"Heterophile" Antigen in Sera of Human Recipients of Renal Allografts (44039)

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Abstract. **It was noted that many sera of patients with renal allograft produce distinct precipitation lines in gel diffusion tests with about 20% of infectious mononucleosis sera. The antibodies in infectious mononucleosis sera were of IgM isotope, but, interestingly, they could be removed by guinea pig kidney homogenate, which indicated that the reactions studied were of the Hanganutziu-Deicher rather than of the Paul-Bunnell type. This contention was strengthened by the fact that positive transplantation sera reacted also with standard serum with Hanganutziu-Deicher antibodies. Thus far, the presence of the antigen in the transplantation sera could not be related to the clinical status of the patients, however, the antigen was noted primarily in those sera that did not contain heterophile transplantation antibodies. It was proposed that the antigen detected in the transplantation sera was an altered tissue antigen released from the grafted organ. Besides, interactions between two serum samples from the same patient were noted in immunodiffusion tests. These reactions occurred very seldom and were unrelated to heterophile transplantation antigens or antibodies.** [P.S.E.B.M. 1996, Vol 213]

For the last 30 years this laboratory conducted extensive studies on heterophile antigens and antibodies (review in Ref. 1). Reactions between these antigens and antibodies were studied primarily in the traditional way, by agglutination of erythrocytes from sheep, cattle, and horses, and also by gel diffusion tests we developed, in which either suspensions or extracts of erythrocyte stromata were employed (2–5). We succeeded in identifying two major specificities of Paul-Bunnell antibodies: BS antibodies combining with bovine as well as sheep erythrocytes and B antibodies combining with bovine erythrocytes though not with those of sheep (6). We also studied reactions of Paul-Bunnell antibodies with pathological sera and detected the presence of Paul-Bunnell antigens in a small proportion of such sera (7).

Interest in heterophile antibodies of the "serum sickness type" (8, 9) was revived by the demonstration that these antibodies may be found, not only in sera of patients who received injections of animal sera, but also in several patients with various pathological conditions (5, 10–12). Accordingly, we proposed to call these antibodies Hanganutziu-Deicher antibodies, after their original discoverers (8, 9), and to abandon the misnomer of "serum sickness antibodies."

We devoted extensive studies to heterophile transplantation antibodies which were detected in human allograft recipients by means of agglutination of rat and bovine erythrocytes as well as in gel diffusion tests with suspensions and extracts of erythrocyte stromata (4, 13–15).

Reactions with guinea pig kidney sharply distinguish Paul-Bunnell antibodies on the one hand from Fossman, Hanganutziu-Deicher, and transplantation antibodies on the other hand in that the latter but not the former are absorbed by suspension of guinea pig kidney. On this basis, we proposed dividing human heterophile antibodies into two major groups (1).

We have been fascinated and puzzled by the origin

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of heterophile antibodies in humans. Some of these antibodies are formed as natural antibodies in response to hidden antigenic stimuli, for example, for bacteria which carry the Forssman antigen. In other instances, production of heterophile antibodies might be stimulated by altered antigens of the patient's own tissues, which emerge as a result of pathological processes and which resemble the structure of antigens physiologically appearing in tissues, first of all on erythrocytes of other species (16). We suggested that this latter mechanism operates in formation of Paul-Bunnell antibodies in infectious mononucleosis and of Hanganutziu-Deicher antibodies in various pathological conditions. Finally, we presented evidence that formation of heterophile transplantation antibodies may result from the response to alien transplantation antigens that share epitopes with antigens appearing in foreign species.

Materials and Methods

Transplantation sera studied originated from the transplantation service of the Buffalo General Hospital, Buffalo, NY. Infectious mononucleosis sera were kindly supplied by the Student Health Service of the University at Buffalo.

