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Short communication

Toll- and NOD-like receptor mRNA expression in canine sino-nasal aspergillosis and idiopathic lymphoplasmacytic rhinitis

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ABSTRACT

The pathogenesis of canine sino-nasal aspergillosis (SNA) and lymphoplasmacytic rhinitis (LPR) remains poorly understood. The innate immune system is implicated in the etiology of human chronic rhinosinusitis. Therefore, we hypothesized that dysfunction in innate immunity could be implicated in the pathogenesis of SNA and LPR.

Expression of messenger RNA (mRNA) encoding Toll-like receptors (TLRs) 1–10 and NOD-like receptors (NODs) 1 and 2 in nasal mucosal biopsies from SNA or LPR dogs was compared with mucosa from healthy controls. Gene expression was quantified using quantitative real-time reverse transcriptase polymerase chain reaction normalized against multiple housekeeper genes.

All TLR and NOD genes were quantified in all samples. SNA was associated with significantly increased expression of TLRs 1–4, 6–10; and NOD2, relative to controls. LPR was associated with significantly increased expression of TLRs 1, 2, 6–8, relative to controls. There was significantly more expression of TLRs 1, 4, 6–10 and NOD2 in SNA dogs than in LPR dogs. The significance of these differences in the pathogenesis of these diseases is yet to be determined.

1. Introduction

The sino-nasal mucosa, particularly the innate immune system, plays a major role in upper airway immunity (Ooi et al., 2008). Recognition of conserved structures present on bacterial, fungal, viral and protozoal microorganisms is possible by pattern-recognition receptors (PRRs). These receptors are expressed by a wide range of immune and non-immune cells, such as respiratory epithelial cells (Sha et al., 2004). The two best-characterized classes of PRRs are the Toll-like receptors (TLRs), which sense the presence of microbial molecules on the cell surface and in endosomes; and the nucleotide binding oligomerization domain (NOD)-like receptors (NLRs), which recognize microbes in the cytosol (Becker and O'Neill, 2007; Franchi et al., 2008). To date, 10 TLRs have been described in man, and NOD1 and NOD2 are the first NLRs reported to act as PRRs (Turvey and Broide, 2010). One of the major functions of the PRRs is to signal the antigen-presenting cell to induce the release of particular chemokines and cytokines, which in turn determine the nature of the acquired immune response mounted against the pathogen that stimulated the PRR. Uncontrolled or inappropriate TLR and/or NLR activation can lead to development of inflammation and damage to the host (Franchi et al., 2008; Shibolet and Podolsky, 2007).

In man, chronic rhinosinusitis (CRS) is considered as an inflammatory rather than an infectious disease, with microbes found in the nasal cavity playing a role in initiating or perpetuating mucosal inflammation (Shin et al.,

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2004). Although the underlying mechanisms are unknown, innate immune processes, including the TLR pathways, may play a role in the pathogenesis of this disease (Lane et al., 2006b).

The most common causative agent of sino-nasal aspergillosis (SNA) is *Aspergillus fumigatus* (Sharp et al., 1991). Because this fungus is ubiquitous and SNA affects only a small proportion of the otherwise healthy canine population, a local immune deficiency in affected dogs has been suggested (Peeters et al., 2006).

Lymphoplasmacytic rhinitis (LPR) is a disease characterized by mild to moderate lymphoplasmacytic to mixed inflammation of the nasal mucosal, for which no cause can be found (Windsor et al., 2004). The pathogenesis of this disease remains unknown, but an aberrant immune response to inhaled organisms or allergens is suspected (Peeters et al., 2007).

Recently, a comparative analysis of gene expression in the nasal mucosa of SNA and LPR dogs revealed distinct cytokine and chemokine profiles in both diseases (Peeters et al., 2007). The mechanisms leading to these distinct inflammatory responses are unknown. Hence, we hypothesized that a dysfunction in innate immunity may play a role in the pathogenesis of both diseases. Therefore, the aim of the present study was to quantify the expression of genes encoding a panel of PRRs (TLRs 1–10, NOD1 and NOD2) in nasal mucosal biopsies from SNA and LPR dogs and to compare these results with those obtained in biopsies from dogs without nasal disease.

2. Materials and methods

2.1. Animals

All animals used in the study were part of a previous study (Peeters et al., 2007). Thirteen SNA, seven LPR and thirteen control dogs were included.

2.2. Tissue samples, RNA isolation and cDNA synthesis

Tissue samples were the same as those used previously (Peeters et al., 2007), and the frozen cDNA remaining from that study was used in the current study.

