Relation of Human Blood-Groups MN to Cancer Cell Surface Antigens and to Receptors for Oncogenic Viruses*

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ABSTRACT

It has been shown by us that the human blood-group MN antigenic determinants are not the products of allelomorphic genes as believed so far, but that N is the precursor substance of M and that the allelomorph to the M gene is amorph. The determinant structure of the N antigen is branched and possesses as non-reducing termini β-D-galactopyranosyl (Gal) and α-N-acetyl-neuraminic acid (NANA) linked to β-Gal. The M substance differs from N only in that α-NANA covers the terminal β-Gal of the N determinant. Vicia graminea anti-N reacts with terminal β-Gal of the N antigen as well as its precursor.

A human blood-group N-like antigen in the cell surface of the TA3 mammary adenocarcinoma (ascites form) has been found by us. The TA3 cancer occurs as the non-strain specific Ha subline and as the strain-specific St subline. This is the first description of an N-like antigen in a non-primate as well as a tumor. This antigen reacts with Vicia anti-N. In serological specificity the Vicia agglutinin is closely related to the Thomsen-Friedenreich anti-T agglutinin present in most human and animal sera. These sera plus complement kill ordinary TA3-St cells and sialidase-treated Ha cells to >95 percent. Untreated TA3-Ha cells are fully resistant even though they absorb cytotoxin. Beta-galactosidase treatment of either Ha or St cells abolishes the killing activity of the sera. The cancer cells absorb anti-T but they lose this capability after exposure to β-galactosidase.

An immunological cross-relationship between the human blood-group MN antigens and the receptor for an oncogenic virus, the avian subgroup B leukemia sarcoma virus has been observed.

Human Blood-Group M and N Substances

The second human blood-group system, the MN system, was discovered by Land-
RELATION OF HUMAN BLOOD-GROUPS MN TO CANCER CELL ANTIGENS

CLASSICAL PATHWAY

Precursor ——> \(\text{If.} \) MM

NEWLY PROPOSED PATHWAY

Precursor ——> Vicia ——> \(\text{If.} \) MM

Figure 1. Genetic pathways leading to the human blood-group M and N specificities. If.—Myxovirus receptor activity.

(NANA) was the first accurate information on the chemical basis of these specificities. Since these substances are excellent receptors for influenza viruses and are inactivated by their sialidases, this was also the first indication that a single gene locus may control both a virus receptor and an isoantigen specificity.\(^{10,11}\)

Recent studies have shown that the human blood-group MN antigenic determinants are not the products of allelomorphic genes, but that N is the precursor substance of M, that M can be readily transformed to N and that the allelomorph to the M gene is amorph,\(^{16,20}\) as depicted in figure 1 which shows both the classical and the newly proposed scheme. Added to it is the myxovirus inhibitory property (If.) discovered by the authors. The structure which reacts with the anti-N specific reagent from *Vicia graminea* seems to be only part of the N antigen and precedes the complete N specific determinant in the biosynthetic pathway. These findings have been corroborated by Wiener et al\(^{21}\) in independent serological studies of blood types of gorillas.

On a chemical basis the situation has been found as depicted in figure 2.\(^{15,19}\) The immunodominant structure of the N antigen is branched and one branch terminates in a non-reducing \(\beta\)-d-galactopyranosyl (Gal)-grouping while the other has a non-reducing terminal \(\alpha\)-NANA linked to \(\beta\)-Gal. There appear to be three moles of N determinant structures\(^{19}\) per mole of N antigen subunit.\(^9\) The M substance differs from the N substance only in that \(\alpha\)-NANA covers the terminal \(\beta\)-Gal of the human N specific grouping.\(^{15,19}\)

**Mouse Mammary TA3 Adenocarcinoma (Ascites form)**

A glycoprotein antigen has been found by us to be closely related to those of the human blood-group MN system in the cell surface of the TA3-Ha subline of the ascites form of a mouse mammary adenocarcinoma.\(^{12}\) This is the first description of an antigen of the blood-group MN system in a non-primate as well as a tumor. Some of the essential characteristics of the mouse mammary carcinoma antigen are compared with corresponding properties of the human blood-group N antigen in table I. Both possess extremely high *Vicia*-agglutinin inhibitory activity and both have Gal and NANA as termini, even though the carcinoma glycoprotein carries these on two different chains\(^2\) while they are probably present in the blood-group N substances on branches of the same chain.\(^{10,17}\)

The TA3-Ha mouse cancer which possesses this blood-group N-like antigen in its surface is closely akin to human adenocarcinomata, not only because of its “spontaneous” origin and minimal deviation from the diploid karyotype and isoantigenic phenotype of its primary host\(^6\) but also because it carries human blood-group specific structures, as do human mammary

![Figure 2](image)

**Figure 2.** Human blood-groups M and N, immunodeterminant structures.
**TABLE I**

CHARACTERISTICS OF MOUSE TA3 MAMMARY ADENOCARCINOMA SURFACE GLYCOPROTEIN I

<table>
<thead>
<tr>
<th>TA3 Carcinoma Glycoprotein</th>
<th>Human Blood-group N Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vicia graminea</strong></td>
<td></td>
</tr>
<tr>
<td>anti-β-inhibitory activity*</td>
<td>1-10</td>
</tr>
<tr>
<td>Terminal carbohydrates</td>
<td>2 different chains:</td>
</tr>
<tr>
<td>α-NNA-β-Gal</td>
<td>α-NNA-β-Gal</td>
</tr>
<tr>
<td>β-D-Gal</td>
<td>β-D-Gal</td>
</tr>
</tbody>
</table>

*Minimum quantity (μg/ml) completely inhibiting agglutination of human NN red cells by 4 doses of *Vicia* agglutinin.