Preparation of Stroma Extract. Blood of oxen was obtained from the local abattoir. It was collected to acid citrate–dextrose solution and preserved at 4°C for not more than 1 week. Erythrocytes were washed three times with phosphate-buffered saline (PBS), pH 7.2. Packed erythrocytes were mixed with an equal volume of 5% (w/v) trypsin solution, incubated at 37°C for 10 min, and washed three times with cold PBS. Then hemolysis was induced by suspending packed erythrocytes in 50 volumes of distilled water and leaving the suspension for 2–16 hr at 4°C. The stromata were washed repeatedly with distilled water and PBS, and thereafter sedimented at 50,000*g*. The extract was prepared by suspending stromata in an equal volume of PBS and exposing them to ultrasound at 20,000 cps for 90 sec. The sonicated preparation was centrifuged at 60,000*g* for 30 min, and the clear supernatant extract was withdrawn. The protein concentration of this extract was about 8 mg/ml. The extract was preserved at 4°C for not more than 2 days prior to testing, or otherwise for a longer time at −20°C.

For double diffusion tests, 0.5% solution of agarose with the addition of 0.01% thimersol was poured into plastic Petri dishes to form layers 2-mm thick. After the agarose solidified, circular wells with 4-mm diameters were cut by means of brass rings. The diffusion distance between antigen and serum wells was usually 4 mm. After the reagents were added to the wells, the Petri dishes were closed by cover lids and left for 3–7 days at 4°C. Then the precipitation lines were inspected and pictures were taken.

Treatment with 2-Mercaptoethanol. Selected sera were mixed with equal volumes of 0.2 *M* solution of 2-mercaptoethanol and incubated for 1 hr at 37°C. They were examined by double diffusion tests in gel without any further treatment.

Results

In conducting studies on human heterophile antibodies by means of gel diffusion reactions against extracts of bovine erythrocytes, we noted coincidentally a reaction between infectious mononucleosis serum Cw and a serum of renal graft recipient WB. Figure 1 shows that the Cw serum produced two precipitation lines with extract of bovine erythrocytes and that one of these lines merged into a reaction of complete identity with the "unexpected" line formed by Cw serum with WB serum. Significantly, this particular serum WB had no heterophile transplantation antibodies and failed to react with the stroma extract. Intrigued by this observation, we tested several serum samples from patient WB, obtained between April 1966 and January 1968, against serum Cw. As shown in Figure 2, of 12 samples tested 7 produced distinct precipitation lines. In each instance the neighboring lines merged into identity reactions.

Patient WB, a 47-year-old Caucasian male, suffered from end-stage kidney disease due to chronic glomerulonephritis. He received a renal allograft in December 1965 and experienced chronic rejection in 1968. He died in March 1968. Interestingly, the antigen in the serum would appear or disappear within a few days, e.g., the samples from December 1, 5, and 12, 1967, were positive, whereas the one from December 19, 1967, was negative but the sample of December 27, 1967, was again positive.

Subsequently, we studied sera from 20 other transplantation patients, testing 1–34 serum samples from an individual patient. We found the antigen precipitated by Cw serum in six patients. Having in mind that only some serum samples from serial bleedings were positive, we selected two patients from whom we had many serum samples available and studied all these samples. Interestingly, as seen in Table I, of 11 samples from patient Cal only one was positive, whereas of 34 samples of patient NR 17 were positive. Reactions with serum Cw showed identity reactions,

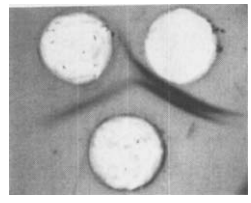

Figure 1. (Lower well) Infectious mononucleosis serum Cw. (upper left well) Transplantation serum WB. (upper right well) Extract of bovine erythrocyte stromata.

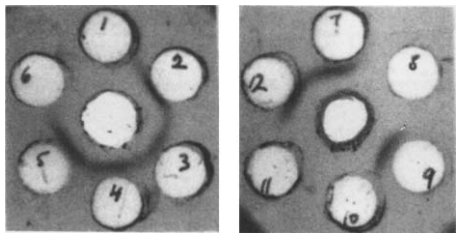

Figure 2. Central wells in both plates: Infectious mononucleosis serum Cw. Peripheral wells: Sequential sera from patient WB: 1, 4/18/66; 2, 1/3/67; 3, 12/1/67; 4, 12/5/67; 5, 12/12/67; 6, 12/19/67; 7, 12/27/67; 8, 1/3/68; 9, 1/16/68; 10, 1/17/68; 11, 1/20/68; 12, 1/23/68.

not only when the various serum samples from the same transplantation patient were studied (Fig. 2), but also when sera of various transplantation patients were examined.

