PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 147, 883-886 (1974)

Correlation of Canine Secretory Alloantigens A and Y with Two Fucolipids Isolated from Dog Intestines (38461)

ALAIN ZWEIBAUM, ELISABETH DUSSAULX, EDWIN L. SMITH, AND JOHN M. MCKIBBIN

Institut d'Immuno-biologie de l'Inserm, Hôpital Broussais, 96 rue Didot, 75674 Paris Cedex 14, France, and Department of Biochemistry, University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294

Previous studies have shown the presence in dogs of a polymorphic immunogenetic system which was defined as the canine secretory alloantigen alloantibody system (CSA) (1). This system involves the presence of alloantigens in most secretions and of the corresponding naturally occurring alloantibodies (2, 3). As in the human ABO system there is a mutually exclusive relationship between the presence of the antigen(s) and the corresponding antibodies. Three antigens have been detected so far: CSA A, which is cross reactive with human blood group A antigen (4), CSA X, and CSA Y (3). Concurrently, biochemical studies performed on dog individual small intestines have led to the isolation of fucose containing glycolipids with A, H-like, and Le^b-like activity (5–7). The purpose of the present study was to investigate the correlation between CSA alloantigens and the glycolipid fractions isolated from dog small intestines.

Materials and Methods. The isolation of the fucolipid fractions was performed on individual small intestine mucosa from six dogs selected for their different CSA types: dog 8(A), 15(X), 18(Y), 2(AX), 10(AY), and 3(XY). CSA typing was done with the immunofluorescence (IF) indirect technique on small intestine frozen sections using natural anti-CSA monospecific alloantisera as previously reported (1, 3). For shipment convenience the mucosa was transformed into dry acetone powder.

The glycolipid fractions were prepared by a method described previously (5, 8, 9). The method involves extraction of fresh tissue with alcohol/ether 3:1 (modified to alcohol:ether:water 3:1:0.24 for use with acetone powder) and purification of the extracted lipids in chloroform solution with aqueous $MgCl_2$. The lipids were then fractionated by silicic acid column chromatography, solvent partition, Florisil column chromatography, dialysis, and finally thin layer chromatography (TLC) using system B (5). This system gives three groups of glycolipids (5), the fucolipids being the slowest migrating compounds. The fucolipids were eluted from the gel, taken to volume and the total sugar content determined by anthrone. An aliquot of the preparation was dried and taken up in saline for immunologic study in a concentration of about 1 mg/ml (using an approximation: 4 μmoles anthrone "galactose" equal 1 mg fucolipid). The fraction from dog 10 (type AY) was additionally made up in a concentration of 2 mg/ml. Another portion of the preparation was examined in TLC with three solvent systems: A, B, and D (5).

The fucolipid preparations were tested on Ouchterlony plates against rabbit antisera specific for the following dog intestinal fucolipids (7): 23F-1 (fucolipid with one molecule of fucose/molecule), 12F-1 (fucolipid with human blood group A activity), 13F-2 and 23F-2 (fucolipids with two molecules of fucose/molecule and with blood group Le^b activity). Additionally the same preparations were tested against monospecific anti-CSA antisera including rabbit and dog anti A, dog and rat anti X, dog and rat anti Y (1). Of all these antisera only rabbit anti A had a high IF titer (1/512). All the other antisera had an IF titer (1/64) (1).

Rabbit antisera specific for the above-mentioned fucolipids were tested with the IF indirect technique on colonic mucosa sections from 12 dogs of different CSA types. IF titers were checked for each antiserum on monotyped CSA sections as described previously (1).

Results. TLC of the fucolipid preparations in solvent system B is shown in Fig. 1. This system is of particular interest in that it characteristically

