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Title	QUANTITATIVE STUDIES ON HANGANUTZIU-DEICHER (H-D) TYPE OF HETEROPHILE ANTIGEN EXPRESSED ON MAREK'S DISEASE (MD) LYMPHOMA AS A TUMOR-ASSOCIATED ANTIGEN
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QUANTITATIVE STUDIES ON HANGANUTZIU-DEICHER (H-D)  
TYPE OF HETEROPHILE ANTIGEN EXPRESSED ON MAREK'S  
DISEASE (MD) LYMPHOMA AS A TUMOR-ASSOCIATED ANTIGEN

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The expression of H-D antigen on MD lymphoma was qualitatively demonstrated by IKUTA et al. (1981). In this report, we established an enzyme immunoassay (EIA) and radioimmunoassay (RIA), and attempted to determine the antigen quantitatively by both methods. The antigenic molecules were previously purified from animal erythrocytes as N-glycolylneuraminic acid-containing glycosphingolipids (GSLs) and glycoprotein. H-D antisera were prepared by immunizing chickens with the GSLs.

The optimal conditions for EIA using the GSL antigen were firstly standardized as follows: 1) 2.5  $\mu$ g of antigen dissolved in ethanol were coated to each well of a microtitration plate by dryness; 2) non-specific sites were blocked by incubation with 1% gelatin or egg albumin solution; 3) the first immune reaction was carried out with the chicken antisera, and the second immune reaction with alkaline phosphatase-labelled rabbit anti-chicken IgG (specific antibody, 15 ng; enzyme, 0.054 units) at 4°C for 12h and 37°C for 2h, respectively. The EIA and RIA using the radioiodinated glycoprotein antigen showed that the H-D antiserum used was specific for all of the antigenic molecules.

For detection of cell antigen in EIA, fixed cells were attached to plate wells precoated with polylysine, and in RIA cell homogenate or extract with Triton X-100 was used as the antigen. The two methods were not so different in sensitivity, however, H-D antigenic activity was hardly detected in MD lymphoma-derived cell lines, transplantable MD tumor-derived cell lines or lymphoid leukemia lymphoma-derived cell lines. This finding indicates a difficulty in performing quantitative studies on H-D antigen expressed on MD lymphoma.