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Biological Characterization of Mouse 'Early' Antibodies

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Summary. The ability of mouse antiserum obtained early after immunization to induce passive cutaneous anaphylaxis (PCA) using a short (4 hours) and a long (72 hours) latent period was studied. It was observed that the ability of an antiserum to induce PCA reactions after a short latent period is not correlated with its ability to induce PCA reactions after a long latent period. Furthermore PCA reactions induced 4 hours after sensitization are not abolished by heating of antisera and are completely prevented by mepyramine whereas PCA reactions induced 72 hours after sensitization are completely abolished by heating but are not prevented by mepyramine. It is suggested that antiserum obtained from mice a few days after a single dose of antigen contains at least two biologically distinct antibodies.

INTRODUCTION

It is known that many species produce antibodies that are characterized by their skin-fixing and heat labile properties. In the rat this antibody is produced early after immunization with *Bordetella pertussis* or Freund's adjuvant. It sensitizes the rat for cutaneous and systematic anaphylaxis and also rat mast cells both *in vivo* and *in vitro*. A γG antibody is found late in immunization which although capable of sensitizing rats for cutaneous and systemic anaphylaxis does not sensitize mast cells (Mota 1963a, b, c, 1964). The presence of a similar 'early' antibody in the mouse was recently described by Mota and Peixoto (1966). The antibody is transient and was differentiated from described mouse IG globulins by its property of remaining fixed to homologous skin for many days and by its thermolability. The present paper reports further experimental results on the biological properties of mouse 'early' antibodies.

MATERIAL AND METHODS

Animals

Female, adult albino mice weighing 18–25 g were used throughout.

Antigens

Crystalline bovine serum albumin (BSA), hen egg albumin (Ea) twice crystallized (Mann Research Laboratories, New York, U.S.A.) and bovine γ-globulin (BGG), fraction II (Pentex Inc., Illinois, U.S.A.) were used as antigens.

Immunization methods

To induce the production of 'early' antibodies the mice were immunized either with a subcutaneous injection of Ea, BSA or BGG adsorbed on aluminium hydroxide or emulsified

in Freund's complete adjuvant or with an intraperitoneal injection of each antigen along with a suspension of *Bordetella pertussis* vaccine, phase I organisms. Each animal received a single dose of 50 μg Ea, BSA or BGG.

Technique for detection and estimation of mouse 'early' antibodies

To study the production of mouse 'early' antibodies groups of mice were immunized according to the schedules already described and were bled 10 or 12 days later. Blood obtained by puncture of the opthalmic plexus was allowed to clot and the serum was separated by centrifugation in a refrigerated centrifuge. For each group of animals the antisera were pooled and the pooled antisera kept frozen until used. Ovary's method for PCA (Ovary, 1958) was used for detecting and estimating mouse 'early' antibodies. The backs of the mice were shaved with an electric hair clipper, care being taken to avoid irritation of the skin. One or two intradermal injections of 0·05 ml antiserum or antiserum dilutions were made on each side of the dorsal skin with a sharp short bevel hypodermic needle and PCA reactions were elicited after a latent period of either 4 or 72 hours. The animals were injected intravenously with 0·5 ml of a 0·25 per cent solution of Evans blue saline (0·85 per cent NaCl) containing 1 mg antigen. Twenty to 30 minutes after antigen injection, the animals were killed with an overdose of ether, the skin was inverted and the lesion diameter was measured on the inner surface of the skin with a transparent ruler. The antibody content was estimated by determining the PCA titre, i.e. the highest dilution of antiserum able to induce PCA. A minimal number of six mice was used for each determination.

Effect of heating

In order to find out the effect of heating on the ability of mouse 'early' antibodies to induce PCA the antiserum was heated for 30 minutes in a water-bath kept at 56°.

Antagonist of histamine

Mepyramine (pyrilamine maleate, U.S.P.) was used as an antihistamine. The drug was injected intraperitoneally (50 mg/kg) 45 minutes before challenge.

