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Glycan microarray profiling of parasite infection sera identifies the LDNF glycan as a potential antigen for serodiagnosis of Trichinellosis

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Abstract

Diagnostic methods for parasite infections still highly depend on the identification of the parasites by direct methods such as microscopic examination of blood, stool and tissue biopsies. Serodiagnosis is often carried out to complement the direct methods, however few synthetic antigens with sufficient sensitivity and specificity are available. Here we evaluated a glycan microarray approach to select for synthetic glycan antigens that could be used for serodiagnosis of parasitic infections. Using a glycan array containing over 250 different glycan antigens, we identified GalNAc β 1-4(Fuc α 1-3)GlcNAc-R (LDNF) as a glycan antigen that is recognized by antibodies from *Trichinella*-infected individuals. We synthesized a neoglycoconjugate, consisting of 5 LDNF molecules covalently coupled to bovine serum albumin (BSA), and used this neoglycoconjugate as an antigen to develop a highly sensitive total-Ig ELISA for serological screening of trichinellosis. The results indicate that glycan microarrays constitute a promising technology for fast and specific identification of parasite glycan antigens to improve serodiagnosis of different parasitic infections, either using an ELISA format, or parasite-specific glycan-arrays.

1. Introduction

Infections with parasites cause worldwide suffering and death, particularly in developing countries. Diagnostic methods for some of these infections still highly depend on the identification of the parasites by direct methods such as microscopic examination of blood, stool and tissue biopsies (Gillespie, 2007). Although these approaches can result in a specific diagnosis, the sensitivity is generally low. Serodiagnosis could be used to complement the direct methods. However, preparation of the parasite antigens is often

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complicated which can compromise reproducibility of the results. Improving serodiagnosis using new technology is required.

Here we evaluated the potential use of parasite glycan antigens for serodiagnosis of parasitic infections. Glycans, linked to proteins and lipids, are abundantly present on the surface and in the excretory/secretory products of parasites, and humoral immune responses to glycan antigens are a dominant feature of parasite infections, as illustrated by studies of helminth infections in mice, humans and primates (Van Remoortere et al., 2001; Eberl et al., 2001; Van Remoortere et al., 2003; Nyame et al., 2003; Schabussova et al., 2007).

To select for helminth glycan antigens that have serodiagnostic potential, we used an approach based on glycan microarray technology. A glycan microarray comprises a library of synthetic or natural glycan molecules that are covalently linked to a solid support such as glass slides (Blixt, O., et al, 2004; Song et al., 2009; Lonardi et al., 2010). The most common application of glycan array technology is analyzing the binding specificity of lectins and antibodies. Such glycan microarrays have been already used to analyze the antibody response to pathogens, such as *Schistosoma* and *Salmonella* (De Boer et al., 2008; Blixt et al., 2008). The application of this technology could lead to novel glycan-based diagnostic tools for the serodiagnosis of parasitic infections.

A common parasitic infection which direct diagnosis is aided by serology is trichinellosis. This is a food borne zoonotic disease caused by nematodes of the genus *Trichinella*. Human infection occurs due to the ingestion of raw or inadequately cooked meat containing parasite larvae, mostly of *T. spiralis* species (Gottstein et al., 2009). The disease in humans can range from asymptomatic infection to a fatal disease, depending on the number of larvae ingested and the host immune status. According to reports from 55 countries worldwide, the yearly total number of trichinellosis cases is estimated to be 10,000, with a mortality rate of 0.2% (Despommier D. et al., 2005).

The direct detection of muscle-stage larvae in muscle biopsies etiologically proves the diagnosis. The disadvantage of this method is that it requires surgical intervention and that the sensitivity of the diagnosis depends on the parasite load and the amount of muscle sample tested (Gottstein et al., 2009). In addition to the clinical history and results from the biopsy, serology by ELISA is used for the detection of specific anti-*Trichinella* antibodies in human sera. Most ELISA assays are based on the use of excretory/secretory (ES) products from the *T. spiralis* muscle larvae (Gottstein et al., 2009). The use of the ES antigen, however, has serious disadvantages since the preparation of this antigen is laborious and requires the use of laboratory animals. Furthermore, micro-environmental factors during culture of the animal-derived larvae may affect antigen quality (Bolas-Fernandez et al., 2009), resulting in standardization problems. Replacement of the ES antigen by synthetic antigens with sufficient sensitivity and specificity could solve these problems.

