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The distribution pattern of α2,3- and α2,6-linked sialic acids affects host cell preference in *Toxoplasma gondii*

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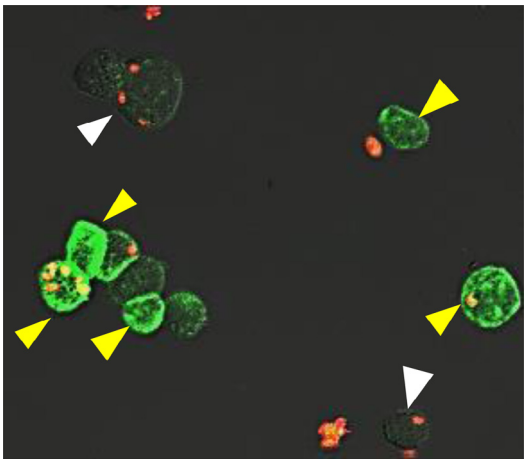
HIGHLIGHTS

- Enzymatic removing of α2,3- but α2,6-linked sialic acid of host cells decreased *T. gondii* tachyzoites adhesion.
- Tachyzoites preferentially attached to α2,3-linked sialic acid-rich cells among cells with variety of sialic acid expressing levels.
- More tachyzoites adhered to α2,3-linked sialic acid-rich cells single cell than α2,3-linked sialic acid-poor cell.

GRAPHICAL ABSTRACT



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ABSTRACT

Tachyzoites of *Toxoplasma gondii*, an obligate intracellular parasite, actively invade almost all types of nucleated cells. However, *T. gondii* tachyzoites preferentially infect particular types of animal tissue cells. The mechanism underlying the host cell preference of *T. gondii* is not yet known. In this study, we found that enzymatic removal of α2,3- but not α2,6-linked sialic acids on the surface of Vero cells decreased *T. gondii* tachyzoite adhesion or invasion to the treated cells. Although Chinese hamster ovary (CHO) cells express only α2,3-linked sialic acid, a genetically modified CHO cell line constructed by transfection with the α2,6-sialiltransferase gene contains subpopulations with a variety of expression patterns of α2,3- and α2,6-linked sialic acids. When *T. gondii* tachyzoites were added to the modified CHO cells, the tachyzoites preferentially attached to cells belonging to a subpopulation of cells that highly expressed α2,3-linked sialic acids. Additionally, multiple regression analysis performed to analyse the relationship between the amount of α2,3-linked/α2,6-linked sialic acids and parasite-expressed fluorescence intensity suggested that more tachyzoites adhered to individual α2,3-linked sialic acid rich-cells than to α2,3-linked sialic acid-poor/null cells. The results of confocal laser microscopy confirmed this finding. These results indicate that the host cell preference of *T. gondii* was, at least partially, affected by the distribution pattern of α2,3-, but almost never α2,6-linked sialic acids on host cells.

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1. Introduction

Toxoplasma gondii is an intracellular apicomplexan parasite responsible for congenital infections and abortion, and opportunistic diseases in immunodeficient individuals. *T. gondii* tachyzoites are able to infect virtually any nucleated cell of warm-blooded vertebrates (Carruthers, 1999). This wide host range suggests that *T. gondii* recognises abundant components on the host cell surface, such as glycan molecules. It is already known that surface-exposed carbohydrates on the host cell surface play an important role in early recognition events by *T. gondii* tachyzoites (Sheiner et al., 2010). It is thought that among the abundant carbohydrate molecules on host cell surfaces, sialic acid, heparan sulphate, chondroitin sulphate and galactose are receptors for *T. gondii* invasion, because removing them from the host cell surface or blocking them decreases the efficiency with which *T. gondii* can infect cells (Carruthers et al., 2000; Monteiro et al., 1998). Despite their extremely wide host cell tropism, *T. gondii* tachyzoites preferentially infect particular types of animal tissue and cells. It is known that monocytes are more susceptible to becoming infected with *T. gondii* than other types of peripheral blood cells (Channon et al., 2000). When *T. gondii* tachyzoites invade human placenta, the syncytiotrophoblast is resistant to *T. gondii* infection while cytotrophoblasts, which are located under syncytiotrophoblast, are selectively infected (Abbasi et al., 2003; Robbins et al., 2012). The host cell tropism observed in certain organs plays an important role in the pathophysiology of toxoplasmosis. However, the mechanism underlying the host cell preference of *T. gondii* for particular host animal tissues is not yet known; neither can host cell preference simply be explained by the presence or absence of particular receptor molecules on the cell surface, because, under certain conditions, *T. gondii* can infect almost all cell types.