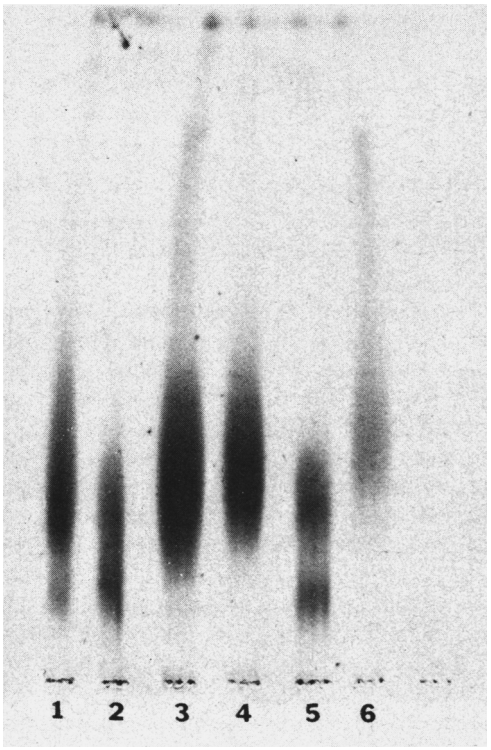

FIG. 1. Thin layer chromatography of the fucolipid preparations from intestine mucosa of typed dogs in System B (Chloroform:methanol:concentrated NH_4OH 40:80:25. Silica Gel G). (1) 10-AY, (2) 18-Y, (3) 2-AX, (4) 15-X, (5) 3-XY, (6) 8-A.

separates the Le^b-like fucolipids from other fucolipids. The analysis by TLC shows that the Le^b-like compound is present in three dogs only: 18(Y), 10(AY), and 3(XY). All these dogs have the CSA Y antigen. Mobilities in system B of fucolipids from three of the intestines containing the Y antigen and those of known Le^b-like fucolipids from dog intestine are shown in Fig. 2.

Results obtained with the different antifucolipid antisera when tested on Ouchterlony plates against each fucolipid preparation from the six dogs under study are reported in Table I. Any precipitin band recorded is designated "+". The results were particularly clear with anti 12F-1 and anti 23F-2. Anti 12F-1 (anti A-like) reacted with all preparations from dogs with CSA A and did not react with any of those from dogs without the CSA A antigen. Likewise anti 23F-2 reacted wiith all preparations from dogs with the CSA Y antigen and did not react with any of those from dogs without the CSA Y antigen. Anti 13F-2 (anti Le^b-like) reacted with fucolipids from only one dog, 3(XY), and did not react with any preparation from dogs not typed Y. Anti 23F-1 reacted only with one preparation which was typed XY. Of all the anti CSA antisera tested against the same preparations, only rabbit anti A reacted with the fucolipids from dog 10(AY). No precipitin band could be recorded against any of the preparations with all the other anti CSA antisera.

Immunofluorescent reactions observed on colonic mucosa goblet cells from 12 CSA typed dogs with anti fucolipid antisera 23F-1, 12F-1, 13F-2, and 23F-2 are reported in Table II. Anti 23F-1 was regularly negative on all sections, whatever their CSA type. Anti 12F-1 gave strong positive reactions on sections from all dogs with the CSA A antigen, and was negative on sections from all the dogs which were lacking the A antigen. Both anti 13F-2 and 23F-2 were strongly positive on all sections from dogs with the CSA Y antigen, but negative on all sections from dogs not typed Y. The IF titer of the three CSA positive antisera was checked on the corresponding CSA typed sections: Anti 12F-1 had an anti A IF titer of 1/512 when tested on colonic sections from dog B59(A); when tested on dog B54(Y) anti 13F-2 had an anti Y IF titer of 1/256, and anti 23F-2 an anti Y IF titer of 1/1000.

Discussion. The results clearly indicate that the CSA A and Y antigens have respectively the

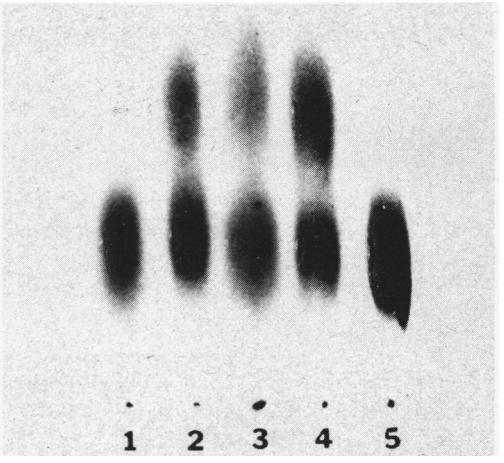

FIG. 2. Thin layer chromatography of the fucolipid preparations from the intestinal mucosa of dogs typed Y and the Le^b-like fucolipids. System B (chloroform:methanol:concentrated NH_4OH 40:80:25. Silica Gel G). (1) 23F-2 (Le^b-like), (2) 10-AY, (3) 18-Y, (4) 3XY, (5) 16F-2 (Le^b-like).