Our studies clearly show that specific parasite glycan antigens can be identified by glycan array analysis of minute amounts of serum from infected individuals. In addition, we showed that an ELISA assay based on neoglycoconjugates carrying the GalNAc β 1-4(Fuca1-3)GlcNAc (LDNF) glycan antigen has a high sensitivity for serodiagnosis of trichinellosis, indicating the potential value of glycan microarray technology for diagnosis of parasite infections.

2. Material and methods

2.1. Human sera

A total of 29 *Trichinella* positive serum samples were tested. Seven of these sera were from the diagnostic laboratory at the RIVM, 12 were from an outbreak in Turkey (2004) that was confirmed to be caused by *Trichinella britovi* (Akkoc et al., 2009) and 10 were from an outbreak in Poland (1991) caused by *T. spiralis* (Pinelli et al., 2001). The sera were tested both in a total Ig *Trichinella*-ELISA using ES antigen and confirmed with a *Trichinella*-immunoblot as previously described (Pinelli et al., 2001). In addition, 90 serum samples from patients that were serologically positive to the following infections were used: ascariasis, leishmaniasis, toxocariasis, echinococcosis, cysticercosis, amoebiasis, toxoplasmosis, borreliosis, and syphilis. Ten positive sera from patients with schistosomiasis and 10 from strongyloidiasis were kindly provided by Dr. J. Van Hellemond from the Rotterdam Harbour Hospital/Erasmus MC, the Netherlands. Ten serum samples from healthy blood donors with no known history of parasitic infection were also used.

2.2. Preparation of *Trichinella spiralis* ES and crude antigen

Preparation of the ES antigen was performed as previously described by Gamble (Gamble, 1985). Briefly, 5 to 7 weeks old male Wistar rats obtained from Harlan-Netherlands were orally infected with 3,000 *T. spiralis* muscle larvae that were recovered by acid-pepsin digestion from chronically infected mice. After 42 days of infection, *T. spiralis* muscle larvae were recovered from infected rats by acid-pepsin digestion, washed and incubated at a concentration of 10^5 larvae per ml, for 19 h at 37°C in 5% CO₂ in RPMI medium supplemented with 1% penicillin/ streptomycin. After incubation, the medium was centrifuged and the supernatant containing the ES antigen was dialyzed and concentrated. The protein concentration was determined by the BCA protein assay (Pierce, Rockford, IL, USA). *T. spiralis* crude antigen was prepared from muscle larvae, in 100mM Tris HCl (pH=8), essentially as described by DeBose-Boyd et al (DeBose-Boyd et al., 1998).

2.3 Glycan array

Glycan array screening was performed at Core H of the Consortium for Functional Glycomics (CFG), Emory University School of Medicine, Atlanta, USA). The glycan array is a microarray containing a library of natural and synthetic glycans with amino linkers printed onto NHS-derivatized glass slides to form a covalent amide linkage. Printed array Version 2.1 containing glycan structures with the CFG numbers # 1-264 was used. The procedure for testing the glycan array as well as all glycan structures used and their corresponding CFG numbers are available at the website of the CFG (<http://www.functionalglycomics.org/fg/>). Glycan-array slides were incubated with human serum (1:100 dilution) derived from parasite-infected or healthy blood donors as indicated, and subsequently with Alexa labeled mouse anti-human IgG secondary antibodies in phosphate buffered saline (PBS) containing 0.5% Tween-20. The samples (100 µl) were applied directly onto the surface of a single slide, covered with a microscope cover slip and incubated in a humidified chamber for 60 min. Slides were subsequently washed by successive rinses in (i) PBS-0.05% Tween, (ii) PBS, and (iii) deionized water, and immediately subjected to imaging. Fluorescence intensities were detected by using a ScanArray 5000 (PerkinElmer) confocal scanner. Image analyses were carried out using IMAGEGENE image analysis software (BioDiscovery, El Segundo, CA). No background subtractions were performed. Data were plotted by using Microsoft EXCEL software.

