Core alpha 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

Core α1→3-fucose is a common modification of N-glycans in parasitic helminths and constitutes an important epitope for IgE from *Haemonchus contortus* infected sheep

Irma van Die[a,*], Veronique Gomord[b], Frans N.J. Kooyman[c], Timo K. van den Berg[d], Richard D. Cummings[e], Lonneke Vervelde[c]

[a] *Department of Medical Chemistry, Research Institute Immunology and Inflammatory diseases, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands*
[b] *European Institute for Peptide Research, LTI-CNRS URA 203, University of Rouen, 76821 Mont Saint Aignan, France*
[c] *Department of Parasitology and Tropical Veterinary Medicine, Institute of Infectious Diseases and Immunology, Utrecht University, P.O. Box 80.165, 3508 TD Utrecht, The Netherlands*
[d] *Department of Cell Biology and Immunology, Research Institute Immunology and Inflammatory diseases, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands*
[e] *Department of Biochemistry and Molecular Biology, University of Oklahoma HSC, Oklahoma City, OK 73190, USA*

Received 21 September 1999

Edited by Hans-Dieter Klenk

Abstract Synthesis of parasite specific IgE plays a critical role in the defence against helminth infections. We report here that IgE from serum from *Schistosoma mansoni* infected mice and *Haemonchus contortus* infected sheep recognizes complex-type N-glycans from *Arabidopsis thaliana*, which contain R-GlcNAcβ1→4(Fucα1→3)GlcNAcβ1-Asn (core α1→3-Fuc) and Xylβ1→2Manβ1→4GlcNAcβ1-R (core β1→2-Xyl) modifications, and honeybee phospholipase A2, which carries N-glycans that contain the core α1→3-Fuc epitope. Evidence is presented that core α1→3-fucosylated N-glycans bind a substantial part of the parasite specific IgE in serum of *H. contortus* infected sheep. These results suggest that the core α1→3-Fuc antigen may contribute to induction of a Th2 response leading to the production of IgE. In addition we show here that N-glycans carrying core α1→3-Fuc and β1→2-Xyl antigens are synthesized by many parasitic helminths and also by the free living nematode *Caenorhabditis elegans*. Since N-glycans containing the core α1→3-Fuc have also been implicated in honeybee and plant induced allergies, this conserved glycan might represent an important common IgE epitope.

Key words: Schistosome; Fucose; Xylose; Glycoprotein allergen; IgE; *Haemonchus contortus*

1. Introduction

Parasitic diseases caused by helminths afflict billions of people worldwide and are among the main causes of morbidity and mortality resulting from infectious disease. Such diseases in animals also have major economic consequences. Recent information about the basic biochemistry and the immunological responses of infected hosts to parasitic helminths and their eggs has led to increased interest in parasite glycoconjugates, since they are the major focus of the immune response (for reviews, see [1–4]). However, only a few helminth carbohydrate structures have sofar been structurally characterized.

A typical feature of helminth infections is the induction of specific IgE [5,6] and this is the result of a T-helper-2 (Th2) response. In the presence of antigen this IgE triggers the activation and proliferation of mast cells and eosinophils (i.e. type I hypersensitivity reactions). The exact implications of these IgE responses, however, are still unclear. Several studies have shown that high parasite specific IgE levels are associated with resistance to reinfection, suggesting a protective role for IgE [6–9]. However, very little is known about the structures of the parasite antigens that induce these IgE responses.

N-glycans carrying a 'core α1→3-Fuc' (R-GlcNAcβ1→4(Fucα1→3)GlcNAcβ1-Asn) or a 'core β1→2-Xyl' (Xylβ1→2Manβ1→4GlcNAcβ1-R) are found on many plant, insect and mollusc, but not mammalian, glycoproteins [10–14], and can contribute to the allergenicity and IgE cross-reactivity between extracts of these organisms [15–17]. Recently it has been reported that the ruminant nematode *Haemonchus contortus* expresses glycoproteins carrying core α1→3-Fuc residues [18], and that both core α1→3-Fuc and β1→2-Xyl epitopes have been observed on egg glycoproteins of the human schistosomes, *Schistosoma mansoni* and *Schistosoma japonicum* [19]. Thus, core α1→3-Fuc and β1→2-Xyl epitopes might contribute to the IgE response observed in helminth infections. To identify the possible presence of such epitopes in other helminths, we have analyzed the glycoproteins of different parasitic helminths, and also of the free living nematode *Caenorhabditis elegans* in Western blots using immunopurified core α1→3-Fuc specific and β1→2-Xyl specific antibodies [20]. The results show that core α1→3-fucosylation and β1→2-xylosylation are common glycan modifications occurring in many different helminths. In addition, we report that glycans carrying core α1→3-Fuc are recognized by IgE antibodies from sheep infected with *H. contortus* and mice infected with *S. mansoni*.

