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PRELIMINARY REPORT

HANGANUTZIU-DEICHER TYPE-HETEROPHILE ANTIGEN-POSITIVE CELLS IN HUMAN CANCER TISSUES DEMONSTRATED BY MEMBRANE IMMUNOFLUORESCENCE

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The possible expression of Hanganutziu-Deicher (H-D)-type heterophile antigen on the cell surface of several types of human cancer tissues was investigated by the membrane immunofluorescence test with anti-H-D serum. Anti-H-D serum of high titer was obtained from chickens immunized with H-D antigen-active glycosphingolipid, N-glycolylneuraminyl-lactosylceramide (equine hematoside). H-D antigen was demonstrated in 3 of 6 gastric cancer tissues, 2 of 3 lung cancer tissues, and 2 of 4 breast cancer tissues examined, but not in 5 colorectal cancer tissues examined.

The production of heterophile antibody in sera of patients receiving therapeutic injections of foreign serum was described by Hanganutziu (1924) and Deicher (1926). Thereafter, this antibody was called serum-sickness or H-D antibodies. This H-D antibody differs in specificity from other kinds of heterophile antibodies, such as Forssman and Paul-Bunnell antibodies. Recently, H-D antibody was detected in sera from almost all patients examined who received the γ-globulin fraction of goat anti-human thymocyte serum (Pirofsky et al., 1973), and also in sera from some patients suffering from various diseases who had never received a therapeutic injection of foreign serum (Kasukawa et al., 1976). More recently, Nishimaki et al. (1979) demonstrated H-D antigen in sera and tissue extracts from

patients with various types of cancer by the hemagglutination inhibition test with human H-D antibody.

H-D antigen has been found in tissues and sera of various animals, but not humans or chickens. Recently, H-D antigen-active glycosphingolipid was purified and identified as N-glycolylneuraminyl (α, 2–3) lactosylceramide (equine hematoside) and N-glycolylneuraminyl (α, 2–3) neolactotetraosylceramide (bovine ganglioside) (Higashi et al., 1977). Results showed that the H-D antigenic determinant requires N-glycolylneuraminic acid at the nonreducing end of the carbohydrate chain of complex carbohydrates. However, chickens cannot synthesize this kind of sialic acid (Klenk and Ulenbruck, 1958). Therefore, chickens were immunized with equine hematoside or bovine ganglioside containing N-glycolylneuraminic acid, and the antisera obtained showed H-D specificity and contained a higher titer of H-D antibody than human patients (Ikuta et al., 1981; Fujii et al., 1982).

Recently, we demonstrated the H-D antigen on Marek's disease virus-transformed chicken lymphoblastoid line cells as tumor-associated cell surface antigen by membrane immunofluorescence (MIF) and complement dependent antibody cytotoxicity tests with chicken anti-equine hematoside or anti-bovine ganglioside serum (Ikuta et al., 1981). In addition, we demonstrated by the MIF test with chicken anti-equine hematoside serum that human lymphoblastoid line cells transformed by Epstein-Barr virus express the H-D antigen on their cell surface (Yonemura et al., in preparation). These results indicate that H-D antigen is newly expressed in the course of transformation of normal cells of H-D antigen-negative species by certain tumor viruses or other factors. This communication reports tests by the same methods for expression of H-D antigen on cells of several types of human cancer tissues.

