Bovine Reaginic Antibody III. Cross-reaction of Antihuman IgE and Antibovine Reaginic Immunoglobulin Antisera with Sera from Several Species of Mammals

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ABSTRACT

Using antisera specific for the heavy chain of human IgE and bovine reaginic immunoglobulin, the degree of cross-reaction amongst sera from pig, rat, rabbit, guinea pig, goat, cow, horse, dog, cat and human was tested. Antihuman IgE antiserum gave strong reactions with pig, rabbit, cow, goat and human sera (100% to 15.1%) and weak reactions with rat, guinea pig, horse, dog and cat sera (10.1% to 3.22%). Antibovine reagin antiserum produced a considerable amount of cross-reaction with sera from pig, rat, rabbit, goat, horse and human (43.6% to 20.1%) with limited reactions with guinea pig, dog and cat sera (13.9% to 9.3%).

RÉSUMÉ

Cette expérience visait à déterminer, à l'aide d'antisérums spécifiques pour les longues chaînes de l'IgE humaine et de l'immunoglobuline réaginique bovine, le degré de réaction croisée entre le sérum des espèces suivantes: porc, rat, lapin, cobaye, chèvre, vache, cheval, chien, chat et homme. L'antisérum spécifique pour l'IgE humaine donna de fortes réactions croisées (100% à 15.1%) avec le sérum du porc, du lapin, de la vache, de la chèvre et de l'homme; il donna par ailleurs de faibles réactions croisées (10.1% à 3.22%) avec le sérum du rat, du cobaye, du cheval, du chien et du chat. L'antisérum spécifique pour la réagine bovine produisit une forte réaction croisée (43.6% à 20.1%) avec le sérum du porc, du lapin, de la chèvre, du cheval et de l'homme; il produisit cependant une réaction croisée plus faible (13.9% à 9.3%) avec le sérum du porc, du chien et du chat.

INTRODUCTION

Reaginic antibody has been described in many species of mammals including man (9), monkey (10), cow (7), sheep (8), dog (17), pig (1), rabbit (11), guinea pig (4), rat (19) and mouse (3). In general, the biological properties of the reaginic antibody are similar in these mammals. However, the similarity of the immunochemical properties of these proteins is not fully understood (2). Although these proteins have similar sedimentation coefficients, molecular weights and ionic charges as characterized by electrophoresis, it is not known to what extent they cross-react. This communication deals with an attempt to ascertain the degree of cross-reaction amongst various mammalian reaginic immunoglobulins by the use of two heavy chain specific antireagin antisera. Thus sera from man, horse, cow, goat, rabbit, pig, dog, cat, guinea pig and rat were tested for their ability to bind to an antihuman IgE antiserum and to an antibovine reagin antiserum. The degree of cross-reaction was measured by radial immunodiffusion and by the ability of the reaginic immunoglobulin to bind to rat mast cells.

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MATERIALS AND METHODS

Sera: Blood was collected by venopuncture or cardiac puncture from at least ten animals of each species. The blood samples were incubated at 20°C for two hours and at 4°C for two hours after which the serum was removed from the clot, centrifuged at 1000 xg for 10 min and stored at −70°C until used.

Antisera: Sheep antihuman IgE antiserum was purchased from McMaster University, Hamilton, Ontario. Rabbit antibovine reaginic immunoglobulin antiserum was prepared by the method of Nielsen and Wilkie (16).

Radiolabelling: Globulin preparations were made of the antisera by precipitation of the sera with saturated ammonium sulfate (50% final concentration). The precipitates were recovered by centrifugation 4000 xg for 10 min at 4°C and resolubilized with phosphate buffered saline, 0.01M $PO_4^=$, 0.15M NaCl, pH 7.2 (PBS). The precipitation procedures were repeated twice with 33% final concentration of ammonium sulfate. The final precipitates were resolubilized and dialized against PBS (200 volumes of original sera volumes). The globulin preparations were then adjusted to a final concentration of 33 mg/ml using an ultraviolet spectrophotometer set at 280 nm and an extinction coefficient of $E_{280}^{1cm} = 13.7$. Aliquots of 100 mg of globulin preparations were labelled with 1.0 mCi carrier-free ^{125}I (Atomic Energy Commission, Ottawa, Ontario) using the chloramine-T method of McConahey and Dixon (14).