RESULTS

ABILITY OF MOUSE ANTISERUM OBTAINED AT DIFFERENT TIMES AFTER SENSITIZATION TO INDUCE PCA 4 OR 72 HOURS AFTER ANTISERUM INJECTION

It was previously shown that mouse antiserum obtained early after a single dose of antigen was able to induce PCA 72 hours after antiserum injection and that this ability was lost after heating the antiserum at 56° for 30 minutes (Mota and Peixoto, 1966). Further experiments have shown that such antisera are also able to induce PCA reactions within a shorter latent period and the previous experiments were duplicated using a short and a long latent period. Thus antisera obtained at different days after sensitization were used to induce PCA allowing either 4 or 72 hours between sensitization and challenge. The results of these experiments are summarized in Table 1. It can be observed that the ability of the same antiserum to induce PCA reactions after a 4 hours latent period is not correlated with its ability to induce PCA reactions after 72 hours. Thus 20 days after sensitization most antisera have lost the ability to induce PCA after 72 hours although

TABLE 1

ABILITY OF MOUSE ANTISERUM OBTAINED AT DIFFERENT TIMES AFTER SENSITIZATION TO INDUCE PCA 4 OR 72 HOURS AFTER ANTISERUM INJECTION

Immunization	Days after sensitization							
	10		20		30		40	
	PCA reactions (mm)							
	4 hours	72 hours	4 hours	72 hours	4 hours	72 hours	4 hours	72 hours
BSA + Al(OH)3	20	15	20	0	15	0	15	0
Ea + Al(OH)3	20	10	10	0	10	0	0	0
Ea + Al(OH)3	20	15	20	10	20	0	15	0
Ea + pertussis	15	12	20	0	18	0	10	0
Ea + pertussis	18	0	20	0	ND	ND	ND	ND
BGG + Freund's	15	0	18	0	20	0	ND	ND

ND, Not done.

keeping their ability to induce PCA within 4 hours. Furthermore some antisera were able to induce PCA only when a 5 hours latent period was used.

EFFECT OF HEATING ON PCA REACTIONS INDUCED 4 OR 72 HOURS AFTER SENSITIZATION

The results obtained in the previous experiments could be interpreted as being due to the presence of two antibodies. Thus it was thought of interest to study the effect of heating on PCA reactions induced 4 or 72 hours after sensitization. The results of these experiments (Table 2) showed that although heating of antiserum destroys its ability to induce PCA reactions 72 hours after sensitization it did not destroy its ability to induce the same reactions when a 4 hours latent period was used.

TABLE 2

EFFECT OF HEATING ON PCA REACTIONS INDUCED 4 OR 72 HOURS AFTER SENSITIZATION

Antiserum	PCA titre			
	Control serum		Heated serum	
	4 hours	72 hours	4 hours	72 hours
Anti-Eal	40	20	40	0
Anti-Ea2	20	18	20	0
Anti-Ea3	50	20	50	0
Anti-BSA1	20	15	20	0
Anti-BSA2	40	15	40	0

EFFECT OF HEATING ON THE PCA TITRE DETERMINED 4 HOURS AFTER SENSITIZATION

If the loss of ability to induce PCA after 72 hours caused by heating were not due to destruction of a heat labile antibody but to denaturation of the tissue site on the antibody molecule it might be expected that some change in the PCA titre determined 4 hours after sensitization might occur. However when mice were injected with different dilutions of

non-treated and heated antiserum and challenged 4 hours later their PCA titre was observed to be identical as one can see in Table 2.

EFFECT OF MEPYRAMINE ON PCA REACTONS INDUCED 4 OR 72 HOURS AFTER SENSITIZATION

It is known that PCA reactions may have different mediators according to the kind of antibody involved in the reactions (Mota, 1963c). Thus it was decided to find out the effect of mepyramine, a specific antagonist of histamine, on PCA reactions induced 4 or 72 hours after antiserum injection. Accordingly mice were sensitized by injecting anti-Ea in the right side of the dorsal skin and 3 days later were injected with the same volume of the same antiserum in the left side of the dorsal skin. PCA was then induced by injecting Ea 4 hours later. Mepyramine was injected intraperitoneally 45 minutes before challenge. Inspection of Table 3 shows that while PCA reactions induced 4 hours after sensitization are completely abolished by pretreatment with mepyramine PCA reactions induced 72 hours after sensitization are not changed by the same treatment.