2.3. Primer and hydrolysis probe design

Primers and probes were designed using Primer 3 (Rozen and Skaletsky, 2000) (www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi.) and *M*-fold using the canine specific GenBank sequences for TLR1 (NM_001146143.1), TLR2 (NM_001005264.2), TLR4 (NM_001002950.1), TLR5 (EU551146.1), TLR6 (EU551147.1), TLR7 (AB248956.1) and TLR9 (NM_001002998.1), as described previously (Table 1) (Peters et al., 2004). In addition, primers pairs which amplified larger portions of these genes were designed for use in evaluation of reaction efficiency (Table 2).

For TLR3 (XM_540020.2), NOD1 (XM_539499.2) and NOD2 (XM_544412.2), the predicted GenBank canine sequences were confirmed by conventional PCR and direct sequencing of PCR products amplified from complementary DNA (cDNA). The amplicon amplified by these primer sets (Table 2) included the target amplicon of the quantitative PCR (qPCR) assay (Table 1).

As no sequences were available in GenBank for the canine TLR8 and 10 genes, qPCR primers (Table 1) were designed using a consensus sequence derived from the available human, rat, horse and cat sequences (Table 2).

Primers and probes were synthesized by Metabion (Martinsried, Germany) and were reconstituted in EB buffer (10 mM Tris–HCl pH 8.4, Qiagen Ltd.) before use. Ribosomal protein L13a (RPL13A), TATA box binding protein (TBP) and ribosomal protein S18 (RPS18) served as housekeeper genes for result normalization as these were the same as used previously (Peeters et al., 2007).

2.4. Conventional PCR and product sequencing

Conventional PCR was carried out using HotStarTaq Master Mix (Qiagen Ltd.) to amplify larger portions of the TLR and NOD genes (Table 2) (Peeters et al., 2007; Peters et al., 2007), and products were separated and purified as previously described. Products from the predicted canine TLR3, TLR8, TLR10, NOD1 and NOD2 cDNAs were sent to the Sequencing Service (University of Dundee, Scotland) for direct sequencing to confirm their identity. These sequences were deposited in GenBank under the following

Table 1
Primer and probe sequences used in the quantitative RT-PCR assays used for quantification of TLR, NOD and housekeeper gene mRNA expression.

Gene	Product length (base pairs)	Forward primer (5′-3′)	Reverse primer (5′-3′)	Probe sequence (5′-3′)
TLR1	114	GCCATCCTACCGTGAACCT	GCACTCAACCCCAGAAACTC	TGCAAAGAGTTTGGCAACATGTCTCA
TLR2	90	TCGAGAAGAGCCACAAAACC	CGAAAATGGGAGAAGTCCAG	TCGTGAAAAGCGAGTGGTGCAA
TLR3	130	GCAACACCCAGCTACACAGA	ATGTGGAAGCCAGACAAAGG	TCACCATGCTCGATCTTTCCCACA
TLR4	146	GTGCTTCATGGTTTCTCTGGT	CCAGTCTTCATCCTGGCTTG	TGCTTCTCGCTTGGCTTGCAA
TLR5	143	TCGTGTTGACAGACGGTTATTT	TCCGGTTGAGGGAAAAGTC	ACGCCTCCTTCCGGGAGCTG
TLR6	109	TCAAGCATTTAGACCTCTCATTCA	CCGTAACTTTGTAGCACTTAAACCT	TGCCCATCTGTAAGGAATTTGGCA
TLR7	100	GCCCTTTTTCTGATGGTGATT	CGCCGATACCCCTTTATTTT	CCACCTCTACTTCTGGGACGTGTGG
TLR8	138	TCAGCTACAATGCACACTACTTCC	ACGCTTCTCAGGTCTTGCTC	TCCTAGGCGGTGCGTCACCC
TLR9	104	ACTGGCTGTTCCTCAAGTCC	AGTCATGGAGGTGGTGGATG	ACCCCGCGGTAACGTCACCA
TLR10	145	TGCCAACAACACATCCTTG	GCAAGCACCTGAAAACAGAA	TGCTTTTGGCCAGAAACCTTGATCA
NOD1	84	GTCACTCACATCCGCAACAC	CCACGATCTCCGCATCTT	TGTCTGGTGGACAACTTGCTGGAGA
NOD2	98	GCACATCACCTTCCAGTGTTT	GGCCCATGACAAATGAAGA	CGGTGACACGTCAGTGCTGAGC
RPL13A	87	GCCGGAAGGTTGTAGTCGT	GGAGGAAGGCCAGGTAATTC	TGTGAAGGCATCAACATTTCTGGCA
TBP	96	CTATTTCTTGGTGTGCATGAGG	CCTCGGCATTCAGTCTTTTC	TCGCTAAGAAGCGTGTACTGGGGATG
RPS18	116	TGCTCATGTGGTATTGAGGAA	TCTTATACTGGCGTGGATTCTG	CACTGAGGATGAGGTGGAACGTGTG