Binding to rat mast cells: Rat peritoneal mast cells were obtained as described by Nielsen and Wilkie (16). Approximately 2 x 10^6 peritoneal cells were incubated in plastic tubes with 10.0 μl of each serum sample for 30 min at 37°C. The contents of all tubes were washed three times in Hank's balanced salt solution (HBSS). Cells incubated with HBSS rather than serum were included to be used as controls. Upon washing 2.0 mg of radiolabelled antireagin globulin (either antihuman or antibovine) were added to each tube, mixed thoroughly with the cells and incubated at 37°C for 30 min. All tubes were again washed three times in HBSS and the final pellets were enumerated for retained radioactivity in Nuclear Chicago gamma counter.

Detection of reaginic immunoglobulin by gel diffusion: A modification of the technique of Rowe (18) was used. Radiolabelled antireagin globulin was used in a radial diffusion test in 2% agarose incorporated in 0.1M phosphate buffer with 0.15M NaCl pH 7.2 which was dispensed onto microscope slides. Holes capable of holding 10 μl serum were cut 2.0 cm apart on the agar coating and filled with serum (10.0 μl). After incubation at 20°C for 48 hours the slides were washed extensively in PBS at 4°C and agarose plugs 1.0 cm in diameter were cut from the area surrounding each serum well. Radioactivity retained as antigen-antibody complexes was enumerated in a Nuclear Chicago gamma counter using plugs cut from areas where no antigen-antibody interactions could have taken place as controls.

RESULTS

Each experiment was performed three times to ascertain the reproducibility of the results. Cross-reaction of various mammalian sera with radiolabelled sheep antihuman IgE globulin produced results depicted in Table I. The counts per minute recorded are the average of three experiments with at least ten sera from each animal species per experiment. The gross count per minute of the antiserum was 14.63 x 10^5 cpm per mg of protein.

From these experiments it is apparent that the sera from pig, rabbit and cow cross-react extensively with antihuman IgE antibody by either measurement (37.5% to 22.4%). Goat and rat sera cross-react to a lesser extent (15.2% to 8.1%) while guinea pig, horse, dog and cat sera probably do not cross-react with antihuman IgE (7.7% to 3.2% cross-reaction). In similar experiments, presented in Table II, using radiolabelled rabbit antibovine reaginic immunoglobulin with a gross count of 6.31 x 10^5 cpm per mg protein it was noted that sera from pig, rabbit, goat, horse and human cross-reacted extensively (43.6% to 28.1%). Some cross-reactions

TABLE I. Cross-reaction Between Various Mammalian Sera and Sheep Antihuman IgE Globulin Measured by Gel Diffusion (GD) and Rat Mast Cell Binding (RMC)

Animal	No. Samples	Mean cpm ± SD		% Cross-reaction	
		GD	RMC	GD	RMC
Pig	10	7260 ± 1340	7000 ± 1300	37.5	34.9
Rat	20	1980 ± 1040	1680 ± 280	10.1	8.1
Rabbit	12	4600 ± 970	4630 ± 910	23.8	22.4
Guinea pig	10	615 ± 210	780 ± 170	3.2	3.7
Goat	10	2910 ± 610	3140 ± 260	15.1	15.2
Cow	20	5350 ± 1470	5260 ± 710	27.6	25.4
Horse	14	650 ± 390	760 ± 285	3.4	3.7
Dog	20	1480 ± 610	1360 ± 470	7.7	6.6
Human	16	19350 ± 3050	20700 ± 3300	100.0	100.0
Cat	10	1280 ± 590	1240 ± 410	6.6	6.0