2. Materials and methods

2.1. Antigen preparations and sera

Honeybee venom phospholipase A2 (PLA2) and cucurbita ascorbate oxidase were purchased from Sigma. Adult *H. contortus* were obtained post mortem from the abomasum of sheep experimentally

*Corresponding author. Fax: (31) (20) 4448144.
E-mail: im.van_die.medchem@med.vu.nl

PII: S0014-5793(99)01508-2

infected with 20 000 L3 larvae. *H. contortus* excretory secretory (ES) products were obtained as described before [21]. Sheep antisera were collected 28 days after infection from two sheep, experimentally infected with the *H. contortus* larvae. The preparation of extracts of adult *Dirofilaria immitis*, *Hymenolepis diminuta*, *Fasciola hepatica*, *S. mansoni*, *S. japonicum* and *Schistosoma haematobium* was described previously [22]. Extracts of *C. elegans* were generated by resuspending the worm pellets in SDS-PAGE buffer, followed by incubation for 10 min at 100°C. Insoluble material was removed by centrifugation. *Trichinella spiralis*, *Toxocara canis* and cercariae of *Trichobilharzia ocellata* were resuspended in 100 μl PBS including the protease inhibitors Pefabloc (1 mg/ml), EDTA (0,5 mg/ml), leupeptin (10 μg/ml), pepstatin (10 μg/ml), aprotinin (1 μg/ml) (Boehringer Mannheim), and homogenized with a polytron. Triton X-100 (1%) was added, and the mixture incubated on ice for 20 min. After addition of SDS-PAGE buffer and incubation for 10 min at 100°C, insoluble material was removed by centrifugation.

2.2. Preparation of antisera

The core α1→3-Fuc and β1→2-Xyl specific antisera were prepared as described previously [20]. Briefly, antiserum raised against horseradish peroxidase was fractionated on an affinity column of honeybee PLA2, resulting in serum fractions specific for core α1→3-Fuc and β1→2-Xyl, respectively. Antisera were tested for their specificity as described.

2.3. SDS-PAGE and Western blotting

Helminth extracts (approximately 20–40 μg of protein) and controls were separated by SDS/PAGE on 15% gels using the Mini-Protean II system (BioRad). Western blotting and antibody reactions were performed essentially as described previously [23]. For detection of core α1→3-Fuc and β1→2-Xyl containing glycoproteins, rabbit polyclonal anti-core α1→3-Fuc or anti-β1→2-Xyl specific antibodies were used as the first antibody and goat anti-rabbit (IgG/IgM) peroxidase conjugate (TAGO, Inc. Immunodiagnostic reagents) as the second antibody. For detection of mouse IgE (Fig. 4) the blots were incubated overnight with 1:2 diluted mouse serum followed by incubation with rabbit anti-mouse IgE peroxidase conjugate (Nordic, the Netherlands) for 1 h at room temperature. Bound antibodies were visualized using 0.6 mg/ml chloronaphtol in TBS containing 0.03% H_2O_2.

2.4. ELISA

ELISA was performed as the ES specific ELISA described previously [24]. Plates were coated with *H. contortus* ES, PLA2, *Arabidopsis thaliana*, *A. thaliana cgl*, and human transferrin. Competitive glycoproteins were added to the sheep serum in a final concentration range of 80 to 0.026 μg/ml. Zero % inhibition was defined as the OD value of serum without competitive glycoproteins, and 100% inhibition as the OD value measured with 80 μg/ml autologous glycoproteins. The monosaccharides D-Glc, L-Fuc, D-Xyl or L-Xyl were added to the sheep serum in a final concentration range of 0.01 to 1.4 M.

