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Sialylated Le^a Blood Group Substances Detected by the Monoclonal Antibody Ca 19-9 in Human Seminal Plasma and Other Organs

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Summary. The glycoprotein fractions of human seminal plasma, amniotic fluid, urine, human saliva and human gastric juice have been found to contain sialyl-Le^a blood group substance, an antigen and which is known to function as a tumor-marker in human pancreatic and gastrointestinal cancer (Ca 19-9). Tumor-associated carbohydrate structures may well occur in large amounts both in normal tissue and in secretions as organ-specific markers. In human seminal plasma typical variations have been found in relationship to the Lewis blood group of the donor. Accordingly, the Ca 19-9 antigen can be regarded as a marker of the main glycoprotein fraction of human seminal plasma, which could be useful as a tool for clinical investigations.

Key words: Seminal plasma, Glycoproteins, Lewis antigen, Organ-specificity, Tumor-marker, Ca 19-9.

Introduction

Human blood group substances have been reported to have close relationship to tumor-associated oncofetal antigens. Deletions in the ABH group system, enhanced reactivity of its precursor H, Ii and type 1 or 2 chains, increased Le^a or Le^b receptors, PP_1 structures in p negative persons, the Forssman-antigen in Forssman negative individuals and A-like antigens in persons of group 0 or B have been described [3]. Recently a monoclonal antibody, Ca 19-9, has been demonstrated to react with sera from pancreatic cancer and gastrointestinal tumor patients [1]. This antibody detects a sialylated Le^a blood group structure which is supposed to be synthesized by a tumor cell. The aim of this report is to show, that this Ca 19-9 antigen also occurs in large amounts in normal human body effusions and secretions without entering the serum.

A typical example is human seminal fluid, from which we have isolated and characterized various glycoproteins [4, 12, 11]. One of these glycoproteins, the main component obtained by phenol/saline extraction of pooled seminal plasma, is rich in L-fucose and in N-acetyl-neuraminic acid. As the monoclonal antibody, Ca 19-9, reacts with structures containing these carbohydrates [7] and because seminal plasma contains Lewis blood group antigens in secreted form, we re-investigated this glycoprotein fraction. The result, which has already been reported [13], showed high Ca 19-9 reactivity, and also in the purified glycoprotein, which must be considered as an organ-specific antigen occurring in a non-tumorous tissue. The aim of this investigation is to demonstrate significant differences in the quantity of this antigen in Le^a, Le^b and Lewis negative persons. Our results have revealed, that the amount of this antigen shows great variation and that this is a Lewis blood group dependent phenomenon, which influences the composition of the human seminal fluid significantly.

Material and Methods

Human Seminal Plasma. Specimens of human seminal plasma were obtained by masturbation from 150 healthy donors and after liquefaction centrifuged at 2,500 rpm for 15 min. After separation of the sperm-cells specimens were stored at −18 °C until being analyzed for Ca 19-9 TM.

Blood. 5 ml of blood were obtained by venipuncture from the same persons as above. Coagulation was prevented by adding sodiumcitrate. All specimens were processed immediately.

Ca 19-9 Radioimmunoassay. All samples of human seminal plasma were analyzed for Ca 19-9 in accordance with the method of DelVillano and collaborators [1]. Test kits were procured from CENTOCOR, Malvern, PA, USA (German agency: Isotopen Diagnostik CIS GmbH, 6072 Dreieich, P.O.B. 102025).

Lewis Blood Typing. Lewis Blood Typing was conducted as described by Behring-Werke, Marburg, FRG. Le^a- and Le^b-antiserum were obtained from the same firm. Erythrocytes of 0.2 ml blood were washed once with saline, containing 0.9% NaCl. After washing, the suspension was centrifuged at 1,000 rpm for 5 min. The supernatant was discarded and 5 ml of saline, containing 0.9% NaCl were

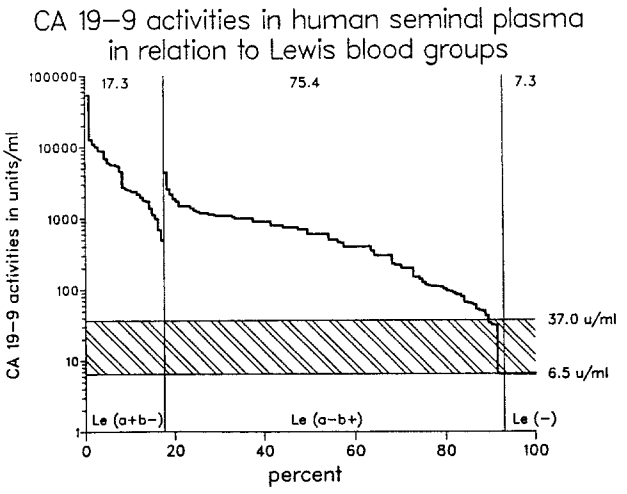

Fig. 1

added. The concentration of the suspension amounted to 2–5% erythrocytes.

