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

G. Uhlenbruck\textsuperscript{1}, U. Höller\textsuperscript{1}, J. Heising\textsuperscript{2}, A. van Mil\textsuperscript{1} and C. Dienst\textsuperscript{1}

\textsuperscript{1} Department of Immunobiology, Medical University Clinic I (Director: Prof. Dr. V. Diehl), Cologne, FRG
\textsuperscript{2} Urological Clinic (Director: Prof. Dr. R. Engelking), Cologne, FRG

Accepted: January 11, 1985

Summary. The glycoprotein fractions of human seminal plasma, amniotic fluid, urine, human saliva and human gastric juice have been found to contain sialyl-Le\textsuperscript{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, I\textsubscript{i} and type 1 or 2 chains, increased Le\textsuperscript{a} or Le\textsuperscript{b} receptors, PP\textsubscript{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\textsuperscript{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\textsuperscript{a}, Le\textsuperscript{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 \(-18\,^\circ\text{C}\) 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 sodium-citrate. 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 DelVilllano 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\textsuperscript{a} and Le\textsuperscript{b} antisera 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...
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 37.0 units/ml. 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 normal seminal fluid glycoproteins are found which are influenced by the Lewis genes. Individuals who are Lea positive, secrete the precursor for Ca 19-9 and have the highest amount of Ca 19-9 substance, due to a conversion of the Lea substance by a sialyl-transferase, whereas Leb 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 Lea active ovarian cyst glycoprotein [9]. However, when the monoclonal antibody Ca 19-9 was introduced, reacting with sialylated Lea 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 Lea 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