

ORIGINAL ARTICLE

Allergen dose dependency of the early- and late-phase cutaneous response in the cynomolgus monkey

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Summary

Background Cutaneous administration of allergen provides a means to confirm an allergic status, investigate the pathogenesis of allergic diseases, and/or provide a mechanism to evaluate the benefit of new potential therapeutics.

Objective Studies were performed to characterize the allergen-induced cutaneous early- and late-phase response (EPR and LPR) in the cynomolgus monkey.

Methods Following intradermal injections of *Ascaris suum* allergen, the cutaneous weal and flare EPR was measured 15 min post-injection, and skin biopsies were collected at 8–24 h to determine the optimal time of LPR occurrence. Biopsies were analysed for epidermal and dermal inflammatory changes.

Results The EPR was dose related with a reproducible, measurable response at 1 : 10 000 and maximal at a 1 : 100 allergen dilution. In contrast, the threshold dose required for a reproducible LPR was much greater requiring a dilution of 6 : 100, suggesting independent mechanisms for the EPR and LPR. The LPR 20 h post-allergen injection induced an inflammatory response in the upper and deep dermis. The response was characterized by a moderate perivascular to diffuse inflammation consisting of mononuclear cells, neutrophils and eosinophils. Dexamethasone, while having no effect on the EPR, reduced dermal inflammation (upper dermis, $P = 0.004$; deep dermis, $P = 0.03$). Similarly, dermal eosinophilia was also reduced (upper dermis, $P < 0.001$; deep dermis, $P = 0.02$).

Conclusion Collectively, the results indicate the dose dependency of the EPR and LPR. Furthermore, our observations indicate the value of the LPR response in the cynomolgus monkey to evaluate new therapeutics for the treatment of allergic diseases such as atopic dermatitis.

Keywords allergen, cutaneous, cynomolgus monkey, early-phase response, late-phase response

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Introduction

Allergic (IgE-mediated hypersensitivity) diseases affect approximately 20% of the population of industrialized countries and cause a broad spectrum of symptomology that manifest as diseases such as allergic asthma, atopic dermatitis, and allergic rhinitis [1, 2]. The controlled application of allergen has been shown to be useful as a clinical and preclinical research tool [3–11]. Administration of allergen via the inhaled, cutaneous, or nasal routes can provide a means to confirm an allergic status, investigate the pathogenesis of allergic diseases, and provide a mechanism to evaluate the benefit of new potential therapeutics.

Injection of allergen into the dermis of sensitized atopic individuals is associated with a rapidly developing early-phase response (EPR) characterized by the appearance of a

weal and flare on the skin, usually peaking within 15–30 min [12]. This EPR is triggered by the cross-linking of IgE on mast cells resulting in the release of chemical mediators such as histamine [13]. The EPR is often followed after several (6–24) hours by a delayed and sustained local inflammation termed the late-phase response (LPR). This secondary LPR is characterized by the infiltration of activated inflammatory cells, particularly eosinophils and $CD4^+$ T cells [11, 13–15]. The EPR and LPR are not limited to the skin. Equally, following allergen inhalation in asthmatics, an early- and late-asthmatic response, with similar inflammatory changes may be induced [3, 7, 16].

The LPR is of interest because of the similarity to the clinical manifestations of chronic allergic diseases such as asthma, and atopic dermatitis. The LPR in the skin and

airways has been used as a model, both preclinically and clinically, to evaluate candidate therapeutics such as corticosteroids, anti-leukotrienes, and anti-IgE therapies [3, 7, 17–21].

Many new protein therapeutics are limited in preclinical evaluation because of poor species cross-reactivity. New preclinical animal models with greater homology to humans will improve the evaluation of protein therapeutics, and are considered immensely valuable to development efforts.

Non-human primates (NHP), with their genetic and physiological similarities to man, provided an opportunity for evaluating protein therapeutics that do not cross-react in rodent species. Several NHP models of allergic disease focusing on the airways have been reported. Rhesus and cynomolgus monkeys have been utilized extensively with *Ascaris suum* protein due to their natural sensitivity to the nematode extract [8–10, 22, 23]. Other examples of allergen challenge models include exposure to house dust mite and birch pollen but only after lengthy sensitization periods [9, 22, 24]. All these models, independent of allergen used, demonstrate prolonged sensitivity of the airways represented by increases in airway hyperresponsiveness, and airway eosinophils. Although less widely evaluated, the dual EPR and LPR in the lung has also been demonstrated following allergen provocation and has been utilized as a model to assess anti-inflammatory therapies [8, 25, 26].