For each blood group one tube was prepared with one drop Le^a- and with one drop of Le^b-antiserum respectively. One drop of the suspension was added to each tube and incubated for 30 min at room temperature. Specimens were centrifuged again at 1,000 rpm for 1 min after incubation. Agglutination was read after mixing up the sediments.

Results

It was demonstrated, that the distribution of Lewis blood groups in this study was in accordance with data published in the literature [8, 10]. From 150 healthy volunteers 26 (17.3%) showed the blood group Le(a+b–), 113 (75.4%) Le(a–b+) and 11 (7.3%) Le(a–b–).

137 (91.3%) specimens of the 150 samples of human seminal plasma showed high activity of Ca 19-9 TM by radioimmunoassay. In 13 (8.7%) cases, the activity was lower than 6.5 units/ml. With reference to Lewis blood groups, the activity of Ca 19-9 in the samples of human seminal plasma varied as follows: Patients of the category "Le(a+b–)" presented Ca 19-9 activity in their seminal plasma, ranging from 500 to 55,000 units/ml. The physiological concentration in human serum varies between 6.5 and 37 units/ml. The activities of Ca 19-9 in the group featuring Le(a–b+) ranged from 32 to 4,500 units/ml. Activity lower than 6.5 units/ml could be shown in all samples of the category "Le(a–b–)" and in two specimens of the category "Le(a–b+)". The results are summarized graphically in Fig. 1.

More than 50% of the patients with Le(a+b–) blood group presented high values of Ca 19-9, filling the interval between 2,000 and 10,000 units/ml. Values higher than 10,000 units/ml or lower than 1,000 units/ml seemed to be rare. In the category "Le(a–b+)" more than 50% of data ranged between 400 and 1,500 units/ml. Extreme values seemed to be higher than 2,000 units/ml or lower than 120 units/ml. In this group two individuals had less than 6.5 units/ml of the marker in their seminal plasma.

According to our results, there is sufficient evidence, that the activity of Ca 19-9 in human seminal plasma is dependent on the Lewis blood group of the donor. In human saliva the occurrence of Ca 19-9 in lower quantity is also dependent on Lewis blood groups (Prof. V. Ginsburg, personal communication).

It may, however, be possible, that in different tissues of the same individual the relationship of Lewis (precursor) substance to Ca 19-9 antigen may vary according to the relationship of the two competing enzymes, fucosyl-transferase and sialyl-transferase.

Discussion

Human seminal fluid represents a typical organ-specific body secretion. This secretion is also composed of human secreted blood group substances [12], the amount of which are influenced by the blood group genes of the ABH/Lewis system. In the normal seminal fluid glycoproteins are found which are influenced by the Lewis genes. Individuals who are Le^a positive, secrete the precursor for Ca 19-9 and have the highest amount of Ca 19-9 substance, due to a conversion of the Le^a substance by a sialyl-transferase, whereas Le^b individuals have a great variation in Ca 19-9 content, because of a competition between the sialyl-transferase and a H gene dependent fucosyl-transferase (see also Fig. 2). Lewis negative individuals do not have any Ca 19-9 antigen because of lack of the precursor. Before, however, discussing these relationships in more detail, the history of the research of this new antigen will be described.

In 1966 we published a communication on amniomucoids, a new class of hexosamine-rich glycoproteins [6]. This glycoprotein fraction was obtained from human amniotic fluid by 90% phenol (v/v) extraction. It was 50% reducing sugar, 10% sialic acid and 20% hexosamines [6].

Remarkable was the lack of blood group activity in the preparations from human origin, although in its sugar analysis it resembled the secreted ABH and Lewis blood group substances from human ovarian cysts [9]. Interesting, however, was the high sialic acid and a high fucose content. Such a phenomenon has also been described in a Le^a active ovarian cyst glycoprotein [9].

However, when the monoclonal antibody Ca 19-9 was introduced, reacting with sialylated Le^a substance as a tumor marker [1, 7], we began to re-investigate all those substances from human body secretions, in which we had found a high fucose content (L-fucose is necessary for the determinant structure of the H and of the Lewis antigens) and a high sialic acid value. First, we described a high Ca 19-9 reactivity in human seminal plasma, from which we purified and characterized the receptor and confirmed that its carbohydrate chain was a sialylated Le^a blood group determinant [13]. Secondly, we detected the Ca 19-9 antigen in a soluble fraction of post-colostral human milk (unpublished results), and finally, we re-investigated our "amniomucoids". These were freshly prepared, because in a sample of the

