major problem at present is that the reactions are not as strong or as fast as obtained with the commercial anti-sera. The anti-M obtained from the cattle sera is being used also by Dr. Margery Gray at the Wisconsin University Hospital, Mr. Earl Edwards at the Wisconsin State Laboratory of Hygiene, and Dr. W. H. Stone of the University of Wisconsin Genetics Department. Dr. Gray has tested blood from 30 different people and has found no non-specific reactions. Dr. Stone and Mr. Edwards report some non-specific reactions when using the anti-sera. They both state that these reactions are removed on further dilution of the anti-sera with saline. It is interesting to report that Dr. Stone also prepared anti-M from the same sera while on leave last year at the California Institute of Technology. His reagent is highly specific but has a lower titer than that prepared here. There are some differences in the absorption procedures which were used and this may account for the slight differences in the titers. A possibility as to the non-specific reactions with the anti-sera obtained here as well as differences in titer may be due to the fact that in this laboratory, the absorptions have been carried out using expired cells obtained from the Red Cross Blood Bank. These expired cells are generally four to seven weeks old when used, and generally 1 to 8 different donor's cells have been used for these absorptions. All of the cells used for the absorptions have been typed for the ABO, Rh, and MN systems. The cells used for the presumptive and definitive tests for the presence of the M antigen have been obtained from the Mt. Sinai Medical Research Foundation in Chicago and have been tested for a total of 18 different antigenic specificities. Additional cells used are from donors at the University of Wisconsin and have been tested for the same number of specificities at the Blood Group Research Unit of the Lister Institute in London. In carrying on the tests, the assumption is being made that there may be other antibodies in the cattle sera which may react with erythrocytic antigenic specificities in the panel cells which have not been tested for and some interesting reactions have been observed in some of the analyses. Further investigation is warranted along these lines.

Testing is now in progress to find a method to make the anti-M react faster with the panel cells.

Research is being conducted to find an anti-N. Results to date on the presence of this antibody in the cattle sera are inconclusive. The N antigen generally seems to be weaker in capacity to form the corresponding antibody and consequently its isolation is more difficult.

One attempt has been made to test for one of the antibodies in the Rh system and further work has been done during this academic year at Central High School, Norwood, Minnesota. Dr. W. H. Stone

has also done some preliminary studies in this area with rather interesting results.

Upon completion of the work at the University of Wisconsin this summer, it is expected that a paper will be written for presentation at the International Congress of Human Genetics to be held in Rome in August, 1961.

LITERATURE CITED

MOURANT, A. E. 1959. *Blood groups and anthropology*. British Medical Bulletin, Vol. 15.

RACE, R. R. and RUTH SANGER. 1958. *Blood groups in man*. (3rd Ed.) Oxford, Blackwell Scientific Publications.

RACE, R. R. and RUTH SANGER. 1959. *Inheritance of blood groups*. British Medical Bulletin, Vol. 15, no. 2.