3. Results

3.1. Specificity of the core α1→3-Fuc and β1→2-Xyl specific antisera

The antisera used in this study were raised against the plant glycoprotein horseradish peroxidase, which carries glycans shown in Fig. 1. Fractionation of this antiserum on an affinity column of honeybee venom phospholipase A2 (PLA2) resulted in serum fractions highly specific for either the core α1→3-Fuc, or β1→2-Xyl, respectively. To confirm their specificity purified antibodies were tested with many different plant and mammalian glycoproteins, containing either a core α1→3-Fuc or a β1→2-Xyl, or no core modification [20]. Furthermore, both antisera reacted strongly with glycoproteins isolated from the leaves of *A. thaliana* (Figs. 2, 4C and D), the N-glycans of which have been fully identified recently and shown to consist of high mannose-type N-glycans and complex-type core α1→3-fucosylated and β1→2-xylosylated N-glycans [25]. No other complex-type modifications have been demonstrated in *A. thaliana* [25]. The antisera did not bind to glycoproteins of the *cgl* mutant of *A. thaliana*, lacking *N*-acetylglucosaminyltransferase I, and thus lacking the expression of the core α3-fucosylated and β2-xylosylated N-glycans [26] (Figs. 2, 4C and D). Importantly, the results in Fig. 2 show that no cross-reactivity was observed of the core α1→3-Fuc antiserum with LPS from *Helicobacter pylori* strain O3, containing Galβ1→4(Fucα1→3)GlcNAc (Le^x, [27]), sialyl-Le^x containing α_1-acid glycoprotein (AGP) derived from patients suffering from rheumatoid arthritis [28], core α1→6-fucosylated human IgG, or neoglycoprotein containing GalNAcβ1→4(Fucα1→3)GlcNAc (LDNF) units, indicating that the core α1→3-Fuc specific antiserum does not recognize other Fucα1→3GlcNAc moieties found in the core or outer antennae of N- or O-linked glycans.

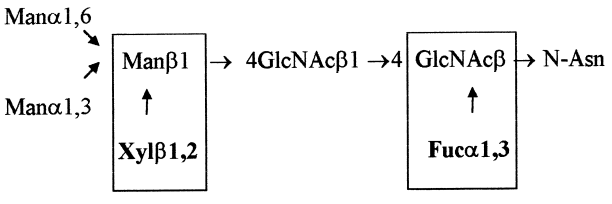

Fig. 1. Structure of a typical plant complex-type N-glycan. The β1→2-Xyl and α1→3-Fuc core antigens are boxed.

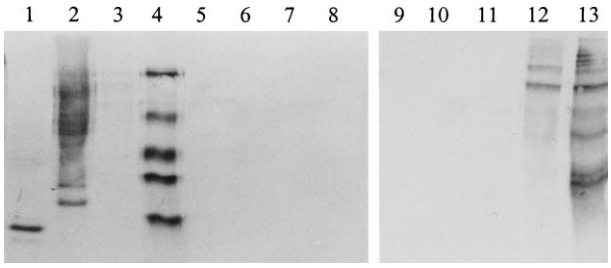

Fig. 2. Reactivity of core α1→3-fucose specific antiserum with different cell extracts and defined glycoproteins. Extracts and glycoproteins were separated by SDS-PAGE and the proteins transferred to nitrocellulose by Western blotting. The blots were incubated with affinity purified core α1→3-Fuc specific antibodies, followed by goat anti-rabbit IgG/IgM peroxidase conjugate. Samples tested were: (1) 0.3 μg honeybee phospholipase A2; (2) 10 μg *A. thaliana* extract; (3) 10 μg mutant *A. thaliana cgl* extract; (4) molecular weight marker (110/84 kDa, 47 kDa, 33 kDa, 24 kDa and 16 kDa); (5) 1 μg cucurbita ascorbate oxidase; (6) 2 μg human IgG; (7) 2 μg human transferrin; (8) 20 μg bovine mammary gland extract; (9) 2 μg BSA-LDNF; (10) 10 μg *H. pylori* O3 LPS; (11) 2 μg AGP from a pool of patients suffering from rheumatoid arthritis; (12) and (13) positive controls for the antiserum.

3.2. Core α1→3-Fuc and β1→2-Xyl residues on protein-linked glycans of different helminths

The presence of core α1→3-Fuc and β1→2-Xyl residues on protein-linked glycans of different helminths was determined by Western blotting, using the affinity purified core α1→3-Fuc and β1→2-Xyl specific antisera [20]. The extracts were derived from adult helminths, except the extract of *T. ocellata*, which was derived from cercariae. In addition to the adult stages, soluble antigens were tested from *H. contortus* (i.e. excretory/secretory (ES) antigens) and *S. mansoni* (soluble egg antigens (SE)). The extracts from most helminths tested contained many glycoproteins that reacted moderate to