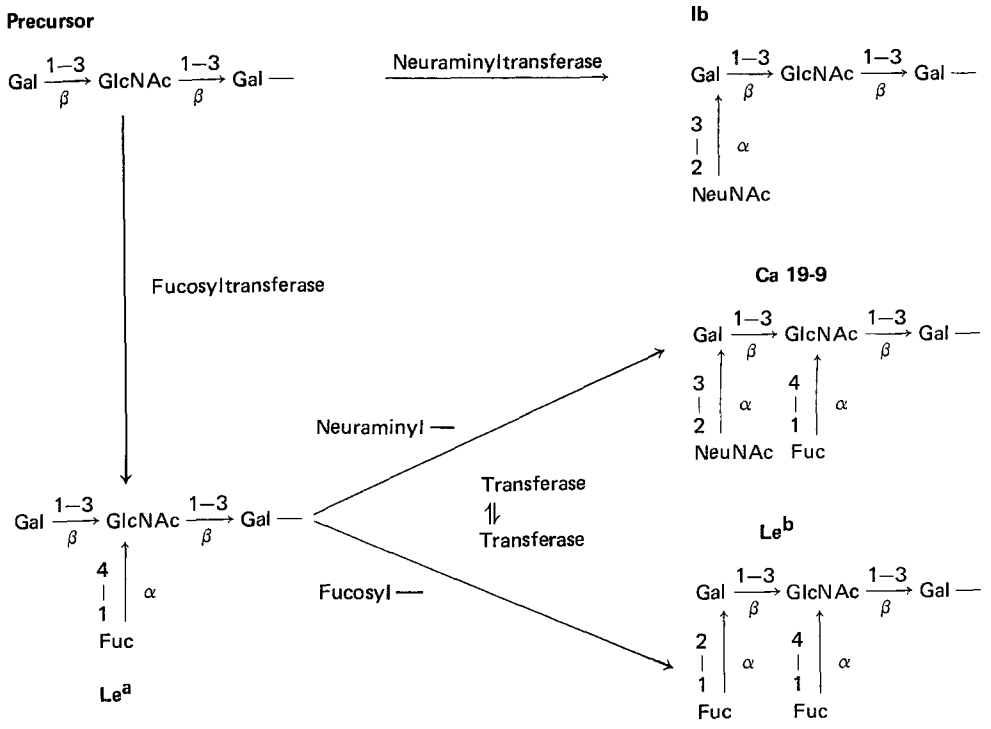

Fig. 2

Table 1. Concomitant and parallel occurrence of blood group Lewis substance and Ca 19-9 antigen (According to [8, 10] and own investigations)

Origin	Le-substance	Ca 19-9 antigen
human saliva	+	+
human gastric juice	+	+
human meconium	+	+
pancreatic secretion	?	+
hum. amniotic fluid	+	+
human urine (phenol/saline extract of dialyzed and concentrated samples)	+	+
post-colostral milk	+	+
human seminal plasma	+	+
ovarian cyst fluid	+	+
old world monkeys (saliva)	+	?
gum arabic	+ (?)	–
Psammechinus miliaris (sea urchin)	?	+
Lobster americanus (stomach)	?	+

above mentioned cyst [9], kindly supplied by Prof. Winifred M. Watkins, the Ca 19-9 activity was relatively low. Since then, we have investigated several ovarian cysts and found some with a very high Ca 19-9 antigen content, although there was no sign of malignancy (unpublished results).

In addition the glycoprotein fraction from the water-layer of the phenol extraction of pooled and individual human amniotic fluids showed an extremely high Ca 19-9 activity as measured with usual radioimmunoassay [1, 7] (mean value: 55,000 units per mg). The activity was lost by neuraminidase-treatment, a procedure, which detected de novo Le^a blood group structures and served as a control. On the other hand the material from bovine amniotic fluid was absolutely negative, although similar in composition.

Our results have now demonstrated, that the tumor marker Ca 19-9, identical with sialylated Le^a structures, is also normally secreted in large amounts as a main glycoprotein constituent of certain human body secretions, such as human seminal plasma, human urine, human saliva, human post-colostral milk and also human amniotic fluid. These body fluids are those, which have Lewis blood group substance as formerly reported [8, 10]. In Table 1 all our results are listed; noteworthy is the occurrence of Ca 19-9 in male and female urine (unpublished results). In these organs, our investigations have confirmed the hypothesis, that sialyl-Le^a substance (identical with the Ca 19-9 antigen) cannot be found in individuals without Lewis blood groups [5]. Accordingly it can be concluded, that a considerable amount of secreted Lewis blood group glycoprotein is normally converted by a fucosyl-transferase-competeting neuraminyl-transferase into sialylated Le^a substance.

This hypothesis, which has also been developed by Prof. Victor Ginsburg for human saliva (personal communication), explains the great quantitative variation of Ca 19-9 in human seminal plasma and is illustrated in Fig. 2. Remarkable is the broad spectrum in Le^b persons. The results suggest, that the individual differences in such glycoconjugates, which are known to belong to the nonspecific immunological defense mechanisms, as barriers for bacterial infections by neutralizing their adhesive lectins, may influence sus-

ceptibility to certain diseases. With regard to the antigenicity of these substances, a relationship to AIDS has already been discussed [5, 13].

Clinical implications, however, have not been made. Therefore it is the aim of this report, to stimulate investigations on Ca 19-9 in human seminal fluid, in order to find out, whether this organ-specific antigen may serve as a useful parameter and marker for certain diseases of the urogenital tract, and in immuno-histological studies (2), of the prostate gland.

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