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The expression of intelectin in sheep goblet cells and upregulation by interleukin-4☆

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Abstract

Upregulation of intelectin (ITLN) transcript and protein has previously been shown in intestinal nematode infections of resistant mice strains with immunolocalisation of protein to goblet cells and paneth cells. In man, intelectin expression has been shown in respiratory tract epithelium, with upregulation occurring in bronchoalveolar lavage fluid of asthmatic individuals. This study describes the expression of intelectin in the respiratory tract of sheep and the immunolocalisation to goblet cells using a novel affinity-purified chicken anti-intelectin peptide antibody. Furthermore we show that when sheep tracheal explants were cultured for 48 h± recombinant sheep IL-4, sheep ITLN transcripts were upregulated compared with controls. Putative roles for intelectin have included an antibacterial role and an alteration of the character of mucus. Our data suggest ITLNs may play an important role in the mucosal response in allergy and parasitic infections.

Keywords: Sheep; Intelectin; Goblet cell; IL-4; Th2 response

1. Introduction

The intelectin (ITLN) family is composed of calcium-dependent galactose binding lectins with homologues known in sea squirts, fishes, frogs and mammals (Chang et al., 2004). The first mammalian intelectin to be described was mouse intelectin-1 in 1998 (Komiya et al., 1998), which was shown to be expressed by small intestinal paneth cells and a role in defence against bacterial infection was suggested.

Human intelectin-1 was subsequently described by separate groups (Lee et al., 2001; Tsuji et al., 2001) and recombinant intelectin-1 was found to bind galactofuranose residues isolated from the bacterium *Nocardia* as well as ribose and deoxyribose, and to a lesser extent, galactose (Tsuji et al., 2001) lending further support for a role of intelectin in innate recognition of bacteria.

Human intelectin-1, also known as human lactoferrin receptor, binds lactoferrin which has anti-microbial, immunomodulatory and anti-inflammatory properties (Suzuki et al., 2001). More recently it has been recognised as omentin and has been isolated from omental fat of patients with inflammatory diseases such as ulcerative colitis (Schaffler et al., 2005) and shown to enhance insulin-mediated uptake of glucose in adipocytes (Yang et al., 2006).

☆ Mouse intelectin-1 and intelectin-2 are not direct orthologues of human intelectin-1 and intelectin-2, respectively. However, this nomenclature is used for consistency with previous literature. Synonyms for mouse intelectin-1 are intelectin, intelectin-a, intelectin-1a and lactoferrin receptor. Synonyms for mouse intelectin-2 are intelectin-b and intelectin-1b.

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Additionally, a novel intelectin variant, mouse intelectin-2, was found to be highly upregulated in intestinal epithelium from the resistant BALB/c mouse strain following infection with *Trichinella spiralis* (Pemberton et al., 2004a). Results were confirmed by RT-PCR and intelectin was immunolocalised to the paneth cells and goblet cells (Pemberton et al., 2004a). There was differential expression of mouse ITLN1 and 2, with constitutive expression of mITLN1 which did not show upregulation on infection. In a gene profiling analysis, significant upregulation of intelectin expression was shown in the caecum of resistant mice infected with *Trichuris muris* (Datta et al., 2005). Subsequently, Artis (2006) confirmed upregulation of ITLN1 and 2 by RT-PCR in this model, and once again intelectin was immunolocalised to goblet cells. In both these models of nematode infection, differential expression of intestinal intelectins has been shown in susceptible and resistant strains of mice, and a potential anti-nematode role has been suggested (Artis, 2006; Pemberton et al., 2004a).

Allergic airway sensitisation in asthmatics, as well as enteric responses to gastrointestinal nematodes, share similarities in that both involve polarisation of T cells and expression of the Th2 cytokines, IL-4 and IL-13 (Datta et al., 2005; Walter and Holtzman, 2005). It is thus of interest that Kuperman et al. (2005) have shown an increase in intelectin expression in mice over expressing IL-13. Furthermore, intelectin has been also shown to be upregulated in bronchial brushings (Kuperman et al., 2005) and bronchoalveolar lavage fluid (Wu et al., 2005) from asthmatics. We now show expression of intelectin in sheep airways, a common model for asthma in man (Abraham et al., 2005; Bischof et al., 2003) and upregulation of ITLN transcription in sheep tracheal explants by recombinant sheep IL-4.

2. Materials and methods

2.1. Antibody preparation and validation

2.1.1. Peptide for commercial antibody preparation

A peptide was chosen for commercial antibody preparation in chickens (Aves Labs, Tigard, OR, USA), based on predicted immunogenicity and high degree of sequence conservation between species, ITLN peptide: TSDDYKNPGY(F/Y)DIQA, corresponding to residues 130–143 of mITLN2. Specific anti-peptide IgY was purified from total IgY by affinity chromatography, using the appropriate peptide immobilised on NHS-activated sepharose (Hi-Trap NHS activated, 1 ml, G.E. Healthcare).

2.1.2. Tissues for validation of antibody

2.1.2.1. Mouse, human and sheep. Mouse: BALB/c mice were chosen as they are known to upregulate jejunal intelectin expression during *T. spiralis* infection (Pemberton et al., 2004a). For *T. spiralis* infection 8–15-week-old BALB/c (B & K Universal, Hull, UK) were infected by gavages with 200–300 muscle larvae per mouse in 0.2 ml of phosphate buffered saline (PBS)/0.1% agar. To check infections were successful, adult worms were isolated from groups of four to five of the mice at 6–7 days after infection. For protein extraction mice were killed 14 days after infection, and jejunum was snap frozen and stored at −70 °C. Jejunal sections were fixed in 4% paraformaldehyde for immunohistochemistry. All experiments were approved by the University of Edinburgh's Biological Services ethical review committee and were performed under license, as required by the United Kingdom's Animals (Scientific Procedures) Act of 1986. Mice were maintained in a conventional environment at the University of Edinburgh.

Human: We have found that LS174T cells, a human colonic mucoid adenocarcinoma cell line, upregulates human intelectin-1 in response to the cytokines IL-4 and IL-13 (manuscript in preparation). Thus LS174T cells (European collection of Animal Cell Cultures, Porton Down, UK) were cultured in Eagle's minimum essential medium (Invitrogen, Paisley, UK) supplemented with non-essential amino acids, 100 U/ml penicillin (Invitrogen), 100 μg/ml streptomycin (Invitrogen), 2 mmol L-glutamine (Invitrogen) and 10% heat inactivated foetal calf serum (Invitrogen) at 37 °C with 5% CO_2. Recombinant human IL-4 (Peprotech EC, London, UK) was made up according to the manufacturer's recommendations and was used at a concentration of 1 ng/ml. Cells were harvested at 72 h, the supernatant was discarded and cells stored at −70 °C for protein extraction.

Sheep: Tracheal mucosa was stripped from freshly culled aged ewes and stored at −70 °C for protein extraction. Trachea and lung sections were fixed in 4% paraformaldehyde for immunohistochemistry.

2.1.3. Western blot

Protein was extracted from jejunum of *T. spiralis*-infected BALB/c mice, IL-4 treated LS174T cells and sheep tracheal epithelium, and run on 12% acrylamide SDS-PAGE mini gels (mini-Protean, Bio-Rad). The gels were blotted (Transblot SD, Bio-Rad) onto polyvinylidenefluoride (PVDF) membrane (Immobilon P, Millipore) and blocked with 50 mM Tris–HCl pH 7.5, 0.15 M NaCl, 1% milk powder, 0.5% Tween 80. Blots