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ScienceDirect
Molecular Immunology 44 (2007) 1623–1630
Molecular Immunology
www.elsevier.com/locate/molimm

Induction of interferon-γ by *Taenia crassiceps* glycans and Lewis sugars in naive BALB/c spleen and peritoneal exudate cells

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Received 7 August 2006; accepted 11 August 2006
Available online 10 October 2006

Abstract

Helminth parasites are known to alter host immune responses and the responsible molecules are a potential source of biological immunoadjuvants. Previously, we have reported strong Th-2 type immunomodulatory properties of *Taenia crassiceps* glycans. In this study, we report interferon-γ (IFN-γ) stimulatory activity of fractionated *Taenia* glycans and Lewis sugars with comparable glycan composition. Our data show that *Taenia* glycans and Lewis X pentasaccharide are potent stimulators of the Th-1 type cytokine IFN-γ. We postulate that the terminal β-(1-4)-galactose residue in Lewis X is associated with IFN-γ stimulation from naive BALB/c mouse spleen and peritoneal exudate cells. Antibodies to toll-like receptors (TLRs) inhibited the Lewis X-induced IFN-γ secretion. Lewis X up-regulated the expression of NF-κB p65 from naive spleen cells and IFN-γ transcription in peritoneal exudate cells. These data demonstrate the ability of Lewis type helminth glycans to modulate host responses in a Th-1 direction via NF-κB p65, IFN-γ and macrophage TLRs.

Keywords: *Taenia crassiceps*; IFN-γ; NF-κB; Lewis X

1. Introduction

It is a generally accepted view that parasitic helminths modulate host immune responses, mostly in a Th-2 direction (Romagnani, 2004; Bach, 2005). Such immunomodulation may manifest as parasite-induced immune suppression and/or a shift in the balance between Th-1 and Th-2 type host immune environments, with either detrimental or beneficial effects to the host. In human and murine schistosome infections, host reactions to parasite-induced early Th-1 and late Th-2 responses appear to correlate with severity of hepatosplenic disease and granuloma development (Brunet et al., 1998, Herbert et al., 2004). Further, infection with schistosomes appear to protect against diabetes and multiple sclerosis (El-Wakil et al., 2002; LaFlamme et al., 2004). In experimental *Tryapanosoma cruzi* infections, parasite-induced immunosuppression is said to be strong enough to prevent experimental autoimmune encephalomyelitis (Tadokoro et al., 2004). *Wuchereria bancrofti* lymphatic filariasis is characterized by parasite-induced immunosuppression associated with an asymptomatic microfilaremic state (Dissanayake, 1989). Our long-term objective is to identify and develop immunomodulatory parasite molecules for immunotherapeutic purposes, as demonstrated by Mclnnes et al. (2003) who showed that the filarial nematode-derived phosphorylcholine-containing glycoprotein ES-62 could be used as a novel therapeutic agent against articular inflammation.

Lewis X (CD 15) family sugars are fucosylated carbohydrates, most commonly present on mammalian cell surface glycoproteins of leukocytes and tumor cells functioning as ligands to cell adhesion molecules (Polley et al., 1991; Kerr and Stocks, 1992; Bevilacqua and Nelson, 1993; Nelson et al., 1993). Parasitic helminths like *Schistosoma mansoni* and *Taenia crassiceps* also express Lewis X sugars and derivatives (Haslam et al., 2001, 2003; Lee et al., 2005; Nyame et al., 1998; Srivatsan et al., 1992). Although a systematic analysis has not been done, helminth Lewis X sugars appear to modulate host responses in a Th-2 direction (Okano et al., 1999, 2001; van Die et al., 1999, 2003; Van der Kleij et al., 2002; Thomas et al., 2003, 2005; Van Dam et al., 1994; van Remoortere et al., 2003; Atochina and Harn, 2005). Whether helminth Lewis sugars modulate host

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doi:10.1016/j.molimm.2006.08.019

responses in a Th-1 direction remains unknown. Definition of the glycan epitopes that drive host responses in a Th-1 or a Th-2 direction has much application in the development of biological immunoadjuvants for immunotherapy (Nyame et al., 2003, 2004).

We have previously reported Th-2 type immunomodulation by *T. crassiceps* glycans, acting via macrophage toll-like receptor-4 (TLR-4) (Dissanayake et al., 2002, 2004, 2005). By MALDI mass spectrometric analysis, we identified terminal glycan structures consisting of Fuc-α-(1-3)-GlcNAc and Gal-β-(1-4)-GlcNAc residues as dominant components of the *T. crassiceps* N-glycans complex (Lee et al., 2005). While the Fuc-α-(1-3)-GlcNAc structural moiety has been considered responsible for driving host immune responses toward a Th-2 type (Garcia-Casado et al., 1996; van Die et al., 1999; Faveeuw et al., 2002, 2003; Tretter et al., 1993; van Remoortere et al., 2003), the role of the Gal-β-(1-4)-GlcNAc residue has not been defined.