2.4 Synthesis of neoglycoconjugates

Neoglycoconjugates presenting LDNF, GalNAc β 1-4GlcNAc (LDN) or N,N',N''-diacetylchitotriose (chitotriose, chi-3, Sigma) were synthesized essentially as described in Tefsen et al, 2009 (Tefsen et al., 2009). In short, N,N'-diacetylchitobiose (chitobiose, chi-2, Sigma) or chitotriose was derivatized with 2,6-diaminopyridine (DAP, Sigma) in DMSO (Sigma), Acetic acid (Sigma) and NaCNBH₃ (Sigma) and purified on a preparative Zorbax NH₂ PrepHT column (250 × 21.2 mm, 7 μ m, Agilent) using a gradient with acetonitrile (Sigma) and 50 mM ammonium formate, pH 4.4 (Sigma) with a column flow of 10 ml. Chi-2-DAP was converted to LDN-DAP using the recombinant enzyme β 1,4-GalNAcT from *Caenorhabditis elegans* (Kawar et al., 2002), and LDN-DAP subsequently was converted to LDNF-DAP using the fusion protein ProtA-FucT-VI as enzyme source (Jost et al., 2005). All DAP-derivatized glycans were purified by Sep-Pak C₁₈ reverse-phase chromatography (Palcic et al., 1988) and dried in a speedvac for subsequent conjugation to BSA. The DAP-derivatized glycans were activated with 3,4-Diethoxy-3-cyclobutene-1,2-dione, (98%, di-ethylsquarate, Sigma Aldrich) in ethanol and coupled to BSA (Sigma) dissolved in conjugation buffer (boric acid (Gibco) and KCl (Fluka); the pH was adjusted with KOH (Merck). By varying the glycan to protein ratio, the amount of glycan antigens coupled to BSA was regulated, resulting in neoglycoconjugates carrying different amounts of LDNF antigen (LDNF2 and LDNF5). Samples were dialyzed in a Slide-A-Lyzer Dialysis Cassette with 10.000 MWCO (Thermo Scientific) against demi-H₂O and neoglycoconjugates were dried in a speedvac and stored at -20°C. The molar ratio of glycans to carrier was analyzed in a 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems) and was as follows: for chi-3-BSA 3.2:1, for LDN-BSA 4.5:1 and for LDNF-BSA 1.8:1 and 5.3:1, respectively.

2.5 ELISAs

Flat-bottomed, 96-well microtiter plates of medium binding (Nunc, Denmark) were coated with neoglycoconjugates at different concentrations in 0.1M Na₂CO₃, pH 9.6 for 1 h at 37°C. After washing, a blocking step was performed by adding BSA 1% for 1h at 37°C. The plates were then washed four times with (PBS), pH 7.2 containing 0.05% (v/v) Tween 20 (PBS/Tween). Serum samples were diluted at 1:10 in 1% BSA (Sigma, St.Louis, MO) in PBS/Tween and added to the wells. After incubation of 1 h at 37°C, the plates were washed and incubated with horseradish peroxidase (HRP)-conjugated to anti-human total-Ig (Sigma) in 4% BSA/PBS/Tween for 1 h at 37°C. The plates were washed again and the substrate containing H₂O₂ (0.05%) and 5-amino-2-hydroxybenzoic acid was added and incubated for 1 h at 22°C. Afterwards, the optical densities (OD) were measured at 450 nm. A cut-off value was calculated as the average OD value from sera (diluted 1:10) from 10 healthy blood donors plus threefold the standard deviation. The OD of the tested serum was divided by the OD of the cut-off value to obtain a ratio. Results from the ELISA were considered positive (+) when the ratio was higher or equal to 1, and it was considered negative (-) when it was less than 1. The procedures for the *Trichinella* ES-ELISA and the *Trichinella* ES-immunoblot have been described previously (Pinelli et al., 2001).

3. Results

3.1 Screening for glycan recognition by antibodies in sera from parasite infected individuals

To identify specific glycan antigens with potential use for serodiagnosis of trichinellosis, the sera of 5 *Trichinella* positive individuals were screened for antibodies recognizing specific oligosaccharides within a large library of glycan antigens, using the glycan-array facility of the CFG (<http://www.functionalglycomics.org>) (Blixt et al., 2004). To enable selection of glycan antigens with specificity for serodiagnosis of trichinellosis, we additionally screened

sera from 5 individuals serologically positive for echinococcosis and 5 for leishmaniasis as well as the sera from 2 healthy blood donors. Figure 1A shows the glycan-array profiles of 2 of these serum samples. IgG in sera of infected individuals and healthy blood donors recognized many different glycan antigens, but the types of glycans recognized differed between the infected versus uninfected individuals. To assess the specificity of glycan recognition in the sera of *Trichinella* positive individuals, we identified all glycan antigens that showed a high (> 10,000 RFU, relative fluorescence units) response in four out of five infection sera, and compared these values to the RFU's of all other sera to the same glycan antigens. This analysis showed that the sera of *Trichinella* positive individuals contain a relatively high level of serum IgG recognizing the glycan antigen LDNF (CFG #91), whereas sera from individuals positive for echinococcosis and leishmaniasis showed a low binding to this glycan antigen (Fig. 1B). Several of the mannose-containing glycan antigens also showed a differential recognition pattern with the sera used in this study (Fig. 1B). Multiple glycan antigens were inconsistently, or not recognized by any of the sera used here, such as the glycan antigen Gal β 1-4(Fuc α 1-3)GlcNAc-R (Le^X, CFG #135) (Fig. 1B). In addition, we observed that several glycan antigens, for example CFG #156, strongly bound to antibodies in all sera, possibly representing a common glycan antigen.