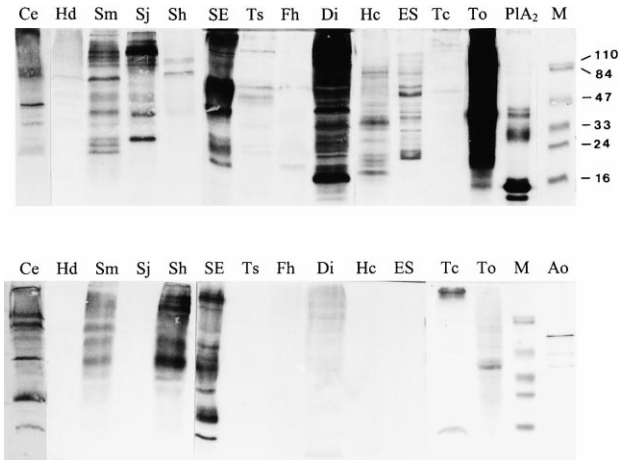

Fig. 3. Western blot analysis for glycoproteins carrying core α3-fucosylated or β1→2-xylosylated N-glycans in different helminths. Extracts of the helminths indicated (20–40 μg) were separated by SDS-PAGE and the presence of glycoproteins carrying core α1→3-Fuc (upper panel) and β1→2-Xyl (lower panel) was identified by immunoblotting with affinity purified polyclonal antisera specific for these core antigens. Extracts tested were derived from adult *C. elegans* (Ce), *H. diminuta* (Hd), *S. mansoni* (Sm), *S. japonicum* (Sj), *S. haematobium* (Sh), *F. hepatica* (Fh), *H. contortus* (Hc), *T. spiralis* (Ts), *T. canis* (Tc) and *D. immitis* (Di). A cercarial extract was used from *T. ocellata* (To). *S. mansoni* soluble egg antigen (SE, 7 μg) and *H. contortus* excretory secretory antigens (ES, 3 μg) were tested. Honeybee phospholipase A2 (PLA2, 1 μg) and ascorbate oxidase (Ao, 0.5 μg) were used as controls.

strongly with the core α1→3-Fuc specific antibodies, whereas reaction of *H. diminuta*, *F. hepatica* and *T. canis* glycoproteins was less abundant (Fig. 3, upper panel). The β1→2-Xyl specific antiserum showed binding to glycoproteins of some of the extracts, i.e. of *C. elegans*, *S. mansoni*, *S. haematobium*, *S. mansoni* SE antigens, *D. immitis*, *T. canis*, and of cercariae of *T. ocellata*, whereas no binding was observed with the other parasite derived samples (Fig. 3, lower panel).

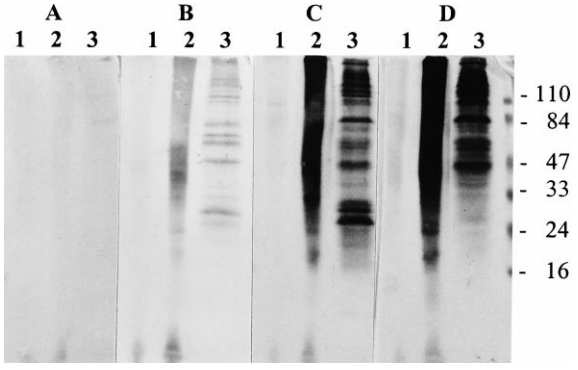

Fig. 4. Cross-reactivity of IgE antibodies from serum of *S. mansoni* infected mice with glycoproteins from *A. thaliana*. Extracts of (1) the *cgl* mutant of *A. thaliana* (10 μg), (2) wild-type *A. thaliana* (10 μg) and (3) *S. mansoni* (20 μg) were separated by SDS-PAGE. Blots were incubated with (A) mouse serum followed by anti-mouse IgE, (B) serum from *S. mansoni* infected mice followed by an anti-mouse IgE. Controls blots were treated with (C) core α1→3-Fuc specific antiserum and (D) β1→2-Xyl specific antiserum.

3.3. IgE in sera of S. mansoni infected mice cross-reacts with A. thaliana glycoproteins

We next sought to determine whether infection of mice with *S. mansoni* results in IgE to core α1→3-fucosylated and/or β1→2-xylosylated N-glycans. Western blots containing proteins of *S. mansoni*, *A. thaliana* [25] and the *cgl* mutant of *A. thaliana* lacking the core antigens [26], were analyzed using different antibodies. IgE from pooled sera of mice experimentally infected with *S. mansoni* binds to several *S. mansoni* proteins, in contrast to IgE from normal mouse serum that showed no reactivity. IgE from the infected mice serum cross-reacted with many proteins of *A. thaliana*, but not with the *cgl* mutant, indicating that the antibodies specifically recognize complex-type glycans carrying a core α1→3-Fuc or β1→2-Xyl residue (Fig. 4). We also could detect a weak binding of the IgE antibodies from infected mice with honeybee PLA2 (data not shown), suggesting that the core-linked α1→3-Fuc is an epitope for IgE of *S. mansoni* infected mice.