TABLE 3

EFFECT OF MEPYRAMINE ON PCA REACTIONS INDUCED 4 OR 72 HOURS AFTER SENSITIZATION WITH MOUSE ANTISERUM OBTAINED EARLY AFTER IMMUNIZATION

Antiserum	PCA (mm)			
	Control		Mepyramine (50 mg/kg)	
	4 hours	72 hours	4 hours	72 hours
Anti-Ea110	20	15	0	20
Anti-Ea210	15	12	0	15
Anti-Ea310	20	18	0	18
Anti-BSA44	18	10	0	12

CAPACITY TO TRANSFER PCA TO GUINEA-PIGS

It is known that some mouse antibodies are able to transfer PCA to guinea-pigs and we decided to find out whether mouse 'early' antibodies would sensitize guinea-pigs for PCA. Thus male guinea-pigs weighing 250–300 g were injected with mouse anti-Ea serum (PCA titre 50) and were challenged 3 or 72 hours later. None of the injected animals presented positive PCA reaction.

DISCUSSION

Many recent experimental data have shown that the various antibodies produced by a given species differ greatly in the kinds of anaphylactic reactions they can mediate (Becker and Austen, 1966). For instance, guinea-pigs produce two types of 7S antibodies, a fast moving γ_1 and slow moving γ_2 which although directed against the same antigenic determinant have different biological activities. Thus $7S\gamma_1$ antibodies are able to transfer passive systemic anaphylaxis or PCA in the guinea-pig while $7S\gamma_2$ is unable to do so (Ovary, Benacerraf and Bloch, 1963). Rats also produce antibodies which differ in their biological properties. One is an 'early' antibody which is heat labile, remains in rat skin for weeks and sensitizes

rat mast cells both *in vivo* and *in vitro*. The other which is found in the sera of hyperimmunized rats is heat stable, remains in skin for a short time and does not sensitize rat mast cells. Both these antibodies produce systemic and passive cutaneous anaphylaxis in the rat. However, anaphylaxis produced with 'mast cell sensitizing' antibody is associated with mast cell damage and is very probably mediated by histamine and 5-hydroxytryptamine whereas anaphylaxis produced with late antibody is not associated with mast cell damage and its mediators are not known (Mota, 1963a, b, c, 1964). Some of these findings were confirmed by Binaghi and Benacerraf (1964), and Binaghi, Benacerraf, Bloch and Kourilsky (1964), who further showed that 'mast cell sensitizing' antibody is a faster moving antibody which migrates ahead of the main population of precipitating antibodies. Furthermore rabbits are also known to produce an 'early' antibody which is thermolabile and appears transiently during immunization (Zvaifler and Becker, 1965). The existence of a similar 'early' antibody in the mouse was only recently observed (Mota and Peixoto, 1966). Our present observations, showing that mouse antiserum obtained early after sensitization can induce PCA reactions that are abolished by heating when a 72 hours latent period is used but not when one uses a 4 hours latent period, can be interpreted in two different ways at least. On the one hand, one can presume that mouse antiserum obtained early after immunization contains two antibodies, one heat stable and one heat labile, which require different latent periods to induce PCA. On the other hand, the assumption may be made that mouse early antiserum contains only one antibody of which the structure necessary for tissue binding can be denaturated by heat. However we think that the following facts suggest the existence of two 'early' mouse antibodies: (a) some antisera although able to induce PCA after 4 hours do not show this ability when a 72 hours latent period is used; (b) the ability to induce PCA after 72 hours disappears a few weeks after sensitization and yet the ability to induce PCA after 4 hours remains; (c) PCA reactions induced 72 hours after sensitization is not abolished by mepyramine while the same reaction induced with a latent period of 4 hours is completely abolished by mepyramine; and (d) furthermore the fact that the PCA titre determined 4 hours after sensitization is not changed by heating does not seem to support the idea of denaturation of the antibody tissue binding site by heating. Preliminary attempts to decide this question by the Ouchterlony technique failed probably because these antibodies do not precipitate. Better immunological studies are required to identify the immunoglobulin fractions present in mouse antiserum early after immunization. The fact that mice can produce different kinds of immunoglobulin has already been observed by Fahey, Wunderlich and Mishell (1964a, b). These authors showed that in addition to γ_{1A} and 19S antibodies mice can also produce two types of 7S antibodies, one faster and one slower moving in type. Furthermore Nussenzweig *et al.* (Nussenzweig, Merryman and Benacerraf, 1964) showed that mice are sensitized for PCA only with $7S\gamma_1$ antibodies whereas guinea-pigs are sensitized with mouse $7S\gamma_2$ antibodies. However these studies have been carried out with sera obtained from hyperimmunized animals whose antibody population is probably different from that present in sera obtained after a single injection of antigen. That this is at least partly true is shown by the existence of a skin-fixing and thermolabile mouse 'early' antibody that disappears in the course of immunization. We suggest that in addition mice also produce an 'early' antibody that is heat stable, remains in the skin for only a short period and can be detected in serum of immunized animals for a long time after immunization. These properties and the fact that the antibody is not able to transfer PCA to guinea-pigs suggest that it may be similar to mouse $7S\gamma_1$ antibody.