In contrast, there are few reports of the cutaneous allergic response in NHP, and those reports of the allergic skin response have focused on the EPR results which, in general, are used qualitatively to assess sensitization to an allergen rather than quantitatively as a model of allergic cutaneous disease [9, 10]. Moreover, to our knowledge, there are no published reports of the cutaneous LPR in monkeys.

In the present study, we have characterized the allergen-induced cutaneous EPR, and for the first time, the LPR in the cynomolgus monkey. Animals showing a natural sensitivity to a protein extract of *A. suum* were selected for study. Studies were performed to establish the allergen dose required to develop the cutaneous EPR and LPR. The inflammatory characteristics of the LPR were characterized in biopsy tissues, and the effect of dexamethasone treatment was evaluated. Collectively, the results indicate the LPR to *A. suum* extract in the cynomolgus monkey is a reproducible model that can be utilized in the assessment of allergic diseases.

Methods

Studies were performed to determine the optimal dose of allergen and time required to establish a measurable early- and late-phase cutaneous response in the monkey. Briefly, animals were anaesthetized, and blood drawn for baseline complete blood count. Various doses of allergen (*A. suum*, 1 : 1 000 000–1 : 10), histamine (25 µg/mL) and

phosphate-buffered saline (PBS) were injected intradermally (i.d.), and the EPR measured at 15 min, as detailed below. Animals were allowed to recover then returned to their cages. In order to assess the LPR, animals were anaesthetized again at a single time-point 8–24 h post-allergen injection and skin biopsy samples were collected and processed for histological and immunohistochemical analysis of tissue inflammation. Preliminary investigations with limited animal numbers were performed to evaluate the timing of the LPR. The time window chosen for investigation was based on literature evidence [8, 16]. In early investigations, biopsies were taken 4 h apart at 8, 12, 16, 20, and 24 h using multiple doses of *A. suum*. For a more thorough evaluation of the LPR, a time-point (20 h) giving a robust reproducible response was chosen for evaluation.

Following the determination of the allergen dose-response and time course of the early and late cutaneous responses, treatment studies were performed to evaluate the effect of acute administration of dexamethasone, a corticosteroid with anti-inflammatory properties, on the allergen-induced response.

Animals

These studies used a colony of 34 adult (6–11 kg) male, wild-caught Mauritius cynomolgus monkeys (*Macaca fascicularis*) with a natural and demonstrated sensitivity to a protein extract of *A. suum*. The monkeys were selected from a larger colony of male cynomolgus monkeys that were originally identified as *A. suum* sensitive by skin testing (1 : 100 dilution). The colony was comprised of only male monkeys to reduce the effects of the female hormone cycles and diminish the associated behavioural issues. Experiments were performed in compliance with the Bayer Healthcare Institutional Animal Care and Use Committee (Berkeley, CA, USA).

Anaesthesia and animal preparation

Animals were anaesthetized for allergen administration and biopsy sampling. On each experimental day, monkeys were anaesthetized with ketamine (40 mg, Ft Dodge, Fort Dodge, IA, USA) combined with medetomidine (0.2 mg, Pfizer, New York, NY, USA) by intramuscular (i.m.) injection (0.6 mL/monkey). Before allergen injection the chest and abdominal area was shaved then cleaned with 70% ethanol and dilute Nolvasan solution™.

Allergen injection

A. suum extract [Lot # XPB33-X10, 1301.8 mg/vial dry weight (dw), Greer Labs Inc., Lenoir, NC, USA] was purchased as a defatted, lyophilized cake. A stock solution (26.04 mg dw/mL) was generated by adding 50 mL of sterile water for

injection to the lyophilized cake. *A. suum* stock solution was diluted further in PBS to achieve 1 : 10 (2.60 mg dw/mL), 6 : 100 (1.56 mg dw/mL), 3 : 100 (0.78 mg dw/mL), 1 : 100 (0.26 mg dw/mL), 1 : 10 000 (2.60 µg dw/mL), and 1 : 1 000 000 (26.00 ng dw/mL) dilutions.