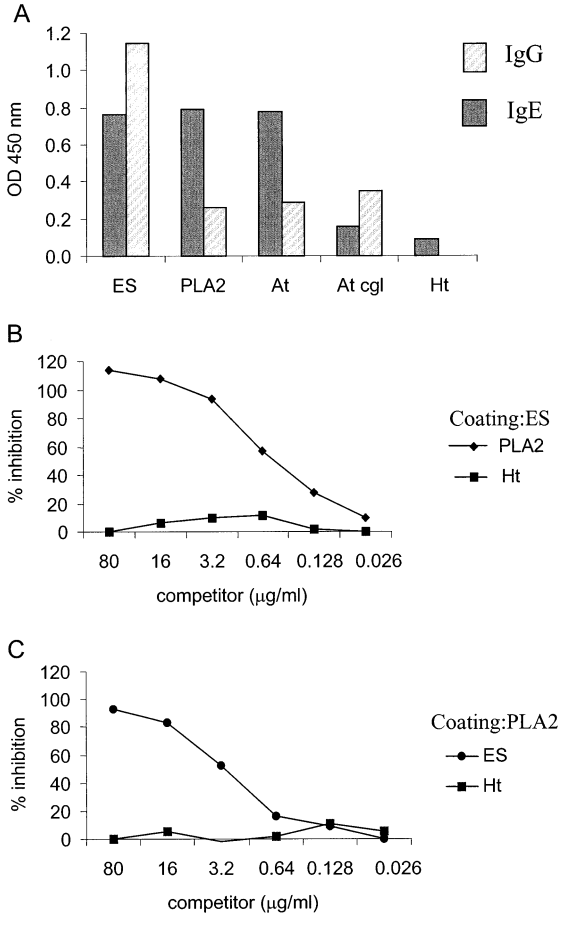

Fig. 5. Cross-reactivity of IgE antibodies from serum of *H. contortus* infected sheep with different glycoproteins measured with ELISA. (A) Serum IgE (solid bars) and IgG (hatched bars) response to *H. contortus* excretory secretory antigens (ES), honeybee phospholipase A2 (PLA2), *A. thaliana* extract (At), *A. thaliana cgl* mutant extract (At cgl) and human transferrin (Ht). (B) Competitive ELISA: plates were coated with *H. contortus* ES antigens, and PLA2 and Ht were used as competitive glycoproteins. (C) Competitive ELISA: plates were coated with PLA2, and *H. contortus* ES antigens and Ht were used as competitive glycoproteins.

3.4. IgE in sera from H. contortus infected sheep recognizes core α1→3-fucosylated N-glycans

Sera from sheep infected with *H. contortus* were analyzed by ELISA. The results in Fig. 5A show that IgE in serum of infected sheep recognizes *H. contortus* ES glycoproteins, honeybee PLA2 and *A. thaliana* glycoproteins, and did not recognize human transferrin or glycoproteins from the *cgl* mutant of *A. thaliana* lacking the core antigens. In contrast, binding of IgG antibodies from the infected sheep sera to *A. thaliana* and PLA2 proteins was hardly detectable, whereas IgG binding was observed to many *H. contortus* ES proteins (Fig. 5A, [29]). These results suggest that IgE from the sera of *H. contortus* infected sheep recognizes the core α1→3-Fuc epitope that occurs on both plant glycoproteins and honeybee PLA2. To further validate this possibility, inhibition experiments were performed (Fig. 5B, C). It was shown that PLA2 could block the binding of IgE from infected sheep to ES. Similarly, ES could block the binding of this IgE to PLA2, whereas human transferrin lacking the core fucose did not block the IgE binding. Binding of sheep IgE to *H. contortus* ES was inhibited to 80% by L-Fuc at a concentration of 1 M, whereas the same concentrations of D-Xyl, L-Xyl or D-Glc showed only 25% inhibition in the same experiment (data not shown). These results demonstrate that an important part of the ES specific IgE in the sera of *H. contortus* infected sheep recognizes core α1→3-fucosylated N-glycans.