In none of the transplantation patients studied was it possible to relate the positivity of the patient's serum to the clinical status. Significantly, however a definite trend was noted that serum samples positive in reactions with Cw were negative in reactions with bovine stroma extract.

Subsequently, we selected transplantation serum NR that formed strong precipitation lines with Cw serum to study reactions with various infectious mononucleosis sera other than Cw. Positive results were obtained with about 20% of these sera and reactions of complete identity were noted (Fig. 3).

Standard absorption tests were conducted in order to obtain insight into the nature of the observed reactions. As could have been expected, absorption of IM serum Cw with bovine erythrocyte stromata abolished completely its reactions with transplantation sera. However, contrary to our expectation, the absorption with homogenized guinea pig kidney also abolished the reactions under study, which indicated that they belonged to the Hanganutziu-Deicher rather than to the Paul-Bunnell group of reactions. Following this observation, we tested transplantation sera positive with Cw serum against our standard serum with Hanganutziu-Deicher antibodies. As exemplified by Figure 4, a strongly positive reaction was noted.

We also studied the mercaptoethanol resistance of the antibodies in Cw serum. As seen in Figure 5, mercaptoethanol treatment destroyed all antibodies combining with transplantation serum WB as well as most though not all antibodies reacting with the extract of bovine erythrocyte stromata. Furthermore, we found that the antigen reacting with serum Cw was remarkably thermostable and was not destroyed by heating the serum at up to 100°C. Also, it was insoluble at 70% ethanol concentration. It closely resembled tissue antigens of glycoprotein nature, which we described in our previous publications (reviewed in Ref. 17).

In conducting the present studies, we made an inadvertent observation on the interaction between two serum samples of patient NR (Fig. 6a) both of which failed to react with infectious mononucleosis serum Cw (Table I). Furthermore, we found that the precipitation line formed between these two specimens was unrelated to heterophile transplantation antibodies; as

Table I. Gel Precipitation Tests with Infectious Mononucleosis Serum Cw and Sequential Serum Samples from Renal Allograft Recipients Cal and NR

Date serum taken	Result of precipitation test[a]
Cal	
5/4/69	−
5/4/69	−
6/24/69	++
6/24/69	−
7/8/69	−
7/25/69	−
8/5/69	−
8/19/69	−
8/26/69	−
9/24/69	−
9/30/69	−
NR	
1/21/70	+
3/6/70	++
3/27/70	−
4/1/70	−
4/13/70	−
4/22/70	−
5/27/70	−
6/10/70	−
6/17/70	−
6/24/70	−
7/8/70	+
7/22/70	−
8/5/70	++
8/12/70	+
9/2/70	+
9/9/70	+
9/16/70	++
9/23/70	−
9/30/70	+
10/7/70	+
10/14/70	−
10/21/70	−
11/6/70	−
11/9/70	++
11/18/70	−
12/16/70	++
12/23/70	−
1/6/71	+
2/3/71	++
3/17/71	−
3/31/71	+
6/9/71	+
6/14/71	−
6/23/71	++

[a] −, negative; +, weak but distinct precipitation line; ++, strong precipitation line.

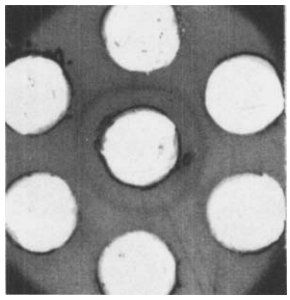

Figure 3. (central well) Transplantation serum NR. (peripheral wells) Six positive infectious mononucleosis sera.

seen in Figure 6b, both transplantation serum samples under study had heterophile transplantation antibodies reacting with bovine stroma extract, while the line formed between the two serum samples showed nonidentity reaction with the lines formed with the stroma extract.