3.2. Detection of LDNF in ES and crude larval antigen

The data from the glycan microarray showed that antibodies in sera from *Trichinella* infected patients recognized glycans containing LDNF, but not other glycan antigens such as Le^X. To evaluate the presence of LDNF glycans on *T. spiralis* ES and/or muscle larvae crude antigen, an ELISA was performed using specific anti-glycan monoclonal antibodies. Anti-LDNF and anti-LDN antibodies recognized antigens in both *T. spiralis* ES and crude larval antigen (Figure 2), indicating the presence of these glycan antigens within *T. spiralis*. By contrast, the monoclonal antibody recognizing Le^X, a dominant glycan antigen within schistosomes (Srivatsan et al., 1992), did not recognize the *T. spiralis* antigens, whereas it recognized neoglycoconjugates carrying Le^X (not shown) suggesting the lack of this glycan in *T. spiralis*.

3.3. Establishment of a sensitive ELISA method using LDNF-neoglycoconjugates

To design a suitable LDNF-neoglycoconjugate for serodiagnosis of trichinellosis, we first determined whether the number of LDNF in the neoglycoconjugate would affect the sensitivity of the total Ig-ELISA by using sera from confirmed *Trichinella*-infected patients. LDNF glycan antigens were coupled to BSA at two different densities, resulting in BSA preparations carrying an average of 2 and 5 LDNF antigens per BSA molecule, LDNF2 and LDNF5, respectively. BSA and neoglycoconjugates (BSA-chitotriose (Chi), BSA-LDN (LDN) and BSA-LDNF (LDNF2 and LDNF5)) were used at concentrations of 5 and 10 μ g/ml. The *Trichinella*-seropositive sera reacted strongly to LDNF5 coated at 5 and 10 μ g/ml, whereas binding to LDNF2 was clearly lower (Figure 3). By contrast, binding of the sera to neoglycoconjugates carrying control glycans (LDN, the precursor glycan of LDNF lacking the fucose moiety, and chitotriose) were below the cut-off value. LDNF5 at a working concentration of 5 μ g/ml was chosen to determine the sensitivity and specificity of this antigen for serodiagnosis of trichinellosis.

3.4. Sensitivity and specificity of the LDNF5 -ELISA

To determine the sensitivity of the LDNF5-ELISA, 29 serum samples from *Trichinella* positive patients were used. *Trichinella* infection was confirmed in these patients by positive biopsy in addition to conventional sero-diagnosis using *Trichinella* ES-ELISA and *Trichinella* ES-Immunoblot analysis. Results indicate that from the 29 *Trichinella*-positive sera tested, 28 sera were positive in the LDNF5-ELISA (Figure 4) indicating that the sensitivity of the LDNF5-ELISA is 96%.

To determine the specificity of the LDNF5-ELISA, sera from individuals that were established seropositive for ascariasis, leishmaniasis, toxocariasis, echinococcosis, cysticercosis, borreliosis, amoebiasis, schistosomiasis, strongyloidiasis, toxoplasmosis and syphilis were used. We determined the number of samples that reacted positively and negatively in the LDNF5-ELISA (Table 1). From the 110 sera tested, 74 were found negative in the LDNF5-ELISA indicating a specificity of 67%. A positive reaction was mainly found in sera from individuals with cysticercosis and strongyloidiasis.