Intradermal injections (40 µL using a 0.5 mL insulin syringe, 28 G, 0.5 in. needle) of allergen *A. suum* extract, PBS (Gibco BRL, Grand Island, NY, USA, Lot # 21031185), and histamine dihydrochloride (25 µg/mL, Sigma, St Louis, MO, USA # D-7250) were administered to the thoracic region of each animal.

Animals were exposed to *A. suum* up to four occasions; each occasion was separated by a minimum of 5 weeks. Animals received no more than two different dose levels of *A. suum* on each dosing occasion. Injection sites were separated at least 12 cm apart on occasions where two doses of allergen were administered simultaneously.

Measurement of weal and flare and clinical score

The area of the weal and flare were measured immediately after and again approximately 15 min after each allergen injection. A modified method was developed which assigned a clinical score of 1.0 (white and flat), 1.5 (pink and raised), or 2.0 (red and raised) and was assigned at the time of measurements [27].

For each time-point, the acute response was assessed as the product of the weal and flare area measured using calipers (length × width), and severity score measured 15 min post-allergen injection. The response was corrected by subtraction of the response immediately following the i.d. allergen injection.

EPR were recorded to assess a positive reaction of each animal to allergen. Histamine was used as an indicator of positive weal and flare skin reaction. Subsequent LPR were followed by placing a reference mark on the skin next to the injection site location.

Biopsy recovery and histopathology preparation

Biopsies were obtained 8–24 h following allergen injection to assess cellular infiltrate at the site of injection during a window of time believed to represent the LPR. Two 3 mm biopsies (Unipunch, Henry Schein, Indianapolis, IN, USA, # 378–5499) were obtained from tissue at the injection site for each concentration of allergen, as well as for the PBS and histamine controls. The formalin-fixed tissues were processed and embedded in paraffin then 5 µm sections were cut and mounted on slides, deparaffinized, and stained with haematoxylin and eosin (H & E) for analysis.

Immunocytochemistry

Tissues randomly selected from the allergen 6 : 100 dilution, 20 h time-point group were processed for immunocyto-

chemical staining. Tissue was stained for CD4 as markers of T cells and CD25 as a marker of T cell activation. Briefly, tissue was cut and mounted on charged glass slides. Next, endogenous peroxidase in the tissue was blocked with H₂O₂ for 5 min, then slides were fixed in 10% neutral buffered formalin for 60 min, rinsed in dH₂O, steamed in pH 10 Tris buffer for 30 min, cooled for 10 min, rinsed in dH₂O, then washed with PBS with Tween 20. Next the slides were processed for CD4⁺ T cell staining (clone 1F6, LabVision, Fremont, CA, USA, #NCL-CD4-1F6) at 1 : 5 (Tris/BSA) for 40 min, Mouse Envision+ for 30 min, diaminobenzidine for 5 min. A similar procedure for CD25⁺ cells (clone 4C9, Novocastra, Newcastle, Upon Tyne, UK, # NCL-CD25-305) was followed at a 1 : 50 dilution as a marker for T cell activation. Positive cells stained brown in colour. Slides were counterstained with H & E.

Administration of dexamethasone

During treatment studies, animals were dosed 1 h before allergen injection with either dexamethasone 21 phosphate (1 mg/kg, i.m. Sigma; D-1159, Lot # 112K1026) or vehicle.

Histopathology analysis

All histopathology analysis was performed blinded by a qualified veterinary pathologist. H & E slides were graded on a 0–4 scale for the severity of the inflammatory response where 1, minimal; 2, mild; 3, moderate; and 4, was defined as a severe response [27, 28]. The tissue was divided into two regions, superficial (or upper) dermis, and deep dermis which included the dermal fat. Each region was scored based on the severity of the inflammation. The inflammatory infiltrate was also described as either predominantly perivascular (surrounding blood vessels) or diffuse (distributed throughout the dermis away from blood vessels). Giemsa stains were applied to additional sections of the skin to clarify the eosinophil response. The numbers of eosinophils were quantified per high-power field for both superficial and deep dermis. Any further histopathological findings such as the presence of oedema were also described. In addition, the number of positive staining CD4⁺ and CD25⁺ cells in the dermis and epidermis were assessed. The positive cells were graded on a scale of 0–4 with one being minimal numbers of positive cells and four large numbers of positive cells.