4. Discussion

We report in this study that IgE from serum of *S. mansoni* infected mice and *H. contortus* infected sheep cross-reacts with *A. thaliana* glycoproteins that carry core α1→3-fucosylated and β1→2-xylosylated N-glycans. In contrast, IgE from these sera did not bind to glycoproteins of the *cgl* mutant of *A. thaliana*, that lack the core antigens and other complex-type glycan modifications [14,25,26]. These results indicate that the core α1→3-Fuc and/or the β1→2-Xyl residues are epitopes for both IgE from *S. mansoni* infected mice and *H. contortus* infected sheep. It has been reported that the human schistosome *S. mansoni* synthesizes glycoproteins carrying both core α1→3-Fuc and β1→2-Xyl residues [19], which was confirmed by our studies using the affinity purified α1→3-Fuc and β1→2-Xyl specific antibodies. Since these core antigens do not occur in mammals they may induce the generation of anti-glycan antibodies during schistosome infection. We cannot deduce from our data whether one or both of the core antigens in *A. thaliana* are involved in binding of IgE from *S. mansoni* infected mice. In contrast, the ruminant nematode *H. contortus* contains glycoproteins carrying core α1→3-fucosylated N-glycans but no evidence was presented for the presence of a β1→2-Xyl ([18], this study). This suggests that the core α1→3-Fuc is likely to be the *A. thaliana* epitope that reacted with IgE from *H. contortus* infected sheep. Evidence is presented using both ELISA and competitive ELISA that the core α1→3-Fuc residue indeed is a major epitope recognized by parasite specific IgE from serum of *H. contortus* infected sheep. Importantly, hardly any IgG reactivity to this core antigen was demonstrable in these sera. In contrast, IgM or IgG antibodies have been demonstrated in sera of parasite infected hosts to other parasite derived carbohydrate components ([30]; Van Remoortere et al., manuscript in preparation). This suggests that the core α1→3-Fuc epitope preferentially triggers a Th2 response leading to the production of IgE, although we cannot rule out the possibility that the core fucose specific IgE is a product rather than an inducer of a Th2 response. Interestingly, several T cell clones have been identified from bee venom sensitized subjects which proliferate in response to honeybee PLA2 but not to its non-glycosylated variants, providing evidence for the involvement of an N-glycan in T cell recognition [31]. It will be important to establish whether the core α-1→3-Fuc antigen is involved in these immunological processes.

Recently it has been demonstrated that vaccination of sheep with ES antigens of *H. contortus* induces protection against challenge infection and that this protection is correlated with IgE rather than with IgG [32]. It thus appears that the core α1→3-Fuc antigen may be an important protective antigen in *H. contortus* infections, and perhaps other helminth infections. To identify the possible presence of core-fucosylated and core-xylosylated epitopes in other helminths we analyzed different species. Our results show that many helminths from different orders of the phyla Platyhelminthes and Nematodes synthesize glycoproteins containing core α1→3-fucosylated N-glycans. In addition several of these organisms also synthesize N-glycans containing β1→2-Xyl. Remarkably, only small amounts of β1→2-Xyl were found on glycoproteins of adult *S. japonicum*, whereas the related *S. mansoni* and *S. haematobium* showed many β1→2-xylosylated glycoproteins. Recent data from Khoo et al. [19] described the presence of β1→2-xylosylated N-glycans in egg glycoproteins of *S. japonicum*. It may be possible that in *S. japonicum* β1→2-xylosylation is stage specific. Alternatively, the plant specific antiserum may be unable to bind some core β1→2-Xyl residues, due to masking of the epitope, e.g. by the presence of multiple core fucose residues [19] or other modifications.

Cross-reactivity has been observed for many years in immunoassays of plant, arthropod and mollusc extracts [33–35]. Part of this cross-reactivity is caused by conserved proteins, as profilins in plants [36], and tropomyosins in crustacea and mollusca [37]. In addition, cross-reactivity between several parasitic helminths, and between helminths and molluscs has been reported [38–40]. The highly antigenic core α1→3-Fuc and/or β1→2-Xyl in N-glycans, that are conserved among many invertebrates and plants, have been shown to contribute to such cross-reactions [35,41]. In some cases it has been established that IgE antibodies from patients allergic for honeybee venom or plant substances such as cereal flour proteins bind to core α1→3-Fuc and/or β1→2-Xyl containing N-glycans [15–17]. Our results indicate that the core α1→3-Fuc structure is commonly found on helminth glycoproteins and that IgE antibodies from helminth infected hosts bind to this glycan epitope. To our knowledge, this is the first description of an antigen that may be important in the induction of both helminth-mediated Th2-type immunity and in allergic reactions. Recognition of putative features common to helminths presumably informs the host immune system that a stereotypic type 2 cytokine response will be more protective than a type 1 or type 0. It has been hypothesized previously that some of these helminth features are shared with allergens and may be responsible for the obviously maladaptive responses made to non-threatening molecules as pollen antigens and bee venom phospholipase [42]. This stresses the importance of the conserved core α1→3-Fuc epitope as a potential 'pan allergen' [41]. Interestingly, several studies have shown an inverse

relationship between exposure to helminth infections and the incidence of allergies [43–45]. It is exciting to consider the possibility that detailed knowledge of the parasite structures involved in Th2 activation and IgE induction and a better understanding of the mechanism by which they evoke these immune responses may lead to novel therapies against both parasitic diseases and atopic disorders.