4. Discussion

The use of glycan antigens in serodiagnosis of helminth infections is an attractive approach and has been successfully applied for the detection of schistosomiasis, even in a field-applicable form as a dipstick (Van Dam et al., 2004; Midzi et al., 2009). In addition to schistosomes, many other helminths express glycan antigens on proteins and lipids at the surface of larvae, adult worms or in the ES products, which in principle could be applied in serodiagnostic approaches. To evaluate the potential of a glycan microarray approach to select for glycan antigens that could be used for serodiagnosis of parasitic infections we focussed on serodiagnosis of *T. spiralis*. Structural analysis of *T. spiralis* glycans showed the presence of unique tri- and tetraantennary N-glycans composed of LDNF (GalNAc β 1-4[Fuc α 1-3]GlcNAc-) antennae capped with β 3-linked tyvelose moieties (Appleton and Romaris, 2001; Van Die and Cummings, 2010). An ELISA based on glycoconjugates containing terminal β -tyvelose-GalNAc-R for serodiagnosis of human trichinellosis showed a sensitivity ranging from 93 to 100 % (Bruschi et al., 2001; Owen et al., 2001; Escalante et al., 2004). However, the use of the β -tyvelose is limited since it is not commercially available and its synthesis is complex. The present study aimed at identifying additional glycan antigens recognized by antibodies from *Trichinella*-infected individuals that could replace the use of ES antigen in the serodiagnosis of trichinellosis.

To select for potential antigenic glycan antigens that are recognized by *Trichinella* infection sera, but not by sera of individuals infected with other parasites, we initially screened a glycan array containing over 250 different glycan antigens with a limited number of positive sera from trichinellosis, leishmaniasis and echinococcosis patients. We found that all *Trichinella*-positive sera recognized the LDNF glycan, in contrast to sera from individuals infected with other parasites or from healthy blood donors. To enable detection of anti-LDNF antibodies in sera of infected individuals we developed an LDNF-ELISA using a neoglycoconjugate, consisting of BSA containing LDNF antigen. The synthesis of this compound was recently described (Tefsen et al., 2009). We showed that the number of LDNF molecules bound to the BSA is crucial for its recognition by antibodies from *Trichinella*-infected individuals. The coupling of an average of 5 LDNF moieties per BSA molecule resulted in a neoglycoconjugate which use in the ELISA resulted in a highly sensitive (96%) assay for the serodiagnosis of trichinellosis.

The specificity of the LDNF5 ELISA however was low (67%) which is not surprising since LDNF is present in different helminths. The presence of LDNF has been demonstrated in *Schistosoma mansoni* (not endemic in Europe), *Dirofilaria immitis* (heartworm of dogs), *Haemonchus contortus* (nematode of ruminants) and *Fasciola hepatica* (endemic in sheep in the Netherlands) (Van Die and Cummings, 2010). In the LDNF5-ELISA, several sera from individuals with cysticercosis and strongyloidiasis showed significant binding, suggesting the presence of LDNF moieties in these helminth species. Surprisingly, only 3 out of 10 sera from established schistosome infections were found positive in the LDNF5-ELISA. LDNF moieties are abundantly observed in different stages of schistosomes (Nyame et al., 2000; Nyame et al., 2003) and the presence of anti-LDNF IgG and IgM has been demonstrated in schistosome infected humans, chimpanzees and mice (Van Remoortere et al., 2003; Nyame

et al., 2003). Whether factors such as the stage of *Schistosoma* infection, the parasite species and load may influence the results here reported, needs to be further investigated.

In this study, we show that the LDNF antigen is recognized by antibodies in sera from individuals infected with *T. spiralis*, and that LDNF5 is a suitable candidate to replace the ES antigen in a first screening for diagnosis of trichinellosis. Increasing the number of LDNF may lead to a further improvement of the sensitivity and/or specificity of the assay. The advantage of using LDNF neoglycoconjugates in a first screening is that enzymatic synthesis of this conjugate is a relatively easy and fast procedure that can be performed in every biochemical research facility.

Our data show that glycan microarrays constitute a promising technology for fast and specific analysis of anti-glycan antibodies in sera from individuals infected with different pathogens. It should be emphasised that the glycan microarray used in this study contains many glycan structures, but the microarray was not designed to present parasite-specific glycan structures. In serological assays which are based on the use of natural antigens, cross-reactivity due to the presence of common glycan antigens is often observed (Aalberse et al., 1981; Wilson and Altmann, 1998; Van Die et al., 1999). To improve the serodiagnosis of parasitic infections, the development of parasite-specific glycan microarrays would be an attractive approach. In such arrays the natural glycans of the parasites are isolated, fractionated and printed as glycan arrays, thus facilitating the identification of glycan antigens specific for one, or a restricted group of parasites by a shotgun glycomics approach (Song et al., 2011). Such an approach is expected to result in the identification of glycan antigens which would allow both a sensitive and specific serodiagnosis of infections by a wide variety of different parasites.

