CONCERNING THE APPEARANCE OF INCOMPLETE ANTIBODIES FOLLOWING BONE MARROW TRANSPLANTATION UNDER CONDITIONS OF RADIATION SICKNESS

(PRELIMINARY REPORT)

M. A. Umnova and M. N. Novikova

Central Order of Lenin Institute of Hematology and Blood Transfusion
(Director, Active Member AMN SSSR A. A. Bagdasarov), Moscow
(Presented by Active Member AMN SSSR A. A. Bagdasarov)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 52, No. 8,
pp. 82-86, August, 1961
Original article submitted June 27, 1960

In the problem of bone marrow transplantation a great deal of attention has been devoted to the question of the so-called secondary or homological disease. The previously advanced theory of its origin as the result of irradiation has been discarded at present. The majority of investigators think that development of the secondary disease is connected with immunological shifts, in their turn connected with the introduction into the recipient's organism of the hemopoietic tissue of an alien antigenic structure. Two theories have been advanced: 1) the secondary disease is connected with the destruction of transplanted bone marrow cells by the recipient's antibodies; 2) the secondary disease is induced by antibodies developed by the transplanted cells against the recipient's erythrocytes.

The first premise was rejected by a number of authors on the ground that the secondary disease sometimes develops against a background of good hemopoiesis and activity of the transplanted cells [1,5].

The second premise, on the contrary, was supported on a number of grounds.

In 1957, Aphof detected in the recipient's blood antibodies against its own erythrocytes. Weyzen and Vos [6], Aphof, Grabar and coworkers [3] found in the organism of a mouse, which had received a transplant of a mouse bone marrow, a mouse gamma-globulin (these data, however, have not been confirmed by a number of authors).

In favor of this theory are Kostrov's experiments [2] who had preliminarily sensitized the donor with the recipient's tissue and obtained a marked pathological effect, whereas transplantation of bone marrow from the donor, sensitized by tissues not akin to the recipient's, did not produce this effect.

This theory is confirmed also by the attempts of Cristoffanini [2] to render the donors tolerant to the homologous bone marrow by introducing during their birth cells identical to the recipient's.

Thus, the problem of the nature of immunological processes in the so-called secondary disease still remains unsolved. Yet, the study of these processes is important to the problem of transplantation of the bone marrow.

On the basis of numerous, including also our (M. A. Umnova), previous works on the value of employing Coombs' test for the elicitation of iso- and auto-immune antibodies, we decided to utilize this test following introduction of bone marrow to animals under conditions of radiation sickness.

Maté reports on the use of Coombs's test in the study of the results of bone marrow injection to the afflicted Yugoslav physicists.

We think that, if the transplanted bone marrow cells are truly capable of developing antibodies against the recipient's erythrocytes, then they can be elicited by means of a direct Coombs test. On the other hand, isoimmune antibodies in an animal-recipient can be elicited by an indirect Coombs test, provided they are formed in response to the introduced cells.

METHOD

As we know, Coombs suggested his test for the elicitation of incomplete antibodies in humans. It is based on introducing into the reaction the precipitating serum against the globulin fraction of human protein. The precipitating

serum causes agglutination of the erythrocytes, provided some antibodies (isoimmune or autoimmune) are fixated on them and because these antibodies are contained in the globulin protein fraction.

First, it was necessary to obtain a precipitating serum against the protein of monkeys. For this purpose rabbits were immunized with the monkey blood serum. In the immunization of rabbits and subsquent processing of the serum we availed ourselves of the experience of M. A. Umnova in obtaining sera for carrying out the Coombs test in humans. Three groups of rabbits, five animals in each group, were immunized by means of various doses of monkey serum injected at various intervals: three injections at 1.5 ml every other day, five injections at 1.5 ml every 5 days, and six daily injections at 0.5 ml. On the 8th-10th day after the last injection, blood was taken from the animals and the serum obtained. Four rabbits died in the immunization process, the rest produced approximately identical results in all three groups.

Since our purpose was to employ the obtained sera for the elicitation of monkey protein in the form of antibodies, fixed on monkey erythrocytes, we tested the serum to ascertain first, whether the immune rabbit serum contains precipitins against monkey proteins (which was our aim) and, secondly, whether it contains heteroagglutinins against monkey erythrocytes, i.e., whether this serum is capable of causing an agglutination of "pure" erythrocytes of the monkey prior to the fixation of antibodies on these erythrocytes.

Sera which did not precipitate monkey protein in a 1:500 dilution (there were four such sera) and sera which, though containing precipitins, had also agglutinins against monkey erythrocytes (four samples) we considered unsuitable for the experiments. As a result of preliminary testing, we selected three sera (No. 4,8, and 12) which had a titer of precipitins against monkey proteins from 1:10,000 to 1:20,000 and which agglutinated monkey erythrocytes in a dilution not higher than 1:3.

These sera were diluted in a physiological solution 5-fold and were used in the experiments. In view of the absence of data on the staging of Coombs test in monkeys, the impossibility of checking these sera with definitely sensitized erythrocytes, and the absence of any related data in the available literature, we employed simultaneously all three sera in our experiments.