In attempts to further characterize the immunomodulatory properties of *T. crassiceps* glycans, particularly the terminal Gal-β-(1-4)-GlcNAc residues (Lee et al., 2005), and to determine whether these residues play a role in Th-1 immune responses, we investigated the cytokine inducing capacity of chromatographically fractionated *Taenia* glycans and homologous Gal-β-(1-4)-GlcNAc-containing natural and synthetic glycans of the Lewis family. Our studies show that certain *Taenia* glycans and Lewis X sugars are potent inducers of IFN-γ from naive BALB/c spleen cells and that the three-dimensional conformation of the terminal Gal-β-(1-4)-[Fuc-α-(1-3)]-GlcNAc is critical for IFN-γ induction. The effector cell recognition of this core structure was at least in part via the toll-like receptors (TLRs) 2, 4, 6 and the TLR homologue, RP105/CD180. The induction of IFN-γ by Lewis X paralleled the expression of NF-κB p65 in these cells. These data provide novel insights to Th-1 type immunomodulation by helminth parasite glycans, acting via IFN-γ and NF-κB p65 induction.

2. Materials and methods

2.1. T. crassiceps metacestode carbohydrates (TCHO) and synthetic glycans

Taenia carbohydrates (TCHOs) were prepared and chromatographically fractionated as described previously (Dissanayake et al., 2004; Dissanayake and Shahin, 2006). Naturally occurring human milk glycans, lacto-*N*-fucopentaose I (L5908), lacto-*N*-fucopentaose II (L6401), lacto-*N*-fucopentaose III (Lewis X pentasaccharide, L7777) and lacto-*N*-difucohexaose I (L7033) were obtained Sigma Chemicals. Synthetic Lewis X pentasaccharide (00-007), Lewis X-PAA-Biotin (01-036), Lewis Y (01-043), α-D-mannose-PAA Biotin (01-005), Lewis X-PAA (04-035), α-D-Gal-PAA Biotin (01-003) and Gal-β-(1-3)-GlcNAc-β-PAA-Biotin (01-020) were obtained from Glycotech (http://www.Glycotech.com). All sugars were used on a dry weight basis, for difficulty in calculating the molar ratios for the polymers.

2.2. In vitro culture of spleen cells for cytokine analysis

All assays were performed with naive cells from 6- to 8-week-old BALB/c mice. Standard 96-well plate cultures were set up with 0.5 million cells per well in a volume of 200 μL. For in vitro stimulation with Lewis sugars, serial dilutions were made from a stock of 10 μg/mL in a volume of 100 μL prior to addition of cells (100 μL, 5×10^6/mL stock). Cultures were incubated at 37 °C for periods from 4 up to 16 h. IFN-γ levels in the culture supernatants were determined by ELISA. In ELISA, cytokine expression was expressed as nanogram/million cells, calculated using a conversion factor with an approximation for curve linearity.

2.3. Inhibition of in vitro IFN-γ secretion by antibodies to TLRs

Naive spleen cells were plated in 96-well plates in 100 μL of RPMI medium supplemented with 10% FCS. Varying amounts (0.1–50 μg/0.5 × 10^6 spleen cells) of anti-TLR antibodies, TLR1 (N-20), sc-8687; TLR2 (D-17), sc-12504; TLR2 (S-16) sc-16237; TLR4 (MTS510) sc-13591; TLR4 C-terminus (sc-16240); TLR6 (E-19), sc-5662 and RP-105 (RP/14), sc-13592 (all from Santa Cruz Biotechnology Inc., CA) in 50 μL RPMI medium/10% FCS were added to duplicate wells. Control wells received 50 μL of the homologous normal goat or rat serum, as appropriate for the goat or rat anti-TLR antibodies listed above. Spleen cells were then added and incubation continued for 2 h followed by 10–25 ng of Lewis X in 50 μL in to each well and the incubation continued for 14–16 h. IFN-γ levels in culture supernates were determined by ELISA as described above.

2.4. Intracellular IFN-γ determination by flow cytometry (FACS)

Intracellular staining for IFN-γ expression was performed with the Cytofix/Cytoperm™ kit (# 555028, Becton Dickinson, Sparks, MD). Spleen cells from 6- to 8-week-old BALB/c mice were washed in PBS/10% FCS and cultured in the presence of Lewis X and Lewis Y at 100 ng/2 million cells in a total volume of 500 μL, for varying time periods up to 4 h. Control cells were incubated with medium alone. The washed cells were then suspended in FCS/5% normal rabbit serum and incubated with FITC conjugated surface markers to CD3, CD21/35 (BD Pharmingen™) and anti-mouse macrophage/monocyte antibody (cat # MCA519F, Serotec) at 4 °C for 35 min followed by Cytofix/Cytoperm solution for 12–16 h at 4 °C. Washed cells were stained intracellularly with PE-anti-IFN-γ antibodies or the isotype matched control PE-IgG1 (#s 554412 and 554685, PharMingen™). The cells were washed in Perm-Wash™ (Becton Dickinson) solution, re-suspended in PBS/5% FCS and flow cytometric analysis performed using a FACSort Flow Cytometer and CELLQUEST software (Becton Dickinson, Sparks, MD).