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References

- Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and *Hymenoptera* venom. *The Journal of Allergy and Clinical Immunology*. 1981; 68:356–364. [PubMed: 7298999]
- Akkoc N, Kuruuzum Z, Akar S, Yuce A, Onen F, Yapar N, Ozgenc O, Turk M, Ozdemir D, Avci M, Guruz Y, Oral AM, Pozio E. A large-scale outbreak of trichinellosis caused by *Trichinella britovi* in Turkey. *Zoonoses and Public Health*. 2009; 56:65–70. [PubMed: 18705658]
- Appleton JA, Romaris F. A pivotal role for glycans at the interface between *Trichinella spiralis* and its host. *Veterinary Parasitology*. 2001; 101:249–260. [PubMed: 11707300]
- Blixt O, Head S, Mondala T, Scanlan C, Huflejt ME, Alvarez R, Bryan MC, Fazio F, Calarese D, Stevens J, Razi N, Stevens DJ, Skehel JJ, Van Die I, Burton DR, Wilson IA, Cummings R, Bovin N, Wong CH, Paulson JC. Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. *Proceedings of the National Academy of Sciences U S A*. 2004; 101:17033–17038.
- Blixt O, Hoffmann J, Svenson S, Norberg T. Pathogen specific carbohydrate antigen microarrays: a chip for detection of Salmonella O-antigen specific antibodies. *Glycoconjugate Journal*. 2008; 25:27–36. [PubMed: 17558551]

- Bolas-Fernandez F, Dea-Ayuela MA, Connolly B, Robinson MW. Micro-environmental conditions modulate protein secretion and infectivity of the *Trichinella spiralis* L1 larva. *Veterinary Parasitology*. 2009; 159:236–239. [PubMed: 19046809]
- Bruschi F, Moretti A, Wassom D, Piergili FD. The use of a synthetic antigen for the serological diagnosis of human trichinellosis. *Parasite*. 2001; 8:S141–S143. [PubMed: 11484339]
- De Boer AR, Hokke CH, Deelder AM, Wuhrer M. Serum antibody screening by surface plasmon resonance using a natural glycan microarray. *Glycoconjugate Journal*. 2008; 25:75–84. [PubMed: 18193481]
- DeBose-Boyd RA, Nyame AK, Cummings RD. Molecular cloning and characterization of an alpha1,3 fucosyltransferase, CEFT-1, from *Caenorhabditis elegans*. *Glycobiology*. 1998; 8:905–917. [PubMed: 9675224]
- Despommier, D.; Gwadz, RG.; Hotez, P.; Knirsch, C. Parasitic diseases. Apple Trees Productions L.L.C; New York: 2005. *Trichinella spiralis*; p. 135-142.
- Eberl M, Langermans JA, Vervenne RA, Nyame AK, Cummings RD, Thomas AW, Coulson PS, Wilson RA. Antibodies to glycans dominate the host response to schistosome larvae and eggs: is their role protective or subversive? *The Journal of Infectious Diseases*. 2001; 183:1238–1247. [PubMed: 11262206]
- Escalante M, Romaris F, Rodriguez M, Rodriguez E, Leiro J, Garate MT, Ubeira FM. Evaluation of *Trichinella spiralis* larva group 1 antigens for serodiagnosis of human trichinellosis. *Journal of Clinical Microbiology*. 2004; 42:4060–4066. [PubMed: 15364990]
- Gamble HR. *Trichinella spiralis*: immunization of mice using monoclonal antibody affinity-isolated antigens. *Experimental Parasitology*. 1985; 59:398–404. [PubMed: 2581801]
- Gillespie, HS. Diagnosis of parasitic infections. In: Cox, FEG.; Wakelin, D.; Gillespie, HS.; Despommier, DD., editors. *Parasitology*. Wiley; 2007. p. 148-156.
- Gottstein B, Pozio E, Nockler K. Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Reviews*. 2009; 22:127–145. [PubMed: 19136437]
- Just F, de Vries T, Knegt RM, Macher BA. Mutation of amino acids in the alpha 1,3-fucosyltransferase motif affects enzyme activity and Km for donor and acceptor substrates. *Glycobiology*. 2005; 15:165–175. [PubMed: 15456734]
- Kawar ZS, Van Die I, Cummings RD. Molecular cloning and enzymatic characterization of a UDP-GalNAc:GlcNAc(beta)-R beta1,4-N-acetylgalactosaminyltransferase from *Caenorhabditis elegans*. *Journal of Biological Chemistry*. 2002; 277:34924–34932. [PubMed: 12167666]
- Lonardi E, Balog CI, Deelder AM, Wuhrer M. Natural glycan microarrays. *Expert Review of Proteomics*. 2010; 7:761–774. [PubMed: 20973647]
- Midzi N, Butterworth AE, Mduluzi T, Munyati S, Deelder AM, Van Dam GJ. Use of circulating cathodic antigen strips for the diagnosis of urinary schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2009; 103:45–51. [PubMed: 18951599]
- Nyame AK, Leppanen AM, Bogitsh BJ, Cummings RD. Antibody responses to the fucosylated LacdiNAc glycan antigen in *Schistosoma mansoni*-infected mice and expression of the glycan among schistosomes. *Experimental Parasitology*. 2000; 96:202–212. [PubMed: 11162373]
- Nyame AK, Leppanen AM, DeBose-Boyd R, Cummings RD. Mice infected with *Schistosoma mansoni* generate antibodies to LacdiNAc (GalNAc beta 1-4GlcNAc) determinants. *Glycobiology*. 1999; 9:1029–1035. [PubMed: 10521539]
- Nyame AK, Lewis FA, Doughty BL, Correa-Oliveira R, Cummings RD. Immunity to schistosomiasis: glycans are potential antigenic targets for immune intervention. *Experimental Parasitology*. 2003; 104:1–13. [PubMed: 12932753]
- Owen IL, Pozio E, Tamburrini A, Danaya RT, Bruschi F, Gomez Morales MA. Focus of human trichinellosis in Papua New Guinea. *The American Journal of Tropical Medicine and Hygiene*. 2001; 65:553–557. [PubMed: 11716113]
- Palcic MM, Heerze LD, Pierce M, Hindsgaul O. The Use of Hydrophobic Synthetic Glycosides as Acceptors in Glycosyltransferase Assays. *Glycoconjugate Journal*. 1988; 5:49–53.
- Pinelli E, Van Der Lugt G, Homan W, Van Der Giessen J, Kortbeek LM. Antigen recognition by IgG4 antibodies in human trichinellosis. *Parasite*. 2001; 8:S168–S171. [PubMed: 11484346]