REFERENCES

BECKER, E. L. and AUSTEN, K. F. (1966). 'Anaphylaxis'. In: *Immunopathology*. Müller-Eberhard and Miescher, (In press.)

BINAGHI, R. A. and BENACERRAF, B. (1964). 'The production of anaphylactic antibody in the rat.' *J. Immunol.*, **92**, 920.

BINAGHI, R. A., BENACERRAF, B., BLOCH, K. J. and KOURILSKY, F. M. (1964). 'Properties of rat anaphylactic antibody.' *J. Immunol.*, **92**, 927.

FAHEY, J. L., WUNDERLICH, J. and MISHELL, R. (1964a). 'The immunoglobulin of mice. I. Four major classes of immunoglobulins 7Sγ2, 7Sγ1, γ1A (β2A) and 18Sγ1M globulins.' *J. exp. Med.*, **120**, 223.

FAHEY, J. L., WUNDERLICH, J. and MISHELL, R. (1964b). 'The immunoglobulins of mice. II. Two subclasses of mouse 7Sγ2-globulins: γ2a and γ2b-globulins.' *J. exp. Med.*, **120**, 243.

MOTA, I. (1963a). 'Mast cell lytic antibody.' *Fed. Proc.*, **22**, 559.

MOTA, I. (1963b). 'Biological characterization of "mast cell sensitizing" antibodies.' *Life Sciences*, **1**, 465.

MOTA, I. (1963c). 'Passive cutaneous anaphylaxis induced with "mast cell sensitizing" antibody. The role of histamine and 5-hydroxytryptamine.' *Life Sciences*, **1**, 917.

MOTA, I. (1964). 'The mechanism of anaphylaxis. I. Production and biological properties of "mast cell sensitizing" antibody.' *Immunology*, **7**, 681.

MOTA, I. and PEIXOTO, J. M. (1966). 'A skin-sensitizing and thermolabile antibody in the mouse.' *Life Sciences*, **5**, 1723.

NUSSENZWEIG, R. S., MERRYMAN, C. and BENACERRAF, B. (1964). 'Electrophoretic separation and properties of mouse antihapten antibodies involved in passive cutaneous anaphylaxis and passive hemolysis.' *J. exp. Med.*, **120**, 315.

OVARY, Z. (1958). 'Passive cutaneous anaphylaxis in the mouse.' *J. Immunol.*, **81**, 355.

OVARY, Z., BENACERRAF, B. and BLOCH, K. J. (1963). 'Properties of guinea pig 7S antibodies. II. Identification of antibodies involved in passive cutaneous and systemic anaphylaxis.' *J. exp. Med.*, **117**, 951.

ZVAIFLER, N. J. and BECKER, E. L. (1965). 'Rabbit passive cutaneous anaphylaxis.' *Fed. Proc.*, **24**, 250.