Discussion and Conclusion

In our previous papers, we showed that many infectious mononucleosis sera contained Hanganutziu-Deicher antibodies in addition to those of the Paul-Bunnell type (18, 19). Interestingly, these antibodies were not described before our reports, and, as a matter of fact, the differential test for infectious mononucleosis was designed to show that the antibodies detected by agglutination of sheep erythrocytes are not removed by guinea pig kidney suspension—that is, that they are not of the Hanganutziu-Deicher (or Forssman) type (20). We did not accept this failure of removal of anti-sheep hemagglutinins by guinea pig kidney suspension as evidence for the absence of Hanganutziu-Deicher agglutinins from infectious mononucleosis sera, but we interpreted this failure as evidence that the agglutinins of the Paul-Bunnell type have considerably higher titer than those of the Hanganutziu-Deicher type. Significantly, Hanganutziu-Deicher antibodies could be readily demonstrated in about 50% of infectious mononucleosis sera by means of their reactions with murine myeloma cells that contained Hanganutziu-Deicher but not Paul-Bunnell antigen (18) or, otherwise, by reactions with Hanganutziu-Deicher antigen isolated by chemical procedures from bovine erythrocyte stromata (19).

In the present study, we showed that a significant

Figure 4. Upper well: Transplantation serum WC. Lower well: Human serum with Hanganutziu-Deicher antibodies, CdR.

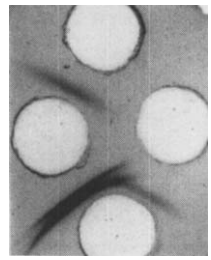

Figure 5. Upper well: Transplantation serum WB. Lower well: Extract of bovine erythrocyte stroma. Left well: Infectious mononucleosis serum Cw mixed with equal volume of PBS. Right well: Infectious mononucleosis serum Cw mixed with equal volume of mercaptoethanol solution.

number of transplantation sera contained an antigen combining with antibodies present in many infectious mononucleosis sera. These antibodies apparently belonged to the IgM isotype since they were mercaptoethanol sensitive. They were of the Hanganutziu-Deicher type rather than of the Paul-Bunnell type since they could be absorbed by guinea pig kidney suspension. This was consistent with the observation that the antigen under study reacted also with our standard serum containing Hanganutziu-Deicher antibodies of the "classical" type (i.e., reacting with N-glycolylneuraminic acid) (21).

In testing sequential serum samples from renal graft recipients, we noted the appearance and disappearance of this antigen under study, sometimes within the period of a few days without any obvious relation to the clinical status of the patient. On the other hand, the appearance of the antigen in the circulation was apparently related to the absence of heterophile transplantation antibodies. This was in agreement with our previous observation (15) that the heterophile transplantation system is related to the Hanganutziu-Deicher rather than the Paul-Bunnell system. In this respect, the antigen detected now in transplantation sera differed sharply from the heterophile antigen found previously in syphilis and leprosy sera since the latter antigen was shown to be related to the Paul-Bunnell system (22).

We were not able to decipher the mechanism responsible for the appearance of the antigen under study in the circulation. We are tempted to assume that this antigen may be an altered tissue antigen re-

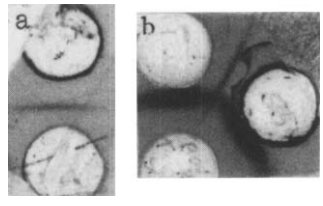

Figure 6. (a) Sera of transplantation patient NR. Lower well, sample of 6/17/70; upper well, sample of 6/24/70. (b) Lower well, extract of bovine erythrocyte stromata; upper well, NR serum of 6/17/70; right well, NR serum of 6/24/70.

leased from the grafted organ as a result of clinical or subclinical rejection episodes.

In our previous immunodiffusion studies, we observed interactions between transplantation sera originating from different patients (23). In the present study, we noticed a precipitation reaction between two serum samples originating from the same patient. Besides the patient whose serum samples were tested in the experiment presented in Figure 6, we observed similar though weaker precipitation reactions between serum samples originating from a few other patients. These autologous serum-serum interactions were not related to the antigen reacting with infectious mononucleosis sera or to the heterophile transplantation system. Future studies will have to provide an explanation for their nature.

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