- Schabussova I, Amer H, Van Die I, Kosma P, Maizels RM. O-methylated glycans from *Toxocara* are specific targets for antibody binding in human and animal infections. *International Journal for Parasitology*. 2007; 37:97–109. [PubMed: 17087964]
- Song X, Lasanajak Y, Xia B, Heimbürg-Molinari J, Rhea JM, Ju H, Zhao C, Molinari RJ, Cummings RD, Smith DF. Shotgun glycomics: a microarray strategy for functional glycomics. *Nature Methods*. 2011; 8:85–90. [PubMed: 21131969]
- Song X, Lasanajak Y, Xia B, Smith DF, Cummings RD. Fluorescent glycosylamides produced by microscale derivatization of free glycans for natural glycan microarrays. *ACS Chemical Biology*. 2009; 4:741–750. [PubMed: 19618966]
- Srivatsan J, Smith DF, Cummings RD. The human blood fluke *Schistosoma mansoni* synthesizes glycoproteins containing the Lewis X antigen. *Journal of Biological Chemistry*. 1992; 267:20196–20203. [PubMed: 1356976]
- Tefsen B, Van Stijn CM, Van den Broek M, Kalay H, Knol JC, Jimenez CR, Van Die I. Chemoenzymatic synthesis of multivalent neoglycoconjugates carrying the helminth glycan antigen LDNF. *Carbohydrate Research*. 2009; 344:1501–1507. [PubMed: 19541294]
- Van Dam GJ, Wichers JH, Ferreira TM, Ghati D, Van Amerongen A, Deelder AM. Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *Journal of Clinical Microbiology*. 2004; 42:5458–5461. [PubMed: 15583265]
- Van Remoortere A, Van Dam GJ, Hokke CH, Van Den Eijnden DH, Van Die I, Deelder AM. Profiles of immunoglobulin M (IgM) and IgG antibodies against defined carbohydrate epitopes in sera of *Schistosoma*-infected individuals determined by surface plasmon resonance. *Infection and Immunity*. 2001; 69:2396–2401. [PubMed: 11254599]
- Van Remoortere A, Vermeer HJ, Van Roon AM, Langermans JA, Thomas AW, Wilson RA, Van Die I, Van Den Eijnden DH, Agoston K, Kerekgyarto J, Vliegenthart JF, Kamerling JP, Van Dam GJ, Hokke CH, Deelder AM. Dominant antibody responses to Fucal α 1-3GalNAc and Fucal α 1-2Fucal α 1-3GlcNAc containing carbohydrate epitopes in Pan troglodytes vaccinated and infected with *Schistosoma mansoni*. *Experimental Parasitology*. 2003; 105:219–225. [PubMed: 14990315]
- Van Die I, Cummings RD. Glycan gimmickry by parasitic helminths: a strategy for modulating the host immune response? *Glycobiology*. 2010; 20:2–12. [PubMed: 19748975]
- Van Die I, Gomord V, Kooyman FN, Van Den Berg TK, Cummings RD, Vervelde L. Core α 1-->3-fucose is a common modification of N-glycans in parasitic helminths and constitutes an important epitope for IgE from *Haemonchus contortus* infected sheep. *FEBS Letters*. 1999; 463:189–193. [PubMed: 10601665]
- Wilson IB, Altmann F. Structural analysis of N-glycans from allergenic grass, ragweed and tree pollens: core α 1,3-linked fucose and xylose present in all pollens examined. *Glycoconjugate Journal*. 1998; 15:1055–1070. [PubMed: 10386890]