Sialic acids, which are potential receptor molecules for *T. gondii*, are a family of sugars comprising one of the most abundant terminal monosaccharides on the surface of cells (Varki et al., 2011). Sialic acids are mostly linked to the penultimate galactose residues of carbohydrate side chains via α2,3- or α2,6-linkages (Traving and Schauer, 1998). The tissue and cell type dependent distribution pattern of α2,3- or α2,6-linked sialic acids determines influenza virus host cell tropism (Ito, 2000). Host cell surface sialic acids are thought to be receptor molecules for *T. gondii* cell entry because the infection rate of *T. gondii* for a sialic acid-lacking mutant cell line derived from Chinese hamster ovary (CHO) cells was lower than that of wild type CHO cells or CHO derived cell lines with other glycosylation mutants (Monteiro et al., 1998). It indicates that sialic acids have bigger impact on *T. gondii* infection than other sugars. However, CHO cells express α2,3-linked sialic acids, not α2,6-linked sialic acids (Lee et al., 1989). It is not yet known whether the tissue and cell type dependent distribution pattern of α2,3- or α2,6-linked sialic acids affects the host cell preference of *T. gondii*. *T. gondii* tachyzoites employ many proteins, mainly microneme proteins, to attach sugar chains to the host cell surface (Cowper et al., 2012). These proteins bind to glycan receptors on the host cell surface, and establish a strong initial attachment (Cowper et al., 2012). One such microneme protein, Toxoplasma microneme protein 1 (TgMIC1), plays an important role in host cell attachment (Cerede et al., 2005) and has high affinity for α2,3-linked sialic acids (Garnett et al., 2009). In contrast, TgMIC1 hardly binds to α2,6-linked sialic acids because α2,6 linkage induces a markedly different orientation in the polysaccharide chain, resulting in a loss of binding between host sialic acid and the sialic acid binding domains in TgMIC1 molecule (Friedrich et al., 2010). However, many microneme proteins mediate *T. gondii* tachyzoite adhesion and the contribution of α2,6-linked sialic acids to *T. gondii* tachyzoite invasion of host cells is not yet known.

In this study, we examined the effect of enzymatic and genetic modification of host cell sialic acid molecules and revealed that the distribution pattern of α2,3-, but not α2,6-linked sialic acids, at least partially, defines the host cell preference of *T. gondii* tachyzoites.

2. Materials and methods

2.1. Cells and parasites

Vero cells were maintained in RPMI-1640 medium supplemented with 7.5% foetal calf serum (FCS) and 20 μg/ml gentamicin and were incubated at 37 °C in a 5% CO_2 incubator. CHO and Lec2 cells were maintained in Ham's-F-12K medium supplemented with 10% FCS and 20 μg/ml of gentamicin with incubation at 37 °C in a 5% CO_2 incubator. CHO-K1-ST6/Gal1 (ST6) cells had been established by transformation with α2,6-sialiltransferase cDNA (Naito et al., 2007). ST6 cells were maintained in Ham's-F-12K medium supplemented with 10% FCS, 20 μg/ml gentamicin, and 1 mg/ml G418. To obtain cell subpopulations with several patterns of α2,3-, but not α2,6-linked sialic acid expression, ST6 cells were passaged several times in the medium without G418. *T. gondii* transgenic tachyzoites (PLK strain, type II) stably expressing green (PLK/GFP) and red fluorescence (PLK/RED) were maintained in Vero cells as described previously (Nishikawa et al., 2008).

2.2. Neuraminidase treatment

Neuraminidase derived from *Salmonella typhimurium* LT2 (New England Biolabs Japan, Tokyo) or from *Arthrobacter ureafaciens* (Roche, Basel) was suspended at 20,000 U/ml with 1 × G4 reaction buffer (50 mM sodium citrate, 100 mM NaCl, pH 6.0) with 100 mg/ml BSA or 200 mU/ml in phosphate buffered saline (PBS), respectively. Vero cells were dissociated from the culture by trypsinisation and 1×10^6 of the dissociated cells were resuspended in 20 μl of *S. typhimurium* LT2 or *Arthrobacter ureafaciens*-derived neuraminidase solutions and then incubated for 4 h or 100 μl for 1 h at 37 °C, respectively.

2.3. Tachyzoite infections and flow cytometry

2.3.1. Vero cells

Vero cells (9×10^5) were treated with neuraminidases as described earlier. A part of the treated Vero cells (3×10^5) were mixed with 9×10^4 PLK/GFP tachyzoites in 1 ml of RPMI 1640 medium supplemented with 7.5% FCS plus 20 μg/ml of gentamicin and then incubated for 2 h at 37 °C. After incubation, to examine the tachyzoite adhesion or invasion rates, the cells were fixed with 4% paraformaldehyde for 10 min at room temperature and the number of fluorescent tachyzoite adhering or invaded cells was counted using flow cytometry (FACSCanto: Becton Dickinson Japan, Tokyo) and calculated frequency of parasite-adhered or invaded cells. To measure amount of α2,3- or α2,6-linked sialic acids on the treated Vero cells, a part of the treated Vero cells (3×10^5 each) were incubated with FITC-conjugated *Maackia amurensis* agglutinin (MAM: J-OIL MILLIS, Tokyo) or FITC-conjugated *Sambucus sieboldiana* agglutinin (SSA: J-OIL MILLIS, Tokyo) at 20 μg/ml in PBS for 1 h on ice in the dark. After staining, 4% paraformaldehyde was used to fix the cells (10 min, room temperature), after which they were resuspended in 1 ml of PBS. The stained and fixed cell suspensions were analysed using flow cytometry.

2.3.2. CHO derived cell lines

CHO, Lec2 and ST6 cells were washed gently once in Ham's-F-12K medium with 10% FCS plus 20 μg/ml of gentamicin. PLK/RED tachyzoites added to the washed cells at a multiplicity of infection (MOI) of 1 were incubated for 2 h at 37 °C. After incubation, to examine the tachyzoite adhesion rates, the cells were fixed with 4% paraformaldehyde for 10 min at room temperature and the number of fluorescent tachyzoite adhering cells was counted using