Acknowledgements: We gratefully acknowledge Ms. A. van Tetering for technical assistance in part of the work. We thank Dr. A.M. Deelder (Dept. of Parasitology, Leiden, The Netherlands) for serum from schistosome infected mice, Dr. H. Bakker (CPRO-DLO, Wageningen, The Netherlands) for extracts of *A. thaliana*, Dr. H. van Luenen (NKI, Amsterdam, The Netherlands) for *C. elegans*, Drs. A.K. Nyame (University of Oklahoma, USA), Y. Rombouts (RIVM, Bilthoven, The Netherlands), and M. de Jong-Brink (Dept. of Neurobiology, VU, Amsterdam, The Netherlands) for parasites and parasite extracts, Drs A. van Remoortere (Dept. of Medical Chemistry, VU, Amsterdam, The Netherlands) for gift of neoglycoprotein, Dr. B. Appelmelk (Dept. of Medical Microbiology, VU, Amsterdam, The Netherlands) for *H. pylori* LPS, and Dr. W. van Dijk (Dept. of Medical Chemistry, VU, Amsterdam, The Netherlands) for AGP. We thank Dr. P. Lerouge (CNRS, Rouen, France) for stimulating discussions and Dr. A.W. Cornelissen (Dept. of Parasitology, Utrecht, The Netherlands) for critical reading of the manuscript. L.V. was supported by a grant from The Technology Foundation (STW, The Netherlands; project: UDG55.3762). Collaboration between Drs. I. van Die and R. Cummings was facilitated by a NATO Collaborative Research Grant (CRG972098).