Figure 1.

Glycan array analysis of anti-glycan antibodies in the sera of parasite infected individuals. Sera from individuals seropositive for trichinellosis (T, n=5), leishmaniasis (L, n=5) or echinococcus (E, n=5), as well as from healthy blood donors (HC, n=2) were 1:100 diluted and analyzed for the presence of anti-glycan IgG antibodies using printed array Version 2.1 of the Consortium for Functional Glycomics.

(A) Examples are shown of an array from an individual seropositive for trichinellosis and from a healthy blood donor. RFU=Relative Fluorescence Units. The RFU for the LDNF glycan (#91) is indicated in both arrays.

(B) Scatter plot of the RFU values for 5 selected glycan antigens, of all 17 sera analyzed. Three glycan antigens were selected that showed differential recognition by sera from parasite-infected individuals and healthy controls (#91, LDNF antigen, GalNAc β 1-4(Fuca1-3)GlcNAc-R; #189, Man α 1-2Man α 1-2Man α 1-3Man-R; #191, Man α 1-2Man α 1-3Man-R). In addition, one glycan antigen was selected that showed a very low binding, and one that showed high binding, respectively (#135, Le^X antigen, Gal β 1-4(Fuca1-3)GlcNAc-R; #156, GlcNAc α 1-3Gal β 1-4GlcNAc-R). CFG monosaccharide symbols used are: green circle: Man; black square, GlcNAc; yellow square, GalNAc; yellow circle, Gal; red triangle, Fuc.

Figure 2. Recognition of glycan antigens in *Trichinella* by specific monoclonal antibodies in ELISA. Anti-Le^x (IgM, Calbiochem), anti-LDN (mAb SMLDN1.1) (Nyame et al., 1999) and anti-LDNF (mAb SMLDNF) (Nyame et al., 2000) antibodies were used for glycan recognition on crude antigen from *T. spiralis* muscle larvae (ML) and excretory/secretory (ES) antigen.

Figure 3.

Analysis of the binding of anti-glycan Ig from serum of individuals seropositive for trichinellosis to different neoglycoconjugates in ELISA. BSA and neoglycoconjugates [BSA-chitotriose (Chi), BSA-LDN (LDN) and BSA-LDNF (LDNF2 and LDNF5)] were coated at concentrations of 5 (white bars) and 10 µg/ml (grey bars). Bars represent the mean binding of five *Trichinella*-positive sera tested. Ratio values equal or higher than 1 (line) are considered positive.

Figure 4.

Plot of the ratio distribution of the *Trichinella*-positive serum samples tested by the ES-ELISA and LDNF5-ELISA for the detection of total Ig. Ratio values equal or higher than 1 (line) are considered positive

TABLE 1

Reactivity of serum samples from patients with different parasitic and bacterial infections in the LDNF5-ELISA. For each infection 10 serum samples (seropositive for the infections indicated) were tested. The numbers indicate the amount of serum samples that showed either a negative or positive result in the LDNF5-ELISA.

Infections	Negative	Positive
Ascariasis	6	4
Leishmaniasis	6	4
Toxocariasis	7	3
Toxoplasmosis	6	4
Echinococcosis	8	2
Cysticercosis	3	7
Amoebiasis	9	1
Schistosomiasis	7	3
Strongyloidiasis	5	5
Borreliosis	7	3
Syphilis	10	0
Total	74	36