Reduced IL-31 receptor alpha splice variant mRNA following allergen challenge in a canine model of atopic dermatitis

Citation for published version:
Craig, N, Ahrens, K, Wilkes, R & Marsella, R 2021, 'Reduced IL-31 receptor alpha splice variant mRNA following allergen challenge in a canine model of atopic dermatitis', Allergy. https://doi.org/10.1111/all.15005

Digital Object Identifier (DOI):
10.1111/all.15005

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Allergy

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Title: Reduced IL-31 receptor alpha splice variant mRNA following allergen challenge in a canine model of atopic dermatitis

To the Editor,

Interleukin 31 (IL-31) signaling is a target for therapy in atopic dermatitis (AD). Twelve isoforms of the cytokine-specific IL-31 receptor alpha (IL-31RA) produced by alternative mRNA splicing are known to have varied or opposing functions, but the physiological relevance of these has been obscured by the use of qPCR assays which are not specific to one isoform.\(^1-3\) If new treatments are to target IL-31RA, it is important to understand the modulation and likely functions of these different isoforms in allergic inflammation.

When Cevikbas and colleagues examined transcription of two groups of IL-31RA isoforms in skin, they found that while overall IL-31RA transcription was reduced, a subset of transcript variants was increased in the lesional skin of AD patients compared with healthy controls.\(^4\) This subset included both functional IL-31RA isoforms and a non-functional isoform lacking part of the cytokine-binding domain, so exactly which of these are up-regulated in lesional atopic skin, and what effects this may have, remains unclear.\(^1,4\)

Dogs (Canis lupus familiaris) spontaneously develop AD which is strikingly similar to its human counterpart, and can be used to answer questions that may be challenging when studying human patients.\(^5,6\)

Canine IL-31RA mRNA splice variants encode only three isoforms, two full-length and one truncated in the extracellular domain, which correspond to human homologs identified in Cevikbas’ up-regulated subset. As in the human full-length IL-31RA, the extracellular region of the canine long IL-31RA isoforms includes a signal peptide and five fibronectin type III (FnIII) domains, the first two of which include the cytokine binding domain in human IL-31RA.\(^1,2\) The short canine IL-31RA is missing the signal peptide and the first FnIII domain, as in its non-functional human homolog.\(^4\) In functional studies of the human homologs of these IL-31RA splice variants by Maier and colleagues, translation of the short isoform was shown to be markedly reduced, and when expressed it was not able to activate STAT signaling in response to IL-31, suggesting a negative influence on IL-31-signalling.\(^1\) The potential role of these transcript variants during allergic inflammation can therefore be clarified by examining their transcription in atopic dogs.

The atopic beagles used in this study have been validated as suitable model for human AD which mimics natural occurring disease, with the advantage that the amount, timing and frequency of allergen stimulation can be controlled and consecutive skin biopsies can be taken.\(^6\) Full-thickness skin biopsies were collected from 10 atopic beagles at baseline (day 0) and at three hours (day 1, early inflammation), three days (peak inflammation) and 10 days (resolution of inflammation) following acute house dust mite (Dermatophagoides farinae) allergen stimulation. 50 mg crude D. farinae (31 mg/ml in phosphate buffered saline pH 7.2) was gently applied to the inguinal skin on days 1, 2 and 3 to provoke a moderate flare of AD which resolved by day 10. On day 1 and 3, biopsies were taken 3 hours after allergen exposure. The severity of inflammation was assessed using a validated scoring system (canine atopic dermatitis extent and severity index; CADESI-03) prior to collection of each biopsy. IL-31RA splice variant transcription
was measured using qPCR assays targeting total IL-31RA transcription, long IL-31RA isoforms mRNA transcript variants X1-4, short IL-31RA isoform mRNA transcript variant X5 or the reference genes CG14980-PB and RPS19. To examine IL-31RA splice variants in chronic inflammation, transcription was similarly examined using archived RNA from skin biopsies taken from two groups of atopic beagles from the same colony: one group of seven dogs following two weeks of chronic allergic inflammation (50 mg D. farinae applied on days 0, 7 and 14 to induce a continuous allergic flare, biopsies were taken 3 hours after allergen on day 14) were compared with another group of nine dogs prior to allergen exposure. Transcription was also examined using biopsies from nine privately owned healthy dogs.

Both long and short IL-31RA isoforms were transcribed in all the canine skin samples. In non-lesional atopic and normal skin, the short IL-31RA isoform was consistently transcribed at a higher level than the long isoforms (Figure 1). This suggests that the short IL-31RA splice variant has an explicit function in normal canine skin, possibly as a mechanism of post-transcriptional regulation to moderate the response to IL-31 through reduced expression of functional receptors.

During both acute and chronic allergic inflammation, transcription of both long and short IL-31RA isoforms was found to be reduced (Figure 2), and there was no longer a significant difference in transcription between them (Figure 1). A corresponding negative correlation was observed between CADESI-03 scores and transcription of total IL-31RA, both transcript variants, and the ratio of short:long IL-31RA isoform transcripts in the consecutive skin biopsies collected at baseline and over the course of acute allergic inflammation (Supplementary Figure 1). There was no significant difference between the CADESI-03 scores associated with the archived baseline and chronic inflammation samples (Figure 2), probably because these samples were collected from different generations of closely related dogs within the same colony. The younger generation, from which the baseline samples were collected, were able to go outside when the pollen count was low, leading to more variation in baseline CADESI-03, which would explain the non-significant difference compared with the chronic inflammation samples.