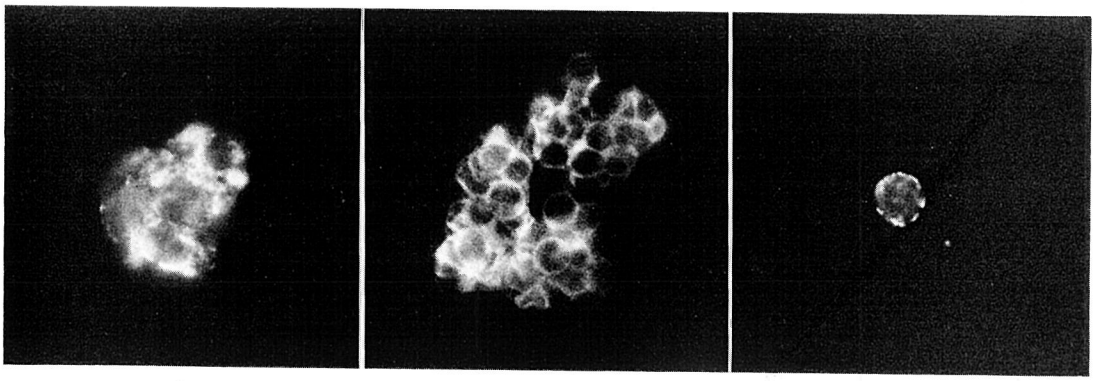
Human malignant cancer tissues were removed from patients with gastric cancer, lung cancer, breast cancer and colorectal cancer by surgery in the Department of Oncologic Surgery of this Institute. H-D antigen-active glycosphingolipid equine hematoside [NeuGc (α, 2–3)Gal(β, 1–4)Glc-Ceramide] was purified from equine erythrocyte stroma (Higashi et al., 1977). Anti-H-D serum was obtained from specific pathogen-free chickens of more than 6 months old that had been immunized intramuscularly with equine hematoside mixed with an equal amount of methylated bovine serum albumin and complete Freund's adjuvant. The chicken anti-equine hematoside serum used agglutinated 0.5% sheep erythrocyte suspension at up to 512-fold dilution in the microplate test as described previously (Ikuta et al., 1981). Fresh tumor tissues were promptly minced, passed through a stainless mesh, and washed three times with PBS. The cell pellet was suspended in 10-fold diluted chicken anti-equine hematoside serum, and incubated for 30 min at 37 C. Then the cells were washed three times with PBS and incubated in FITC-conjugated rabbit anti-chicken IgG serum for 30 min at 37 C. The cells were again washed three times with PBS, and then a drop of cell suspension was placed on a coverslip and promptly examined by fluorescent microscopy.

As summarized in Table 1, H-D antigen-positive cells were detected in 3 of 6 gastric cancer tissues, 2 of 4 breast cancer tissues, and 2 of 3 lung cancer tissues. No reaction was

TABLE 1. *Demonstration of H-D antigen-positive cells in human cancer tissues by the MIF test*

Cancer tissues[a]	Positive/Total	%
Gastric cancer	3/6	50
Breast cancer	2/4	50
Lung cancer	2/3	67
Colorectal cancer	0/5	0

[a] Tumor tissues were minced, passed through stainless mesh, and then treated with chicken anti-equine hematoside serum.



A B C

FIGURE 1. Fluorescent cells in human cancer tissues detected by the MIF test with chicken anti-equine hematoside serum. Cells from gastric cancer tissue (A), breast cancer tissue (B) and lung cancer tissue (C), were treated with the antiserum, and then with FITC-conjugated anti-chicken IgG serum.

observed with control serum from unimmunized specific pathogen-free chickens. The fluorescence was seen as patches or rings on most cells, as shown in Fig. 1. No fluorescence was observed in 5 colorectal cancer tissues examined. Moreover no reaction was detected on acetone or methanol-fixed cells of tissue sections cut with a cryostat. Marek's disease lymphoblastoid line cells fixed with acetone or methanol also did not give a reaction with anti-equine hematoside serum, although these living line cells have the antigen (unpublished data). Therefore, it is unknown whether the H-D antigen-positive cells detected in the cancer tissues by the MIF test are actually cancer cells, although it seems likely that they are because all the cells in tumor tissues that gave a positive reaction in the MIF test were relatively large.

Only H-D serum from patients has been used so far to examine the localization and expression of the antigen on cells or tissue sections (Kasukawa et al., 1976; Nishimaki et al., 1979). But, human H-D serum is no ideal for this purpose, because few sera contain sufficient H-D antibody activity to give a precipitin reaction with H-D antigen-active substances, and different H-D sera have different affinities for H-D antigen-active compounds (Naiki and Higashi, 1980). Therefore, we tried to prepare antisera specific to H-D antigen and containing a high titer of antibody by immunizing chickens with H-D antigen-active glycosphingolipid. In this work we demonstrated a close relation of H-D antigen with human malignant cancer tissues by the MIF test with this anti-H-D serum. In addition, H-D antigen may associate with certain cancer tissues, since the H-D antigen was not detectable in the colorectal cancer tissues examined although it was frequently found in lung, gastric and breast cancer tissues. This work showed that H-D antigen is expressed on human cancer cells as cell-surface antigen as it is on Marek's disease virus-transformed cells in chickens. This finding is of particular interest because it suggests a possible role of the antigen in tumor immunity. Studies on the immunological significance of H-D antigen using chickens with Marek's disease as a model, are now in progress.

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