Statistical analysis

Histology scores were presented as median and compared using either Wilcoxon's signed rank test or Mann–Whitney non-parametric test (as indicated). Eosinophil numbers were presented as the mean and analysed utilizing unpaired or paired Student's *t*-test, when data were considered normally distributed (Kolmogorov–Smirnov test). Weal and flare data were compared by one-way ANOVA and Dunnett's post-test.

All statistical analysis was performed using Prism Graph Pad, version 4.0.

Results

Weal and flare early-phase response

Preliminary studies were performed to evaluate the allergen dose required for the EPR and LPR. *A. suum* allergen induced a weal and flare EPR developing rapidly, peaking within 15–30 min in all monkeys. The response to allergen, measured at 15 min was dose dependent. There was no significant evidence of weal and flare formation at an allergen dilution of 1 : 1 000 000 [19.2 mm², –11.3–49.6 confidence interval (CI), $n = 4$, $P > 0.05$] when compared with PBS injection. Increasing the allergen dose to a 1 : 10 000 dilution resulted in a measurable EPR (66.11 mm², 43.1–89.1 CI, $n = 10$, $P < 0.01$ vs. PBS), with a maximal response at 1 : 100 allergen dilution (130.11 mm², 101.9–159.3 CI, $n = 12$, $P < 0.01$ vs. PBS) compared with the PBS (4.73 mm², 0.5–9.0 CI, $n = 30$) control injections. Further increasing the allergen dose to dilutions of 3 : 100, 6 : 100, and 1 : 10 had no additional effect on the size of the weal and flare EPR (Fig. 1). Histamine used as a positive control generated a weal and flare response comparable to 1 : 100 allergen dilution in all monkeys.

Late-phase response

The EPR in the histamine and lower dose (1 : 1 000 000, 1 : 10 000) allergen groups, faded within 1–2 h of

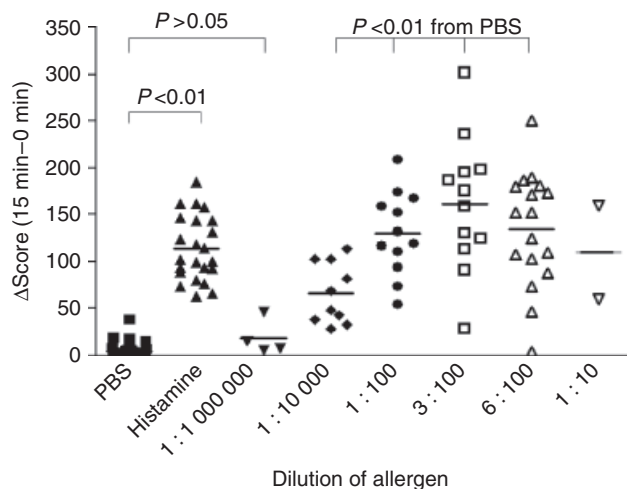


Fig. 1. Intradermal injection of *Ascaris suum* extract produced a dose-dependent weal and flare early-phase response (EPR). The response was assessed as the product of the weal and flare area (length × width), and severity score measured 15 min post-allergen injection, corrected by subtraction of the response immediately following allergen injection. An allergen dose of 1 : 10 000 dilution resulted in a measurable EPR ($P < 0.01$), with a maximal response at 1 : 100 allergen dilution ($P < 0.01$). Data shown as mean, one-way ANOVA and Dunnett's post-test ($n = 2$ –30/group).

allergen administration, with no evidence of a LPR. At an allergen dilution of 1 : 100, a dose sufficient to induce a robust EPR, evidence of an LPR was largely absent ($n = 12$). Because LPR was not observed at the 1 : 100 dose, allergen dose was further increased (3 : 100, 6 : 100) to a level producing a maximal EPR. At these dilutions, the erythema and oedema was more persistent being evident 24 h later in some animals. All animals ($n = 2$) that received the 1 : 10 dilution had systemic reactions characterized by dyspnoea. Further analysis at this dose level was discontinued.