The reduced transcription of all canine IL-31RA isoforms in the skin during allergic inflammation is counterintuitive, and suggests that the influence of IL-31 in excess or during inflammation would be undesirable. This is supported by the observed effects of IL-31 on organotypic human 3D models generated from primary normal human epidermal keratinocytes and primary dermal fibroblasts, which resulted in a thin epidermal layer and disturbed barrier function. Reduced IL-31RA transcription during allergic inflammation would complement the negative feedback provided by suppressor of cytokine signaling 3 (SOCS3) following stimulation with IL-31. SOCS3 is known to be upregulated in response to IL-31 signaling in both transfected HeLa cells expressing IL-31RA and in human monocyte-derived dendritic cells, and is also upregulated in the skin of both humans and dogs during allergic inflammation. SOCS3 has been shown to strongly inhibit IL-31RA-induced activation of STAT3 and may also promote degradation of the IL-31RA protein in transfected HeLa cells. Negative feedback via SOCS3 could therefore act in synergy with reduced IL-31RA transcription to prevent excessive IL-31 signaling once
inflammation has been initiated. Another function for downregulating IL-31RA which has been proposed is that it could promote TH2-type inflammation by enhancing the influence of oncostatin M: Bilsborough and colleagues have previously suggested that the exacerbated TH2-type inflammation in IL-31RA knockout mice may be due to increased availability of OSMRβ to bind with GP130 to make oncostatin M receptor. Oncostatin M has been shown to be produced by T cells in the skin of humans with AD, and to stimulate a gene expression profile in cultured human keratinocytes which is associated with inflammatory infiltration, angiogenesis and hyperplasia as seen in AD.

The stimulus for reducing IL-31RA transcription could be changes in local conditions during inflammation, with reduced transcription reflecting the shift in the local cytokine milieu during TH2-type inflammation away from interferon gamma (IFNγ), which has been shown to stimulate IL-31RA transcription in keratinocytes.

One area of future study would be to confirm that the short canine IL-31RA splice variant suffers from the same reduced translation and inability to propagate IL-31 signaling as its human homolog. This is highly likely as both arise from a 5' insertion encoding an in-frame premature stop codon. Another potential line of research would be to examine whether IL-31RA splice variant transcription can be used as a predictive or diagnostic tool for AD, or is involved in the observed variation in efficacy or rebound pruritus observed following use of anti-IL-31 monoclonal antibody or JAK inhibitor therapeutics in dogs.

This study used a canine atopic model to help clarify the roles of IL-31RA splice variants in the skin during allergic inflammation. Preferential splicing to the presumed non-functional short IL-31RA isoform was identified in healthy skin, whereas during allergic inflammation both functional and non-functional IL-31RA transcription was reduced and this preferential splicing was no longer evident. Regulation of IL-31RA transcription and alternative-splicing to a non-functional IL-31RA isoform are potentially two separate mechanisms by which IL-31 signaling in the skin may be differentially regulated during allergic inflammation or in its absence, respectively.

References:


Authors: Nicky Craig*, Kim Ahrens, Rachel Wilkes, Rosanna Marsella

Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

*Present address: The Roslin Institute, The University of Edinburgh, Easter Bush, Midlothian, UK

Correspondence: Rosanna Marsella, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, 2015 SW 16th Avenue, Gainesville, Florida, USA. Email: marsella@ufl.edu

This study was self-funded. The authors have no conflict of interest to disclose.

Figure 1. Transcription of IL-31RA splice variants in canine skin (N0, arbitrary fluorescence units calculated using LinRegPCR) normalized to reference genes. Long (●) and short (▲) IL-31RA isoform mRNA in skin biopsies from (a) 10 atopic 2-year-old beagles at baseline and over the course of acute allergen exposure and resolution of inflammation, (b) nine atopic 2-year-old beagles prior to allergen exposure (baseline) and seven atopic 7-year-old beagles following two weeks allergen challenge (chronic) and (c) nine normal dogs of varied ages and breeds (p-values < 0.05 annotated).

Figure 2. IL-31RA splice variant transcription in acute and chronic allergic inflammation (N0, arbitrary fluorescence units calculated using LinRegPCR). Transcription of (a) total, (b) long and (c) short IL-31RA isoforms normalized to reference genes, (d) the ratio of short:long IL-31RA isoform transcripts in skin biopsies, and (e) clinical scores (CADESI-03) at the time of biopsy from (i) 10 atopic 2-year-old beagles at baseline and over the course of acute allergen exposure and resolution of inflammation, and (ii) nine atopic 2-
year-old beagles prior to allergen exposure (baseline) and seven atopic 7-year-old beagles following two weeks allergen challenge (chronic) (p-values < 0.05 annotated).