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Short communication

Gene transcription abnormalities in canine atopic dermatitis and related human eosinophilic allergic diseases

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ABSTRACT

Canine atopic dermatitis (AD) is clinically similar to human AD, implicating it as a useful model of human eosinophilic allergic disease. To identify cutaneous gene transcription changes in relatively early inflammation of canine AD, microarrays were used to monitor transcription in normal skin ($n = 13$) and in acute lesional AD (ALAD) and nearby visibly nonlesional AD (NLAD) skin ($n = 13$) from dogs. Scanning the putative abnormally transcribed genes, several potentially relevant genes, some abnormally transcribed in both NLAD and ALAD (e.g. IL6, NFAM1, MSRA, and SYK), were observed. Comparison for abnormally transcribed genes common to two related human diseases, human AD and asthmatic chronic rhinosinusitis with nasal polyps (aCRSwNP), further identified genes or gene sets likely relevant to eosinophilic allergic inflammation. These included: (1) genes associated with alternatively activated monocyte-derived cells, including members of the monocyte chemotactic protein (MCP) gene cluster, (2) members of the IL1 family gene cluster, (3) eosinophil-associated seven transmembrane receptor EMR1 and EMR3 genes, (4) interferon-inducible genes, and (5) keratin genes associated with hair and nail formation. Overall, numerous abnormally transcribed genes were observed only in canine AD; however, many others are common to related human eosinophilic allergic diseases and represent therapeutic targets testable in dogs with AD.

1. Introduction

Both human and canine atopic dermatitis (AD) are prevalent, and severe AD is particularly debilitating. Moreover, naturally occurring canine AD is clinically most similar to human AD – typically early onset, common environmental exposures and IgE reactivity (e.g. against house dust mite), Th2-predominant inflammation with ancillary eosinophil infiltration (i.e. "Th2 eosinophilic" inflammation), and complicating Staphylococcus and Malassezia infections. Some common molecular changes have also been reported in canine and human AD. For example, reduced ceramide (Reiter et al., 2009; Shimada et al., 2009; Yoon et al., 2011), increased CCR4, IL4, and S100A8 (Maeda et al., 2002; Nuttall et al., 2002; van Damme et al., 2009), and altered antimicrobial peptide levels (van Damme et al., 2009) have been shown in canine as well as human AD. Th2 eosinophilic inflammation at other epithelial surfaces also occurs in human allergic diseases such as asthma and eosinophilic esophagitis. The similarities between canine AD and

Abbreviations: aaMDCs, alternatively activated monocyte-derived cells; aCRSwNP, asthmatic chronic rhinosinusitis with nasal polyps; AD, atopic dermatitis; ALAD, acute lesional atopic dermatitis; CADESI-2, Canine Atopic Dermatitis Extent and Severity Index-2; Fc, fold change; MCP, monocyte chemotactic protein; NCBI GEO, National Center for Biotechnology Information Gene Expression Omnibus; NLAD, nonlesional atopic dermatitis.

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related human eosinophilic allergic diseases indicate that canine AD should be a useful model to better understand and develop treatments for both dogs and humans.

Identifying common molecular networks between canine AD and human eosinophilic allergic diseases would provide the opportunity to test the importance of such networks in associated inflammation. A popular screening approach capable of identifying disease-associated molecular networks in an unbiased manner involves analysis of gene transcription using high-density oligonucleotide arrays, often referred to as microarrays. However, only one article using microarray technology to analyze gene transcription in canine AD skin has been reported, and this article presented limited data (Merryman-Simpson et al., 2008). Because identifying and controlling events relatively early in the inflammatory process is appealing, both acute lesional and nearby visibly nonlesional AD skin from dogs were analyzed here and compared to healthy skin from dogs using microarrays. Subsequent comparisons of these data to human AD (Olsson et al., 2006; Plager et al., 2007) and asthmatic chronic rhinosinusitis with nasal polyps (aCRSwNP) (Plager et al., 2010) data were performed to identify molecular networks common to canine AD and human eosinophilic allergic disease.

