Further characterization of the binding properties of two monoclonal antibodies recognizing human Tn red blood cells

Albert M. Wu\textsuperscript{a,*}, June H. Wu\textsuperscript{b}, Hsiang-Wei Kuo\textsuperscript{a} & Anthony Herp\textsuperscript{a}

\textsuperscript{a}Glyco-Immunochemistry Research Laboratory, Institute of Molecular and Cellular Biology, and
\textsuperscript{b}Department of Microbiology and Immunology, Chang-Gung University, Kweishan, Taoyuan 333, Taiwan

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Summary

The terminal α anomic GalNAc residue is an essential sugar for the Tn glycoyte, human blood group A determinant, and Forssman antigen. In a previous study [King M.J., Parson S.F., Wu A.M., Jones N., Transfusion 31: 142–149, 1991], we defined two monoclonal antibodies (MoAbs, BRIC66 and BRIC111) reacting with human Tn red blood cells. However, more advanced studies of these two MoAbs were hampered by the lack of availability of Gal/GalNAc related glycotopes. In order to use these antibodies as powerful probes to elucidate structural changes during life processes, we have characterized in detail the combining sites of these two MoAbs using enzyme-linked immunosorbent (ELISA) and inhibition assays with an extended glycan/ligand collection. From the results, it has been established that BRIC66 demonstrated multiple specificities and its reactivity towards glycotopes was defined as: GalNAc\textsubscript{α1β1}Ser/Thr (Tn) > GalNAc\textsubscript{α1β1}Gal\textsubscript{β14}Glc > GalNAc\textsubscript{α1β1}Gal > GalNAc > Gal or Glc. Another MoAb, BRIC111, mainly bound Tn-glycophorin. The best ligand for this MoAb was Tn-containing glycopeptides (M.W. < 3.0 \times 10^3 Da) from asialo ovine salivary mucin (OSM), which was approximately 70 and 58 times more active than GalNAc and monomeric GalNAc\textsubscript{α1β1}Gal, respectively, suggesting that the active glycotopes present in glycophorin for BRIC111 binding also exist in OSM. The N-acetyl group at carbon-2 and configuration at carbon-4 of the α anomic GalNAc are required for the binding of either MoAb. Identification of these binding properties should aid in the selection of these MoAbs and the conditions required for biological studies and clinical applications.

Abbreviations:

The mammalian carbohydrate structural units in glycans used to define the binding properties of MoAb are: T = Gal/β1→3GalNAc, Thomsen-Friedenreich disaccharide; T\textsubscript{a} = Gal/β1→3GalNAc\textsubscript{α1β1}Ser/Thr; Tn = GalNAc\textsubscript{α1β1}Ser/Thr; A = GalNAc\textsubscript{α1β1}Gal, human blood group A specific disaccharide; A\textsubscript{α} = Gal-

*To whom correspondence should be addressed. Fax: +886-3-2118456 (Lab.); +886-3-2118700 (Col.); e-mail: amwu@mail.cgu.edu.tw
Introduction

The terminal α anomic GalNAc residue (GalNAc1-→) is an essential sugar for three glycotopes - the carbohydrate determinants of Tn, human blood group A, and Forssman antigens. The Tn (GalNAc1-→Ser/Thr) sequence is the simplest carbohydrate chain that has been proposed as a marker of cancerous tissues [1–5]. Tn was also found in many mammalian salivary mucus glycoproteins (gps) [6–11], especially in those of armadillo, hamster, and ovine origins. At the surface of the human red blood cell membrane, the Tn transformation indicates an acquired disorder characterized by the exposure of normally cryptic GalNAc residues α-linked to the Ser or Thr of membrane sialoglycoproteins [12]. The Tn syndrome is the result of a selective deficiency of the 3-β-D-galactosyltransferase involved in the biosynthesis of the T structure: Galβ1-→3GalNAc1-→Ser/Thr [13]. The Tn antigen was also found at the cell surface of granulocytes, platelets, and B and T lymphocytes in patients with the Tn syndrome [14]. The Forssman antigen (GalNAc1-→3GalNAcβ1-→3Galα1-→4Glcβ1-→N’ceramide) commonly occurs as a heterophilic antigen and is not thought to be present in most humans. The Forssman antigen was found in significant quantities in several forms of human cancer, including gastric, colon, and lung cancers [15–19]. This antigen is one of the tumor-associated glycolipid antigens with blood group A-like epitopes. Since the terminus of the antigen shares a sugar residue, GalNAc1-→, with the blood group A terminal saccaride as well as the Tn antigen, the unusual enhancement of activity of the blood group A-like antigen has been associated with carcinogenesis. An assay for this antigen in tissue sections and in circulating plasma would be of value in detecting colon cancer [20].

In a previous study, the binding properties of two GalNAc1-→ monoclonal antibodies (MoAbs), BRIC66 (IgM) and BRIC111 (IgG1), were analyzed using hemagglutination, inhibition tests, and radioimmunoassay. It was found that these MoAbs agglutinated human Tn red blood cells and reacted with Tn glycoporphin [21]. However, the study was hampered by the lack of availability of Gal/GalNAc related glycotopes. In order to use these antibodies as powerful probes to elucidate structural changes during the life processes, it is necessary to understand the recognition factors involved in their antigen interactions. For this reason, the combining sites of these two MoAbs were further characterized with an extended glycan/ligand collection using an enzyme-linked immunosorbent assay (ELISA) and inhibition of antibody–glycan interaction. From the results, it was found that BRIC66 had broad specificities for various GalNAc1-→ related glycotopes and BRIC111 bound mainly with Tn-glycoporphin. The carbohydrate specificity of BRIC111 should be closely related to some of the Tn residues of ovine salivary glycoprotein. Special structural features of polyvalent and cluster forms of Tn should play a major role in binding. These two MoAbs showed poor activity with GalNAcβ1-→ and with Galα1-→ related derivates, indicating that α anomic conformation, N-acetyl group at carbon-2 and configuration at carbon-2 and carbon-4 of GalNAc are required for the binding of either MoAb. The binding properties of these two MoAbs were compared, which should aid in the selection of these MoAbs and the conditions required for biological studies and clinical applications.

Materials and methods

Monoclonal antibodies (MoAbs)

The methods for cell fusion and cloning were essentially as previously described [22]. The MoAb BRIC 66 (IgM) was cloned using spleen cells from a mouse immunized as described by Messeter et al. [23] with blood group A-active ovarian cyst