The count per minute represents the mean of the samples quantitated ± one standard deviation. The % cross-reaction is the numerical relationship between the mean of the homologous reaction (100%) and the mean of the cpm obtained from the sera of that species

TABLE II. Cross-reaction Between Various Mammalian Sera and Rabbit Antibovine Reaginic Immunoglobulin Measured by Gel Diffusion (GD) and Rat Mast Cell Binding (RMC)

Animal	No. Samples	Mean cpm ± SD		% Cross-reaction	
		GD	RMC	GD	RMC
Pig	10	2470 ± 830	2320 ± 280	42.8	37.4
Rat	20	1160 ± 610	1320 ± 140	20.1	21.2
Rabbit	12	2410 ± 520	2260 ± 170	41.7	36.5
Guinea pig	10	535 ± 200	520 ± 180	9.3	8.0
Goat	10	2510 ± 805	2660 ± 340	43.6	42.9
Cow	20	5770 ± 1060	6200 ± 400	100.0	100.0
Horse	14	1810 ± 640	1680 ± 580	31.4	28.0
Dog	20	800 ± 610	920 ± 140	13.9	14.2
Human	16	1620 ± 790	1870 ± 360	28.1	30.2
Cat	10	750 ± 260	820 ± 170	13.0	13.2

The count per minute represents the mean of the samples quantitated ± one standard deviation. The % cross-reaction is the numerical relationship between the mean of the homologous reaction (100%) and the mean of the cpm obtained from the sera of that species

were noted with rat, dog and cat sera (21.2% to 13.0%), while guinea pig sera gave the least counts per minute in either assay (9.3% and 8.0%).

It is interesting to note that the antibovine reagin cross-reacted to approximately the same extent with human sera as did antihuman IgE with bovine sera.

DISCUSSION

It has been shown that absorption of sera of several mammalian species with antihuman IgE antiserum resulted in the reduction of reaginic activities of these sera. The sera tested include those from the pig (1), monkey (10), rat (12) and dog (6). In addition, conflicting results have been reported with absorption studies of guinea pig sera (4, 13) and rabbit sera (11, 21).

In this study, evidence is produced to support the reported cross-reaction between pig serum and antihuman IgE antiserum. It was also found that rabbit, goat and bovine sera could cross-react extensively with antihuman IgE. This partly supports earlier work from our laboratory which indicated that antihuman IgE could bind to bovine reaginic immunoglobulin *in vitro* (15) but not *in vivo* (16). Further, it would appear that rat serum cross-reacts to some extent with antihuman IgE, while horse, dog, cat and guinea pig sera do not. In the case of the guinea pig, perhaps it is due to some of the reaginic activity being attributable to at least one of the subclasses of IgG as reported by Bloch (2).

Using an antibovine reaginic immunoglobulin, it was noted that sera from pig, rabbit, goat, horse and man cross-reacted extensively while rat, guinea pig, dog and cat sera did so to a lesser extent.

For comparison, Vaerman (20) showed that antihuman IgA cross-reacted with cat, cow, goat, sheep, pig, horse and hedgehog serum IgA, while guinea pig, hamster, rat, mouse and chicken IgA did not.

The data presented in this communication does not allow a true comparison of cross-reactivity amongst reagins of the various species as the serum reagin levels are not known. It does, however, indicate that antiserum to bovine reaginic immunoglobulin has antigenic determinants in common with more mammalian sera tested than does human IgE. This observation is in agreement with those of Esteves and Binaghi (5) who reported extensive determinant recognition amongst pig, cow, sheep and goat IgG which all shared antigenic determinant(s) with human IgG. It would therefore appear that reaginic immunoglobulin like the immunoglobulins of other classes share common antigenic determinants among reasonably closely related species. Animals further removed phylogenetically, such as the rodents, cross-react to a much lesser degree.

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