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Miniature Swine Model of Atopic Dermatitis—Assessment of *In Vivo* and *In Vitro* Activity of Recombinant Porcine Interleukin-4 and Interleukin-13

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ABSTRACT

Atopic dermatitis (AD) is a common skin condition that clinically presents as erythematic and pruritic skin. While multiple factors contribute to the pathophysiology of the disease, skin-barrier dysfunction,[1, 2] and prominent Th2-mediated immune responses are characteristic of AD. interleukin-4 (IL-4) and interleukin-13 (IL-13) are thought to be contributing factors to the pathogenesis of AD,[3] and treatments targeting IL-4 and IL-13 mediated pathways have shown to be beneficial in human patients diagnosed with AD.[4]

While multiple animal models of AD exist,[5, 6] species-specific limitations can deter the translational efficacy of therapies developed for humans. Miniature swine are frequently used the study the toxicity/safety of dermally applied products,[7] therefore a model of AD in miniature swine would be beneficial for pre-clinical efficacy tests of such medications.

MATERIALS AND METHODS

In vitro activity of recombinant porcine interleukin-4 (rpIL-4) and recombinant porcine interleukin-13 (rpIL-13) was quantified using an ELISA for phospho-signal transducer and activator of transcription 6 (pSTAT6), and flow cytometry for pSTAT1, pSTAT3, and pSTAT6. In brief, peripheral blood mononuclear cells were isolated from female Hanford miniature swine and treated with rpIL-4 or rpIL-13 (IBI Scientific) for a period of 10 to 30 minutes. Upon completion of cytokine treatment, cell lysates were prepared for ELISA analysis or fixed in preparation for staining and analysis using flow cytometry.

In vivo activity was assessed in multiple phases. During Phase 1, one animal received a single dose of vehicle (1% porcine serum in 1x phosphate buffered saline [PBS]), histamine (1 mg/dose site), rpIL4 (5 to 20 µg/dose site), rpIL-13 (5 to 20 µg/dose site), rpIL-4/rpIL-13 combination (2.5-20/2.5-20 µg dose site) to assess dermal irritation. During Phase 2, animals received daily intradermal injections of vehicle or rpIL-4 (2 to 6 µg/dose site) for a period of five days. During Phase 3, one animal received daily intradermal injections of rpIL-13 (2 to 6 µg/dose site) for a period of two days. Photographs were taken of the dose sites to characterize the reactions at approximately 5, 20, and 60 minutes post dose. Phase 2 and Phase 3 animals were euthanized, and dose sites were collected and preserved in 10% neutral buffered formalin (NBF) for hematoxylin and eosin (H&E) staining and histopathological analysis.

RESULTS

Peripheral blood mononuclear cells (PBMC) isolated from female Hanford miniature swine demonstrated approximately a four-fold increase in STAT6 phosphorylation when challenged with rpIL-4, but not rpIL-13, when measured by ELISA (**Table 1**). STAT6 phosphorylation demonstrated a two-fold increase when treated with rpIL-4 when measured by flow cytometry, but STAT1 and STAT3 phosphorylation were not significantly increased in rpIL-4- or rpIL-13-treated cells (**Figure 1**).

Table 1. Phospho-STAT6 in PBMC Extracts Mean Adjusted Concentration (A.U.)

Time Point (Minutes)	Vehicle	rpIL-4 100 ng/mL	rpIL-4 200 ng/mL	rpIL-13 100 ng/mL	rpIL-13 200 ng/mL
10	1.104	4.348	4.450	1.778	1.814
30	1.207	3.801	4.031	2.058	1.673
60	1.106	3.661	3.731	1.472	1.279

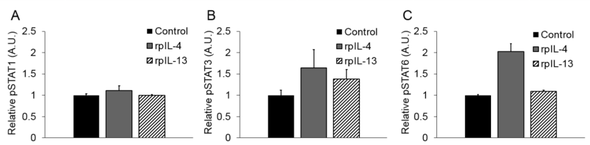

Figure 1. A) pSTAT1, B) pSTAT3, or C) pSTAT6 levels in control, rpIL-4 (100 ng/mL), or rpIL-13 (100 ng/mL). Values were normalized to mean V-R/V-L ratios of control samples. Graphs represent mean ± S.E.M, n=3 per treatment.

When female Hanford miniature swine received a single intradermal dose of rpIL-4 or rpIL-13, erythema and edema were not different from vehicle control dose sites (**Figure 2**). However, repeat intradermal injections for a period of five days did elicit increased erythema and edema in rpIL-4 dose sites relative to vehicle control, but not rpIL-13 dose sites (**Figures 3 and 4**). The peak irritation was observed approximately five minutes after dose administration, similar to histamine injections in miniature swine. Interestingly, perivascular and dermal lymphocytes were observed in ~25% to 38% of rpIL-4 and rpIL-13 dose sites, but were not present in the vehicle control sites. Perivascular eosinophils were observed in ~25% of the rpIL-4 dose sites, but not vehicle or rpIL-13 dose sites. However, these differences were minimal, and did not demonstrate lesions typical of atopic dermatitis. Representative photographs for the histopathology of sites dosed with rpIL-4 and rpIL-13 are shown in **Figures 5 to 10**.

