Natural Antibody against Thomsen-Friedenreich Antigen in Sera of Patients with Carcinomas and Infectious Diseases

Masao KUSAMA1, Kaoru KUSAMA2, Satoru HAYASHI3, Tatsuto SUNOUCHI2, Ling CHU2 and Itaru MORO2

(Received 2 April and accepted 22 July 1993)

Key words: natural antibody, Thomsen-Friedenreich antigen, infectious disease, enzyme-linked immunosorbent assay

Abstract

The levels of natural antibody against Thomsen-Friedenreich (TF) antigen in sera of patients with various cancers and infectious diseases were examined by an enzyme-linked immunosorbent assay (ELISA) and compared with those of healthy donors. The levels of antibody against TF antigen in sera of patients with adenocarcinomas such as gastric, pancreatic and colorectal cancers were lower than those of patients with hepatoma, pyelonephritis and pneumonia. These findings may reflect the expression of TF-antigen in adenocarcinoma tissues.

Introduction

Thomsen-Friedenreich antigen [TF-antigen, β Gal (1-3) α GalNAc-] is a cryptic carbohydrate chain related to MN type blood group substances in humans[1-3]. The presence of TF-antigen has been detected in several kinds of Gram-negative bacteria such as Escherichia coli and in their lipopolysaccharides (LPS)[3]. Natural antibody against TF-antigen (anti-TF antibody) is detectable in the serum of humans and various animals, and even in other biological fluids such as human saliva and bovine milk[4]. Decreased levels of anti-TF antibody in sera of cancer patients compared with those in normal serum have been described[5,6]. In this study, we examined the levels of anti-TF antibody in sera of healthy donors and patients with various cancers and infectious diseases using an enzyme-linked immunosorbent assay (ELISA).

Materials and Methods

Sera from 95 healthy donors, 28 cancer patients (9 gastric carcinomas, 7 pancreatic carcinomas, 3 colorectal carcinomas and 9 hepatocellular carcinomas) and 4 patients with infectious diseases (2 pneumonia and 2 pyelonephritis) were collected by venipuncture and kept at −80°C until use.

The sera were subjected to ELISA as follows: Each well of a 96-well microplate (NUNC) was coated with 10 μg/ml asialoglycophorin (Sigma Chemical Co.). After washing with phosphate-buffered saline (PBS, pH 7.4), each well was incubated with PBS containing 1% human serum albumin to block non-specific binding. Diluted serum from a healthy donor as a standard solution or diluted samples were added to the wells and incubated for 1 h at room temperature. After washing with PBS containing 0.05% Tween 20 (PBS-Tween) 10 times, each well was incubated with peroxidase-labeled affinity-purified goat anti-human IBM (KPL, × 10,000) for 1 h. Washed wells were incubated with 0.1 M citrate-phosphate buffer (pH 5.0)
containing 1 mg/ml o-phenylenediamine and 0.3 μl/ml hydrogen peroxide at room temperature for 20 min. The reaction was stopped by addition of 25 μl of 2 M H₂SO₄. Optical density was measured at 492 nm by a microplate photometer (Corona Electric Co. Ltd.). Diluted human serum from a healthy donor was used as a standard solution, and the amount of anti-TF antibody in 10-fold-diluted normal human serum was estimated to be 100 units/ml. To confirm specific reaction between asialoglycophorin and anti-TF antibody, an inhibition assay using synsorb-T was performed.

Results and Discussion

Figure 1 shows the calibration curve of the ELISA for anti-TF antibody using normal human serum. Inhibition assay revealed that more than 90% of the value was decreased by adding synsorb-T to serum, suggesting the specific binding of anti-TF antibody to asialoglycophorin. Figure 2 shows the results of serum anti-TF antibody levels of healthy donors and patients with various cancers and infectious diseases. The levels of 95 normal sera ranged from 85 to 620 units/ml and the mean values were 273.7 ± 161.8 units/ml. The concentration of serum anti-TF antibody was decreased in sera of patients with gastric (155.2 ± 123.1 units/ml), pancreatic (113.6 ± 99.5 units/ml), colorectal (63.0 ± 14.7 units/ml) and hepatocellular (239.4 ± 160.1 units/ml) carcinomas. On the other hand, high levels of anti-TF antibody were detected in sera from patients with infectious diseases (436.0 ± 39.5 units/ml). It has been reported that large amounts of TF antigen are expressed in adenocarcinoma tissues[7-9] and that the amounts of anti-TF antibody in the sera of patients with lung, pancreatic, breast and bladder cancers are decreased[5,6]. In this study, the levels of anti-TF antibody were decreased in sera of patients with cancer, especially adenocarcinoma. These results suggest that lower levels of anti-TF antibody in serum may be a common phenomenon in adenocarcinoma patients and reflect the expression of TF antigen in cancer tissues.

References


