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Human blood group A and H glycolipids in porcine plasma

Evidence for acquisition of the erythrocyte antigens from plasma

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Human blood group A- and H-antigenic glycosphingolipids were isolated from pooled porcine plasma. The structures of the A-active hexa- and H-active pentaglycosylceramides of lactoseries (type 1 sugar chain) were the same as those in porcine erythrocytes. These results endorse biochemically the previous observations that the A and H antigens on porcine erythrocytes are taken up from plasma.

AH blood groups; Glycolipid; Lactoseries; (Pig plasma)

1. INTRODUCTION

One of the important roles of plasma glycolipids is to act as blood group antigens through transferring antigenic glycolipids from plasma to erythrocytes, as was demonstrated in the Lewis system [1,2]. Similarly to Lewis antigens in man, some of porcine blood group antigens including human type-A and -H on erythrocytes are acquired from plasma [3], although the antigens have not been chemically characterized. In a previous study, we identified human blood group A- and H-antigenic glycolipids belonging to lactoseries in porcine erythrocyte membranes [4]. Therefore, if the antigenic glycolipids present in porcine erythrocytes are found in the plasma as well, this will endorse acquisition of the erythrocyte antigens from plasma. The present study deals with isolation and characterization of the antigenic glycolipids in porcine plasma.

2. EXPERIMENTAL

The ratio of a solvent mixture is expressed by volume. Fresh, pooled, citrated, porcine blood was centrifuged. The supernatant plasma was saturated with ethanol to a final concentration of 70% followed by filtration. The residue was extracted with 70% ethanol at 70°C for a few minutes. First and second filtrates were combined, and evaporated to dryness. The dried materials were subjected to removal of phospholipids [5], and extracted 3 times with $CHCl_3$/CH_3OH (2:1). From the combined extracts, neutral glycolipids were separated by repeated silicic acid chromatographies as described previously [4,6]. All analytical methods including proton nuclear magnetic resonance (PMR) spectrometry, glycosidase treatment, hemagglutination inhibition test and solid phase immunostaining were the same as those described previously [4].

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3. RESULTS

Major glycolipids isolated from porcine plasma were mono- to tetraglycosylceramides with predominant Gb_4Cer as was demonstrated previously [7,8]. In addition, two minor glycolipids having longer carbohydrate chains, which comigrated with blood group A and H glycolipids from porcine erythrocytes [4], were purified at about 2.9 mg and 1.6 mg per 10 liters, respectively. When two glycolipids were examined for human blood group antigenicity by hemagglutination inhibition, the respective lipids exhibited the A activity at 0.15 ng/μl and H activity at 38 ng/μl. Results of the immunostaining are shown in fig.1. No type B glycolipid was detected in porcine plasma. Upon treatment of the plasma A glycolipid with α-*N*-acetylgalactosaminidase, α-L-fucosidase, β-galactosidase and then β-*N*-acetylhexosaminidase followed by thin-layer chromatography developed with $CHCl_3$/CH_3OH/H_2O (60:35:8; solvent 1), the lipid was converted to glycolipids migrating as penta-, tetra-, tri- and diglycosylceramides, respectively (data not shown). The A and H glycolipids were analyzed by PMR spectrometry referring to previous spectra for signal assignment and molar composition [9–11]. The spectra of the A (fig.2a) and H (fig.2b) glycolipids were identical with respective ones from the porcine erythrocytes [4]. Protons at H-1 (4.616 ppm) and H-3 (3.849 ppm) of GlcNAc, which are characteristic of lacto-core skeleton, were observed in both glycolipids.

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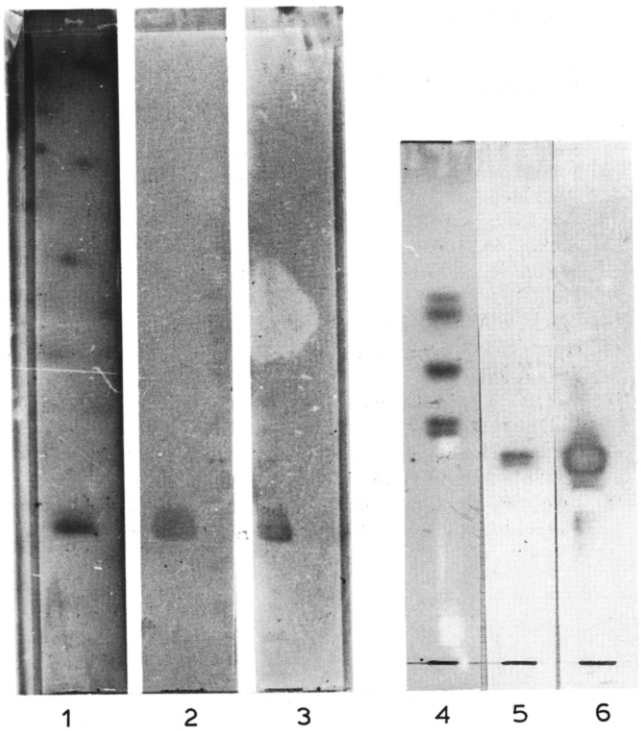

