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## Antigen Induced Histamine Release from Platelets of Rabbits Producing Homologous PCA Antibody. (31371)

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Humans, guinea pigs, rats and mice produce antibodies which are capable of sensitizing homologous species for anaphylaxis. The human antibody (reagin) is capable of sensitizing human leukocytes so that on the addition of antigen histamine is released(1). There is no requirement for added serum or plasma factors, such as complement, for histamine release. The corresponding guinea pig and rat antibodies are able to sensitize their respective mast cells and, here also, it has been demonstrated that complement is not needed(2).

Recently Zvaifler and Becker(3) described an antibody in rabbits appearing as early as 6 days after immunization which sensitizes rabbits for passive cutaneous anaphylaxis (PCA). The antibody was not a $\gamma$G or $\gamma$M immunoglobulin and had no detectable precipitating or complement fixing properties. Onoue *et al*(4) described a similar rabbit skin sensitizing antibody and ascribed it to 7S$\gamma$A immunoglobulin.

All the previous studies of *in vitro* release of histamine from rabbit platelets involved rabbit $\gamma$G antibodies and the presence of fresh plasma was found to be obligatory. In this study it will be shown that sensitized rabbits whose plasma contains rabbit PCA antibody also have platelets which even after thorough washing are capable of releasing histamine on the addition of antigen in the absence of added plasma.

*Materials and methods. Preparation of dinitrophenylated proteins.* Bovine gamma globulin (BGG) and bovine serum albumin (BSA) was dinitrophenylated by reacting with 2,4 dinitrobenzene sulfonate as described by Eisen(5). The conjugated antigens contained approximately 35 moles of DNP per mole of BGG and 15 moles of DNP per mole of BSA. As work progressed it was found that the DNP-BSA conjugate became contaminated and was capable of releasing histamine from platelets of unimmunized rabbits presumably because of its endotoxin content. Subsequent DNP-BSA material was filtered and stored at -20°C in small quantities.

*Immunization of rabbits.* One ml of the DNP-BGG in Freund's complete adjuvant (1.4 mg AgN/ml) was injected in 0.25 ml volumes into each foot pad. Beginning on the fifth day after immunization the animals were bled daily from the marginal ear vein. The blood was collected into ice cold polypropylene tubes which contained sufficient sodium heparin to yield a final concentration of 7 units of heparin per ml of whole blood. In most instances the blood was used for histamine release as soon as possible after collection. In those experiments where release of histamine from whole blood was compared with that from washed platelets, the whole blood was stored in an ice bath until used for release. To obtain washed platelets, a known volume of whole blood was centrifuged at 3000 rpm for 15 minutes, the plasma removed and the platelets washed 3 times with 3 to 5 times their volume of ice cold Tyrode's solution. After washing, the sediment was suspended up to the original volume of whole blood and used for histamine release. The suspension, although it contained platelets, leukocytes and red cells, is referred to as "washed platelets"

since essentially all the histamine release comes from the platelets(6).

*Histamine release.* Whole blood or washed platelets were added to siliconized tubes containing 20 or 40 $\mu$g DNP-BSA nitrogen and sufficient Tyrode's solution for a final volume of 2.5 ml. After 60 minutes incubation at 37°C, the mixture was centrifuged and the supernatant assayed for histamine. The total histamine content was obtained by incubating whole blood or washed platelets with mercuric chloride at a final concentration of $1 \times 10^{-3}$M.

The extraction and condensation of histamine to form a fluorescent product were essentially that described by Shore *et al*(7), modified so as to use smaller amounts of extractants.

*Passive cutaneous anaphylaxis.* Plasma was collected daily from immunized rabbits and kept at 4°C so all bleedings would be tested at the same time, in the same animals. The plasmas were injected into shaved albino rabbits. Two-tenths of an ml of various plasma dilutions were injected intradermally in a random pattern into various rabbits. The animals were challenged 72 hours later by intravenous injection of 1.5 ml of DNP-BSA (2.76 mg N/ml) and 1.0 ml of 5% Pontamine Sky Blue. All tests were performed in at least 2 rabbits. The resulting reactions were recorded and measured 30 to 60 minutes after challenge.

*Results.* The conditions for the production of PCA antibody determined previously(3) were used in this investigation. Twenty-four rabbits, immunized with DNP-BGG in groups of 4, were bled daily usually starting with the 5th day after immunization. Each bleeding was tested for PCA antibody and histamine release.

Table I lists the results obtained from these 24 rabbits. The results demonstrate that only 11 of the 24 rabbits immunized (45.8%) produced detectable PCA antibody. This is in agreement with the results of Zvaifler and Becker who demonstrated that PCA antibody was produced in less than one-half of the animals immunized.