References

[1] Cummings, R.D. and Nyame, A.K. (1996) FASEB J. 10, 838–848.
[2] Van Dam, G.J. and Deelder, A.M. (1996) in: Glycoproteins and Disease (Montreuil, J., Vliegenthart, J.F.G. and Schachter, H., Eds.), pp. 159–182, Elsevier Science B.V., Amsterdam.
[3] Cummings, R.D. and Nyame, A.K. (1999) Biochim. Biophys. Acta, in press.
[4] Dell, A., Haslam, S.M., Morris, H.R. and Khoo, K.-H. (1999) Biochim. Biophys. Acta, in press.
[5] Jarrett, E.E.E. and Miller, H.R.P. (1982) Prog. Allergy 31, 178–233.
[6] Hagan, P. (1993) Parasite Immunol. 15, 1–4.
[7] Ahmad, A., Wang, C.H. and Bell, R.G. (1991) J. Immunol. 146, 3563–3570.
[8] Dunne, D.W., Butterworth, A.E., Fulford, A.J., Kariuki, H.C., Langley, J.G., Ouma, J.H., Capron, A., Pierce, R.J. and Sturrock, R.F. (1992) Eur. J. Immunol. 22, 1483–1494.
[9] King, C.L., Xianli, J., Malhotra, I., Liu, S., Mahmoud, A.A. and Oettgen, H.C. (1997) J. Immunol. 158, 294–300.
[10] Van Kuik, J., Hoffmann, R.A., Mutsaers, J.H.G.M., Van Halbeek, H., Kamerling, J.P. and Vliegenthart, J.F.G. (1986) Glycoconjug. J. 3, 27–34.
[11] D'Andrea, G., Bouwstra, J.B., Kamerling, J.P. and Vliegenthart, J.F.G. (1988) Glycoconjug. J. 5, 151–157.
[12] Kubelka, V., Altmann, F., Staudacher, E., Tretter, V., März, L., Hård, K., Kamerling, J.P. and Vliegenthart, J.F.G. (1993) Eur. J. Biochem. 213, 1193–1204.
[13] Van Kuik, J.A., Van Halbeek, H., Kamerling, J.P. and Vliegenthart, J.F. (1985) J. Biol. Chem. 260, 13984–13988.
[14] Lerouge, P., Cabanesmacheteau, M., Rayon, C., Fischettelaine, A.C., Gomord, V. and Faye, L. (1998) Plant Mol. Biol. 38, 31–48.
[15] Weber, A., Schröder, H., Thalberg, K. and März, L. (1987) Allergy 42, 464–470.
[16] Tretter, V., Altmann, F., Kubelka, V., März, L. and Becker, W.M. (1993) Int. Arch. Allergy Immunol. 102, 259–266.
[17] Garcia-Casado, G., Sanchezmonge, R., Chrispeels, M.J., Armentia, A., Salcedo, G. and Gomez, L. (1996) Glycobiology 6, 471–477.
[18] Haslam, S.M., Coles, G.C., Munn, E.A., Smith, T.S., Smith, H.F., Morris, H.R. and Dell, A. (1996) J. Biol. Chem. 271, 30561–30570.
[19] Khoo, K.-H., Chatterjee, D., Caulfield, J.P., Morris, H.R. and Dell, A. (1997) Glycobiology 7, 663–677.
[20] Faye, L., Gomord, V., Fitchette-Laine, A.C. and Chrispeels, M.J. (1993) Anal. Biochem. 209, 104–108.
[21] Schallig, H.D., Van Leeuwen, M.A. and Hendrikx, W.M. (1994) Parasitology 108, 351–357.
[22] Nyame, A.K., Debose-Boyd, R., Long, T.D., Tsang, V.C.W. and Cummings, R.D. (1998) Glycobiology 8, 615–624.
[23] Agterberg, M., Van Die, I., Yang, H., Andriessen, J.A., Van Tetering, A., Van den Eijnden, D.H. and Ploegh, H.L. (1993) Eur. J. Biochem. 217, 241–246.
[24] Kooyman, F.N.J., Van Kooten, P.J.S., Huntley, J.F., MacKellar, A., Cornelissen, A.W.C.A. and Schallig, H.D.F.H. (1997) Parasitology 114, 395–406.
[25] Rayon, C., Cabanesmacheteau, M., Loutelier-Bourhis, C., Salliot-Maire, I., Lemoine, J., Reiter, W.-D., Lerouge, P. and Faye, L. (1999) Plant Physiol. 119, 725–733.
[26] Von Schaewen, A., Sturm, A., O'Neill, J. and Chrispeels, M.J. (1993) Plant Physiol. 102, 1109–1118.
[27] Simoons-Smit, I.M., Appelmelk, B.J., Verboom, T., Negrini, R., Penner, J.L., Aspinall, G.O., Moran, A.P., Fei, S.F., Shi, B.S., Rudnica, W., Savio, A. and De Graaff, J. (1996) J. Clin. Microbiol. 34, 2196–2200.
[28] Havenaar, E.C., Dolhainm, R.J.E.M., Turner, G., Goodarzi, M.T., Van Ommen, E.C.R., Breedveld, F.C. and Van Dijk, W. (1997) Glycoconjug. J. 14, 457–465.
[29] Schallig, H.D. and Van Leeuwen, M.A. (1997) Parasitology 114, 293–299.
[30] Nyame, A.K., Leppanen, A.M., Debose-Boyd, R. and Cummings, R.D. (1999) Glycobiology, in press.
[31] Dudler, T., Altmann, F., Carballido, J.M. and Blaser, K. (1995) Eur. J. Immunol. 25, 538–542.
[32] Kooyman, F.N.J., Schallig, H.D.F.H., Van Leeuwen, M.A.W., MacKellar, A., Huntley, J.F., Cornelissen, A.W.C.A. and Vervelde, L. (1999) Parasite Immunol. 21, in press.
[33] Aalberse, R.C., Koshte, V. and Clemens, J.G.J. (1981) Int. Arch. Allergy Appl. Immunol. 66, 259–260.
[34] Faye, L. and Chrispeels, M.J. (1988) Glycoconjug. J. 5, 245–256.
[35] Aalberse, R.C. (1997) Allergy Immunol. 15, 375–387.
[36] Valenta, R., Duchene, M., Ebner, C., Valent, P., Sillaber, C., Deviller, P., Ferreira, F., Tejkl, M., Edelmann, H., Kraft, D. and Schneider, O. (1992) J. Exp. Med. 175, 377–385.
[37] Leung, P.S.C., Chow, W.K., Duffey, S., Kwan, H.S., Gershwin, M.E. and Chu, K.H. (1996) J. Allergy Clin. Immunol. 98, 954–961.
[38] Dissous, C., Grzych, J.M. and Capron, A. (1986) Nature 323, 443–445.
[39] Rasmussen, K.R., Hillyer, G.V. and Kemp, W.M. (1985) J. Parasitol. 71, 692.
[40] Thors, C. and Linder, E. (1998) Parasite Immunol. 20, 489–496.
[41] Wilson, I.B.H., Harthill, J.E., Mullin, N.P., Ashford, D.A. and Altmann, F. (1998) Glycobiology 8, 651–661.
[42] Finkelman, F.D., Shea-Donohue, T., Goldhil, J., Sullivan, C.A., Morris, S.C., Madden, K.B., Gause, W.C. and Urban, J.F.J. (1997) Annu. Rev. Immunol. 15, 505–533.
[43] Godfrey, R.C. (1975) Clin. Allergy 5, 201–207.
[44] Lynch, N.R., Hagel, I., Perez, M., DiPrisco, M.C., Lopez, R. and Alvarez, N. (1993) J. Allergy Clin. Immunol. 92, 404–411.
[45] Bell, R.G. (1996) Immunol. Cell Biol. 74, 337–345.