TABLE I. Precipitation Reactions (Ouchterlony) of Fucolipids Extracted from Intestinal Mucosa of CSA Typed Dogs with Various Anti Fucolipids Antisera.

Dog number	CSA type	Anti 23F-1	Anti 12F-1	Anti 13F-2	Anti 23F-2
8	A	–	+	–	–
15	X	–	–	–	–
18	Y	–	–	–	+
2	AX	–	+	–	–
10	AY	–	+	–	+
3	XY	+	–	+	+

same immunological activity shown by the A-like and Le^b-like dog fucolipids. This is supported by the specific precipitation reactions observed with anti 12F-1 (anti A-like antiserum) against fucolipids isolated from small intestines of dogs with the CSA A antigen, and with anti 23F-2 (anti Le^b-like) against fucolipids of dogs with the CSA Y antigen. This correlation between CSA A and Y, and fucolipids with A and Le^b-like activity, is furthermore supported by the IF reactions obtained with the various anti fucolipid antisera on colonic sections from CSA typed dogs. The negative results observed on Ouchterlony plates with the anti CSA antisera, with one exception for rabbit anti A, are probably related to the low anti CSA titers of these antisera. The discrepancy observed with anti 13F-2, which is only positive on one of the fucolipids preparations of the three dogs typed Y, may be related to the relatively low titer of this antiserum (IF titer: 1/256) as compared to the high titer of anti 23F-2 (IF titer on Y sections: 1/1000). Finally, in addition to immunologic correlation of the Y antigen with the Le^b-like fucolipids, there is also correlation in TLC between fucolipids from the dogs with the Y antigen and known Le^b-like intestine fucolipids.

The results obtained in this study are still inconclusive in regard to the CSA X antigen: no precipitation reaction could be observed with any of the antifucolipid antisera in the fucolipid fraction isolated from dog 15 (X), and no IF positive reaction could be observed with any of the same antisera in colonic sections from CSA X dogs. It is possible that other specificities, different from the three CSA specificities, are present in dogs. This is supported by the fact that anti 23F-1 reacts with the fucolipids from dog 3(XY). In addition it appears from an examination of the fucolipid preparations in other solvent systems that more fucolipids than the maximum two antigens of the CSA system exist in some dogs.

The correlation of CSA A and Y alloantigens with dog fucolipids with A and Le^b-like activity is of particular interest since A and Y antigens,

TABLE II. Immunofluorescent Reactions of the Colonic Goblet Cells of CSA Typed Dogs Tested with Various Anti Fucolipids Antisera.

Dog number	CSA type	Anti 23F-1	Anti 12F-1	Anti 13F-2	Anti 23F-2
B59	A	–	+	–	–
C20	A	–	+	–	–
C11	AX	–	+	–	–
C10	AX	–	+	–	–
C8	AY	–	+	+	+
C9	AY	–	+	+	+
C22	AY	–	+	+	+
C7	X	–	–	–	–
C21	X	–	–	–	–
B54	Y	–	–	+	+
C27	Y	–	–	+	+
C28	Y	–	–	+	+

and consequently A and Le^b-like fucolipids, have been extensively studied in regard to their tissue distribution (1), genetics (1) and correlation with the other immunogenetic systems of the dog (10). Furthermore, the presence in dog of antigens whose activity is correlated to the activity of similar human antigens suggests that the dog is an experimental model of great value for the study of the biological function of such substances.

Summary. Fucolipid preparations isolated from individual small intestine mucosa from six CSA typed dogs were tested against various anti fucolipid antisera. The fucolipids from dogs with the CSA A antigen reacted exclusively with the anti A-like fucolipid antiserum. The fucolipids from dogs with the CSA Y antigen reacted exclusively with the anti Le^b-like antisera. Likewise anti A-like fucolipid antiserum gave specific immunofluorescent reactions on colonic mucosa goblet cells of dogs with the CSA A antigen only, while the anti Le^b-like fucolipid antisera gave positive reactions only on colonic mucosa goblet cells of dogs with the CSA Y antigen.

The authors are indebted to Dr. Ray Hiramoto for the preparation of the fucolipid antisera and to Mr. David Law for valuable technical assistance. Supported by University of Alabama Medical Center Faculty Research Grants 82-6111 and 82-6117 and American Cancer Grant IN 66K.

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Received June 28, 1974. P.S.E.B.M., 1974, Vol. 147.