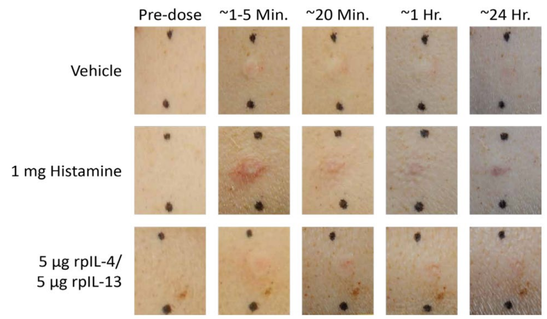

Figure 2. Representative images of dermal irritation when administering a single intradermal dose of vehicle, histamine, or rpIL-4/rpIL13 in Hanford miniature swine. Note a distinct bleb was observed in all three dose sites, but erythema and edema was most prominent in histamine dose sites at five minutes, and was decreased at later time points. There were no observable differences when dosing with a combination of rpIL-4/rpIL-13 compared to vehicle control. No differences were observed in sites dosed with only rpIL-4 or rpIL-13 at varying concentrations (data not shown).

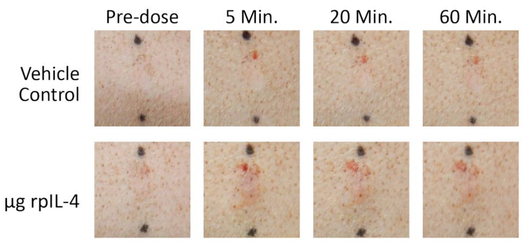

Figure 4. No observable differences were seen on Day 5 of the study when dosed with vehicle or 5 µg rpIL-13.

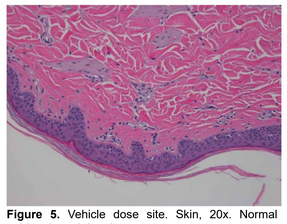

Figure 5. Vehicle dose site. Skin, 20x. Normal epidermis and dermis.

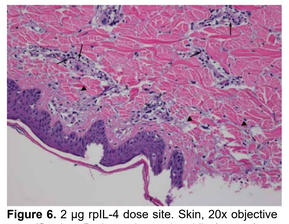

Figure 6. 2 µg rpIL-4 dose site. Skin, 20x objective magnification. Perivascular eosinophils (arrows) are present surrounding capillaries in the superficial dermis. Faintly basophilic material (edema) separates collagen fibers (arrowheads).

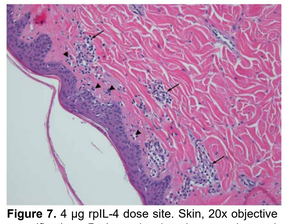

Figure 7. 4 µg rpIL-4 dose site. Skin, 20x objective magnification. Perivascular lymphocytes (arrows) and superficial dermal lymphocytes (arrowheads) are present in the dermis.

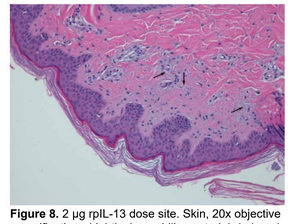

Figure 8. 2 µg rpIL-13 dose site. Skin, 20x objective magnification. Lightly basophilic material (edema) surrounds capillaries and separates collagen fibers in the superficial dermis (arrows).

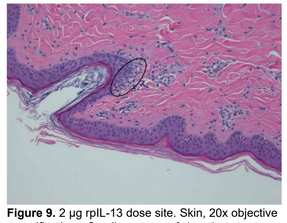

Figure 9. 2 µg rpIL-13 dose site. Skin, 20x objective magnification. Small groups of lymphocytes are present at the dermal-epidermal junction (circled).

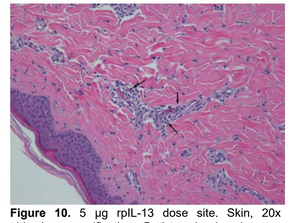

Figure 10. 5 µg rpIL-13 dose site. Skin, 20x objective magnification. Perivascular lymphocytes (arrows) are present surrounding capillaries in the superficial dermis.

DISCUSSION

This study demonstrated *in vitro* and *in vivo* activity of rpIL-4 in Hanford miniature swine, while rpIL-13 did not appear to demonstrate similar effects. These results demonstrate that rpIL-4 can induce signal transduction via STAT-6 phosphorylation in miniature swine PBMC, and ultimately lead to mild inflammation when injected intradermally. The histopathology suggests that intradermal injection of rpIL-4 and rpIL-13 may be capable of recruiting lymphocytes to dermal tissues. While five days of repeat intradermal injections of either rpIL-4 or rpIL-13 did not result in the characteristic lesions associated with atopic dermatitis, histopathology findings demonstrate the potential for biological activity of both cytokines. It may require a longer dosing period, greater dose concentration, or an increase in dosing frequency to create a porcine model of atopic dermatitis.

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