This cancer has been transferred through many generations and 2 sublines have occurred, the Ha subline which has lost its strain-specificity and the St subline which still possesses it. Also the Ha subline is much more invasive than the St subline. Many cancer cells, including those from humans, carry a glycoprotein coat which, according to the hypothesis of Currie and Bagshawe, may protect them from immunological surveillance. We found the *Vicia*-specific glycoprotein to also occur in the ascites fluid of TA3 inoculated mice, but not in any tissue of healthy mice.

The studies reported here may contribute to an understanding of the striking difference in malignancy of these two closely related cancers.

As shown in table I, both the human blood-group N substance and TA3-Ha tumor glycoprotein possess high and specific inhibitory activity towards the *Vicia graminea* agglutinin. The specificity of this plant agglutinin is directed towards an immunodominant β-D-galactopyranosyl structure. The serological specificity of the *Vicia* agglutinin is closely related to the anti-T agglutinin of Thomsen and Friedenreich, which occurs in the sera of all adult humans and most animals, including mice.

The effect of heat-inactivated human sera in the presence of complement on the Ha and St sublines of the mouse mammary glands has therefore been investigated by us. Cells of both sublines adsorb anti-T. Some of the most striking findings are shown in table II which demonstrates that the cancer cells of the St subline are killed, while those of the Ha subline are completely resistant. They become fully susceptible after RDE treatment, i.e. after removal of their terminal NANA. If there was a concomitant treatment with β-galactosidase, the killing activity of human and other mammalian sera was abolished. In addition, substances with B1 → 3 or 1 → 4 linked Gal inhibit the cytotoxic effect. This clearly indicates the human cytotoxic serum factor, like the *Vicia* agglutinin, is directed towards a β-Gal structure.

The relation of the human cytotoxic serum factor to the anti-T agglutinin can be distinctly seen in table III where it is shown that RDE-treated erythrocytes which carry uncovered T-antigen absorb cytotoxin.

**TA3 St Cells**

<table>
<thead>
<tr>
<th>Serum Absorbed With</th>
<th>TA3-St Cells RDE-treated % Killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated erythrocytes</td>
<td>90-100</td>
</tr>
<tr>
<td>RDE-treated erythrocytes</td>
<td>96</td>
</tr>
</tbody>
</table>

**TA3-Ha Cells**

<table>
<thead>
<tr>
<th>Serum Absorbed With</th>
<th>TA3-Ha Cells RDE-treated % Killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated erythrocytes</td>
<td>90-100</td>
</tr>
<tr>
<td>RDE-treated erythrocytes</td>
<td>96</td>
</tr>
</tbody>
</table>

**Anti-T Titer (reciprocal)**

| Untreated erythrocytes | 32 |
| RDE-treated erythrocytes | 4 |

**TABLE II**

KILLING OF TA3 MOUSE ADENOCARCINOMA BY HUMAN CYTOTOXIC SERUM FACTOR PLUS COMPLEMENT

<table>
<thead>
<tr>
<th>TA3 Carcinoma Glycoprotein</th>
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*Minimum quantity (μg/ml) completely inhibiting agglutination of human NN red cells by 4 doses of *Vicia* agglutinin.
in parallel with anti-T; furthermore, both activities can be eluted from RDE-treated red cells as shown in the last line of table III. In addition, anti-T and the related agglutinins from *Vicia* and *Arachis* are absorbed by TA3-Ha cancer cells and this capability is abolished by treatment of the tumor cells with β-galactosidase (table IV). These experiments also demonstrate that even intact TA3-Ha cells bind the cytotoxic. However, we found that the terminal NANA interferes with the fixation of complement.

**Relation MN Substances to Receptor for Oncogenic Fowl Virus**

The authors have found during studies of the human blood group M and N antigens a cross-relation of these antigens to a cell surface structure, the R₄ antigen of chicken cells, believed to be closely related to or identical with a receptor for an oncogenic fowl virus presumed to be the avian subgroup B leukemia sarcoma virus. We have been able to isolate and to inactivate the R₄ antigen with RDE. Furthermore, it has been shown that the blood group MN antigens preincubated with certain Rous sarcoma virus strains prevented subsequent chicken fibroblast infection by these viruses. The substances were not toxic to the fibroblasts (to be published). The anti-infectious effect of the MN antigens is given in table V.

**Table V**

**Neutralization of Rous Sarcoma Virus (RSV)**

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Percent Inhibition of Plaque Formation by RSV of Subgroup A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human blood-group N antigen</td>
<td>94.3</td>
<td>72.5</td>
</tr>
<tr>
<td>Human blood-group N antigen</td>
<td>96.6</td>
<td>90.5</td>
</tr>
</tbody>
</table>

These observations as well as those on the TA3 adenocarcinoma are likely to have an important bearing on human oncology.

**Acknowledgments**

The authors are grateful to Prof. G. Klein and Dr. J. F. Kodington for donation of the TA3 carcinoma sublines and to Mr. I. Banatwala and Mrs. H. Tegtmeyer for excellent technical assistance.

**References**