TABLE I. Correlation Between Histamine Release and PCA Antibody Production.

| Histamine release | PCA | | Total histamine release |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 11 | 2 | 13 |
| Negative | 0 | 11 | 11 |
| Total PCA | 11 | 13 | |

Using whole blood, it was possible to demonstrate an antigen induced histamine release of greater than 20% in 13 of the 24 rabbits immunized (54.2%). Of the 11 rabbits that demonstrated PCA antibody all (100%) gave histamine release while of the 13 rabbits that showed no PCA antibody production only 2 rabbits (15.4%) demonstrated histamine release. Although these 2 rabbits released histamine, it should be noted that the release was only slightly more than 20% and then only on one or two of the 6 days on which histamine release was tested.

These results demonstrate a definite correlation between the production of PCA antibody and antigen induced histamine release. While it is possible to obtain antigen induced histamine release in a small number of rabbits in the absence of demonstrable circulating PCA antibody it is not possible to obtain PCA antibody production without accompanying histamine release.

The time course of production of PCA antibody and the appearance of antigen induced release of histamine were roughly similar. PCA antibody appeared in the serum by the 6th to 7th day following immunization. Antigen induced histamine release was occasionally detectable as early as the 5th day, but was not consistently present before the 6th and 7th day. Maximal antigen induced histamine release occurred between the 7th and 14th day. Maximal PCA titers were reached between the 7th and 9th day. This general, but incomplete corrrelation between the time course of the two activities suggested the possibility that platelets were being sensitized *in vivo* with PCA antibody, and the presence of free antibody in the plasma was not entirely responsible for the observed histamine release.

To test this possibility, 31 separate bleedings from 8 rabbits were compared for the ability of whole blood and washed platelets separated from an aliquot of the same whole blood to give antigen induced histamine re-

TABLE II. Comparison of Histamine Release from Whole Blood and Washed Platelets.

| Histamine release from washed platelets | No. of exp |
|---|---|
| Greater than whole blood | 16 |
| Equal to " " | 7 |
| Less than " " | 8 |

lease. Table II tabulates the results of these comparative experiments. It is apparent that in the 31 bleedings tested the washed platelets are capable of the same or better histamine release than whole blood in over 74.2% of the bleedings. Furthermore, in every bleeding tested the washed platelets showed some histamine release while 8 bleedings of the 31 using whole blood showed no histamine release at all.

*Discussion.* The finding that 45.8% of the immunized rabbits produced PCA antibody is in agreement with the results of Zvaifler and Becker(3) who demonstrated that less than one-half of the immunized rabbits were able to produce PCA antibody. In all rabbits where circulating homologous PCA antibody was detectable it was possible to obtain antigen induced histamine release from their platelets.

The ability to obtain antigen induced histamine release in the absence of plasma is completely different from the absolute necessity for free plasma when histamine is released from platelets by the interaction of antigen with γG antibody. This lack of a requirement for added plasma is what has been observed in the histamine release obtained from human leukocytes sensitized with human reagin, rat mast cells sensitized with rat mast cell sensitizing antibody and guinea pig mast cells sensitized with guinea pig γ1 antibody. This suggests that the rabbit PCA antibody is capable of sensitizing rabbit platelets in the same fashion that the functionally similar human reagin, rat mast cell sensitizing antibody and γ1 guinea pig antibody are able to sensitize the human leukocyte, rat and guinea pig mast cell, respectively.

Obviously, further work is required to substantiate this suggestion and to rule out the presence of serum components adsorbed on the platelets. The limited duration of activity of the PCA antibody in plasma as well as the relatively small number of rabbits producing this antibody necessitates that future work be concerned with 1) conditions for production of this antibody in greater concentration and in a larger proportion of immunized animals and 2) conditions for *in vitro* sensitization of normal platelets with PCA antibody. Work toward fulfillment of these objectives is currently planned.

*Summary.* The results obtained with 24 rabbits show a definite correlation between antigen induced histamine release from rabbit platelets and PCA antibody production. In all cases where there was PCA antibody production there was also antigen induced histamine release, while only 15.4% of the rabbits showed antigen induced histamine release when there was no PCA antibody production. When a comparison between antigen induced histamine release from whole blood and washed platelets was made, it was found that in 74.2% of the experiments washed platelets exhibited the same or better release than whole blood. It is concluded that rabbit PCA antibody is capable of sensitizing rabbit platelets and that free plasma factor and free antibody are not necessary for antigen induced histamine release.

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