Fig.1. Solid phase immunostaining of A- and H-antigenic glycolipids from porcine plasma. The A glycolipid (lanes 1–3), the H glycolipid (lanes 5 and 6) and standards (LacCer, Gb_3Cer and Gb_4Cer at lane 4) were chromatographed on thin-layer plates developed with solvent 1. Lanes 1, 4 and 5 were visualized with an orcinol-H_2SO_4 reagent. Lanes 2 and 3 were treated with anti-A antiserum and *Dolichos biflorus* lectin (followed by anti-*D. biflorus* IgG), respectively. Lane 6 was treated with *Ulex europaeus* II lectin and with anti-*U. europaeus* II IgG. Then, the lipids on lanes 2, 3 and 6 were visualized with 4-chloro-1-naphthol solution containing H_2O_2 after treatment with protein A-conjugated peroxidase.

The above results are consistent with the chemical structures of GalNAcα1-3(Fucα1-2)Galβ-3GlcNAcβ1-3Galβ1-4Glcβ1-1Cer for the A glycolipid, and with that lacking GalNAc from the A glycolipid for the H glycolipid.

4. DISCUSSION

Porcine blood group antigenicity is grouped into more than 10 systems, each of which is classified into plural subtypes based on serological reactivity [3]. They include the antigenicities similar to human type-A and -H. In pigs with human type-A or -H blood group, the antigenicity is detected in digestive secretions [12] and serum [13] but not on red blood cells at birth. The A and H antigenicity on the red cells can be detected during the first month of life. Acquisition of the A antigenicity onto porcine red cells from plasma was demonstrated [3], and the A antigenicity was detected in a lipid fraction from porcine serum [14]. Therefore, our present and previous [4] results strongly support that the porcine A and H glycolipids on erythrocyte are transferred from plasma, endorsing biochemically the previous serological observations. Although lactoseries glycolipids including ABH antigens have been

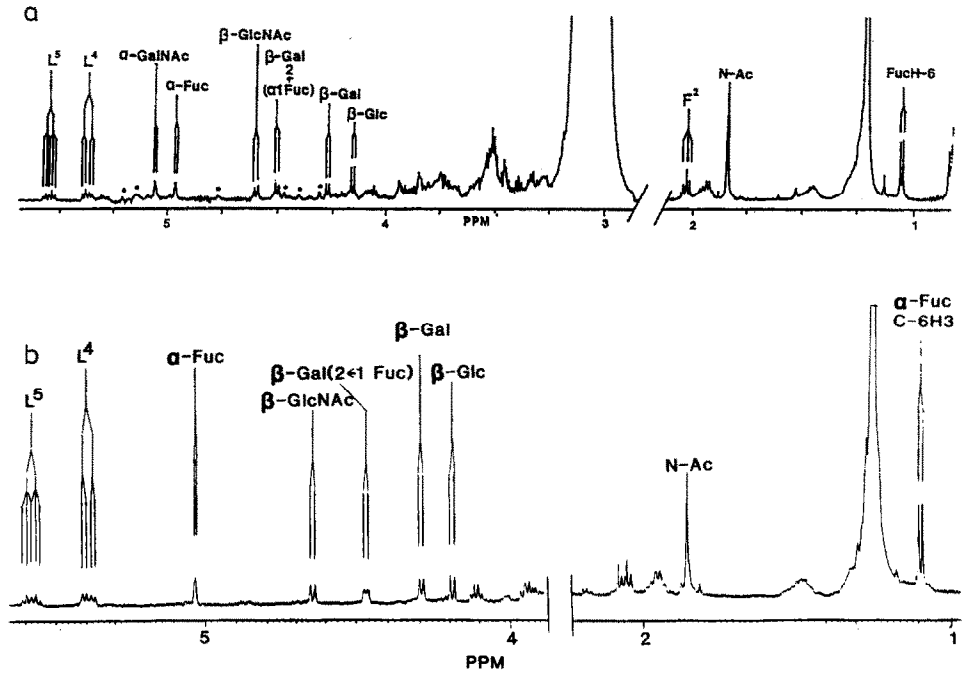

Fig.2. PMR spectra of the porcine antigenic glycolipids. The spectra of the A (a) and H (b) glycolipids were taken in hexadeuterodimethylsulfoxide containing 2% D_2O at 90°C in a Varian JNM-GX500 spectrometer (frequency, 500 MHz; sweep width, 5 kHz). L and F, protons at long chain base and fatty acids, respectively. Peaks marked by asterisks in (a) are due to hydroxy protons [4].

characterized in such glandular tissues as gastrointestines but few in other tissues (reviewed in [15,16]), minor A glycolipids with lacto-core were recently demonstrated in human Le^{a-b-} erythrocytes [17] and plasma [18]. Thus, it is likely that ABH and Lewis blood group glycolipids belonging to lactoseries in man and pig originate from glandular tissues.

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