RESULTS

We had under observation five monkeys (Macaca Rhesus) weighing 1.5 to 2 kg which had been exposed in a cobalt apparatus to a lethal dose of irradiation (609 r). An acute radiation sickness developed in the monkeys. Two of them (controls) perished on the 14th-17th day, the other three received bone marrow obtained from the same species of monkeys. Bone marrow was injected intravenously, 20 ml on the average which contained approximately 1-1.5 billion nuclear cells. Prior to injection, a test was made on the compatibility between recipient's serum and the donor's blood on a flat surface, as it is generally done prior to blood transfusion. No incompatibility was observed. The erythrocytes in all monkeys were examined via direct Coombs test, and the blood serum was tested in relation to the erythrocytes of healthy monkeys, bone marrow donors in particular, by means of the indirect Coombs test.

Observations were carried out prior to irradiation, after irradiation, prior to the injection of bone marrow and finally at various periods after its injection.

The experimental results were as follows. In control monkeys the result of the direct and indirect Coombs test proved to be negative. In one of the irradiated monkeys (No. 54) which had been injected bone marrow on the 2nd day following irradiation, the direct and the indirect Coombs tests were negative. The monkey developed a pronounced anemia which subsequently disappeared gradually.

In the 2nd irradiated monkey (No. 42) the indirect Coombs test was negative which indicated, as in the first case, the absence of antibodies in relation to the introduced cells. As regards the direct Coombs test, on the 16th day after bone marrow injection it produced a positive result. This monkey also survived, but it developed a stable anemia which showed no tendency of abating during the 25 days of observation.

Of special interest were observations of the 3rd monkey (27). The direct Coombs test prior to irradiation and after bone marrow injection was negative. The indirect test, carried out prior to bone marrow injection with the recipient's serum and the erythrocytes of the donor from whom subsequently bone marrow was obtained for injection, proved to be positive. This fact indicated the hitherto unknown phenomenon that in the monkey blood serum incomplete antibodies were present and that in this case the donor's blood was incompatible in relation to the experimental monkey-recipient. Indeed, this monkey in contrast to the other two responded with a reaction to the injection of bone marrow.

Subsequently, the result of the indirect test remained constantly positive. In addition, on the 12th day after the injection of bone marrow the direct Coombs test with the erythrocytes of this monkey produced a markedly positive result.

In this monkey the symptoms of acute radiation sickness were most pronounced. The animal died on the 15th day. Toward the end, the number of erythrocytes decreased from 4.5 million to one million, and the Hb quantity - from 12.4 to 2.6 gm%, the principal reduction of red blood indices showing a precipitate decline between the 9th and 14th day.

In addition to the above-stated we consider it important to cite the results of our initial experiments on dogs. Here, we also employed the direct and indirect Coombs tests on dogs which had been exposed to a 600 r of X-ray irradiation with a subsequent injection of homologous bone marrow. As a reagent, we used the precipitating serum against the dog's proteins. The serum was tested and processed according the same scheme as the precipitating anti-monkey serum.

In experiments with two dogs, we staged the direct and indirect Coombs tests prior to bone marrow injection and at various periods after it.

Interesting data were obtained with dog No. 16. The direct Coombs test remained negative for 26 days, but afterwards positive results were obtained with the direct test on a flat surface, and with the indirect test between the serum of the dog and the erythrocytes of the donor and other healthy dogs. The indirect test titer rose during the observation period from 1:2 to 1:8. It is interesting to note that these results coincide with observations on the amount of properdin (Chertkov) where the titer proved to be reduced during the same period of time (these data require a special analysis).

The results of conducted experiments lead to the conclusion that Coombs test proved to be suitable for the study of immunological shifts in monkeys and dogs following the injection of bone marrow.

The precipitating sera, needed for this purpose, were obtained through immunization of rabbits with blood serum of the same experimental animals.

By means of the indirect Coombs test we were able to elicit in one of the dogs a formation of incomplete isoantibodies following bone marrow transplantation. Also complete isoantibodies were elicited in the same case. The direct Coombs test was negative.

The indirect Coombs test in monkeys showed no formation of immune isoantibodies, but in one monkey we found incomplete (apparently normal) antibodies in regard to the donor, because after the injection of bone marrow symptoms of biological incompatibility appeared.

On this basis we may suggest that in experiments on the injection of bone marrow in monkeys (and possibly in other animals), in the selection of a donor, it is necessary to use the indirect Coombs test in addition to the usually employed incompatibility test.

The use of the direct Coombs test in monkeys enabled us to elicit in two cases antibodies fixated on their own erythrocytes in the experimental monkeys. These antibodies were very clearly elicited in cases where the bone marrow injected in the monkey contained antigens incompatible with the latter.

It can be assumed that in our cases the antibodies, elicited with the direct Coombs test on the erythrocytes of monkeys-recipients, originated as a result of immunological activity of the transplanted cells and that the most active producents of antibodies were the cells which were most alien (incompatible) to the recipient's organism.

In view of the small quantity of conducted observations the present report should be considered as a preliminary one.

LITERATURE CITED

1. C. C. Congdon, 21st Congress of Physiologi. Science, Buenos Aires, (1959), 119.
2. Cosgrove, Blood, 14, (1959), p. 1252.
3. Cristoffanini, Blood, 14, (1959), p. 1256.
4. P. Grabar, J. Courcon and P. L. Ilberg, et al., C. R. Acad Sci. (Paris 1957), 245, p. 950.
5. T. Makinodan, Cited C. Congdon.
6. J. J. Trentin, Proc. Soc. exp. Biol. (N.Y.), 96, (1957), p. 139.
7. W. Weyzen and O. Vos, Nature 180, (1957), p. 288.