2. Materials and methods

2.1. Canine subjects and skin specimens

Thirteen dogs with AD diagnosed based on a characteristic history, clinical signs (Favrot et al., 2010), and exclusion of other pruritic skin disorders as deemed necessary were recruited. In addition, 13 normal dogs with no sign of atopic or other disease were enrolled. The study was approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol number 0601A79512; University of Minnesota Assurance of Compliance number A3456). Canine Atopic Dermatitis Extent and Severity Index-2 (CADESI-2) scores or pruritus intensity using a visual analog scale from 0 to 10 were assessed to estimate overall AD severity (Table S1). Skin biopsies (8 mm) from dogs with AD were collected from sites of acute lesional skin and nearby visibly nonlesional skin (about 10 cm apart) that had not been topically treated with glucocorticoids or other immunosuppressants for at least three weeks before specimen collection. Oral immunosuppressants were also avoided for at least four weeks before skin biopsy collection, and other oral medications were minimized, and if used, are listed in Table S1. Immediately after collection, each skin biopsy was placed in room temperature RNAlater (Ambion, Austin, TX) for 2 min with mixing, transported on ice, and then a small portion of the biopsy was removed for formalin fixation. The larger portion of skin in RNAlater was held at 4 °C overnight and stored at −20 °C until total RNA isolation.

2.2. Total RNA isolation and high-density oligonucleotide microarray analysis

After removal from RNAlater, each skin specimen was frozen in liquid nitrogen, placed in ice-cold TRIzol (Invitrogen, Carlsbad, CA), and immediately shattered and homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). Total RNA was isolated using the TRIzol Plus RNA Purification System (Invitrogen) according to the manufacturer's protocol. Purified total RNA was supplied to the Mayo CTSA Microarray Core facility, assessed for integrity using the Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA), and used for Affymetrix Canine 2 Genome (May 2005 version) high-density oligonucleotide microarray analyses according to Affymetrix-established protocols. To compensate for potential variability among separate microarray analyses, individual, non-pooled total RNA samples were isolated and analyzed in batches containing different specimen types (i.e. Normal, NLAD, and ALAD). Hybridized microarrays were scanned in the Affymetrix GeneArray Scanner. The resulting image files were analyzed to generate files with the x, y coordinates and average fluorescence intensities of each microarray cell. The 39 files with a .CEL extension (deposited at the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO)) were analyzed using Affymetrix Microarray Suite to quantify and scale the fluorescence data. Data analyses to identify differential transcription used GeneSpring GX 9.0 software (Agilent). Molecular network analyses of the identified differentially transcribed genes used Ingenuity Pathways Analysis software (Ingenuity Systems, Mountain View, CA).

To identify abnormal gene transcription common to canine AD and two related human eosinophilic allergic diseases, AD and aCRSwNP, a comparison based on identical Gene Symbols using Microsoft Office Access software was performed. Briefly, AD microarray data sets from Olsson et al. (2006) (GSE6012 in NCBI GEO; lesional AD skin, $n = 10$, and normal skin, $n = 10$) and from our group (GSE5667 in NCBI GEO; minimally lesional AD skin, $n = 6$, and nearby visibly nonlesional AD skin, $n = 6$, and normal skin, $n = 5$) (Plager et al., 2007) were imported separately into GeneSpring GX 9.0. After an initial low stringency comparison between AD and normal samples within each data set (fold change (Fc) ≥ 1.4 and unpaired nonparametric Mann–Whitney U test, $p \leq 0.22$), subsequent analyses for differentially transcribed genes between AD and normal samples at Fc ≥ 2.0 and corrected p-value ≤ 0.1 (unpaired nonparametric test with Benjamini–Hochberg False Discovery Rate correction) were performed. A union set (i.e. a combined list) from the two AD to normal skin comparisons was generated. A similar list of differentially transcribed genes from the comparison of aCRSwNP to normal/rhinitis nasosinus tissue (Fc ≥ 2.0 and corrected p-value ≤ 0.05) was also used (Plager et al., 2010). Finally, a union set of the differentially transcribed genes identified here by comparing ALAD and Normal, NLAD and Normal, or ALAD and NLAD skin from dogs was generated (Fc ≥ 2.0 and corrected p-value ≤ 0.1), and this union set list from canine AD skin was compared to the Gene Symbol lists of differentially transcribed genes from human AD or aCRSwNP.

3. Results and discussion

Eosinophilic allergic diseases are prevalent, and severe disease can be debilitating. Unfortunately, highly