Initial pilot experiments demonstrated increased inflammation from very minimal at 8 h to mostly mild at 12 h to some moderate responses as investigations progressed in time to 24 h. At the earlier time-points, 8–12 h, the cellular examination uncovered a few mainly perivascular neutrophils, and a rare appearance of eosinophils and mononuclear cells in the superficial dermis. In the deeper dermis a more minimal to mild dispersed inflammation was observed consisting of similar cell types. By later time-points (16–24 h) the inflammation was more moderate in severity and characterized by more dispersed perivascular cells in both the superficial dermis and deeper dermis. Further evaluation of biopsies 20 h post-injection revealed a moderate inflammatory response in the upper dermis and a mild/moderate response in the deep dermis when compared with PBS (upper dermis $P = 0.031$, deep dermis $P = 0.031$, $n = 5$) (Fig. 2a). At 20 h, the inflammatory response was more dispersed than the earlier time-points, and was characterized by perivascular to diffuse inflammation consisting of a mix of neutrophils, with a larger infiltration of mononuclear cells and eosinophils (Fig. 2b and c). Eosinophil counts per high-power field from the upper dermis were significantly increased ($P = 0.03$, $n = 4$) as compared with PBS, while the increase in eosinophils in the deeper dermis were not significant (Fig. 3). Further preliminary immunohistochemical characterization of the inflammatory response demonstrated a trend towards increased dermal CD4⁺ T cells at 20 h ($P = 0.057$ vs. PBS, $n = 4$). This was paralleled by a trend in increased CD25⁺ cells ($P = 0.057$ vs. PBS, $n = 4$).

Treatment with dexamethasone

Following the determination of allergen dose and timing of the EPR and LPR, studies were performed to evaluate the effect of acute administration of dexamethasone on the allergen response at a dilution of 6 : 100. Dexamethasone (1 mg/kg, i.m.) administered 1 h before allergen challenge had no effect on the EPR (data not shown). In contrast, dexamethasone significantly ($P = 0.004$ and 0.03, $n = 5$ –6) reduced dermal inflammation when compared with vehicle in the upper dermis and deep dermis,

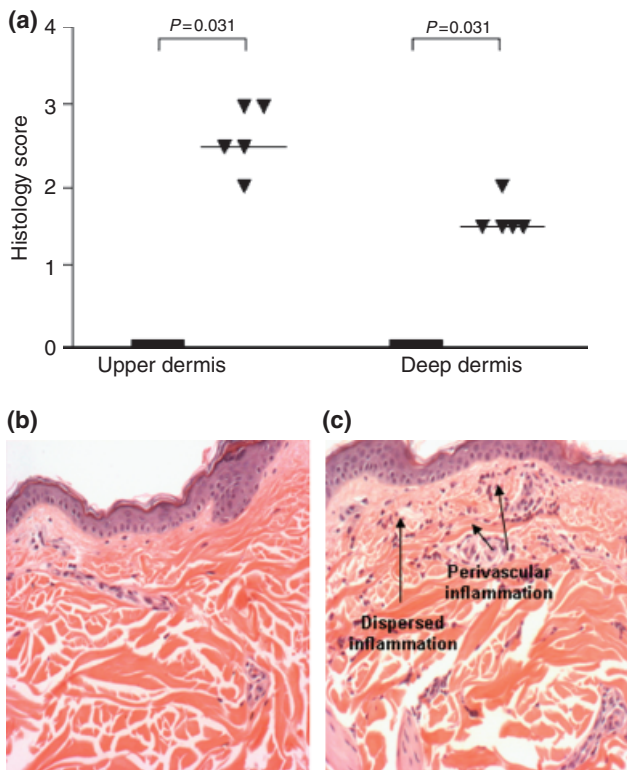


Fig. 2. Histological assessment of the late-phase response 20 h following allergen (6:100) injection (i.d.) induced a moderate inflammatory response in the upper dermis and a mild response in the deep dermis that was significantly different (upper dermis $P=0.031$, deep dermis $P=0.031$) compared with PBS (a). Haematoxylin and eosin (H & E) slides were graded on a 0–4 scale for the severity of the inflammatory response, where 1, minimal; 2, mild; 3, moderate; and 4, defined a severe response. PBS (■), *Ascaris suum* extract (▼). Data shown as median, Wilcoxon signed rank test ($n=5$ /group). Allergen (*A. suum* extract 6:100, i.d.) exposure produced a LPR in the dermis at 20 h characterized by perivascular to diffuse inflammation consisting of mononuclear cells, neutrophils and eosinophils. PBS (b), *Ascaris* (c). Biopsies were fixed in formalin, and sections stained with H&E (magnification is $\times 200$).

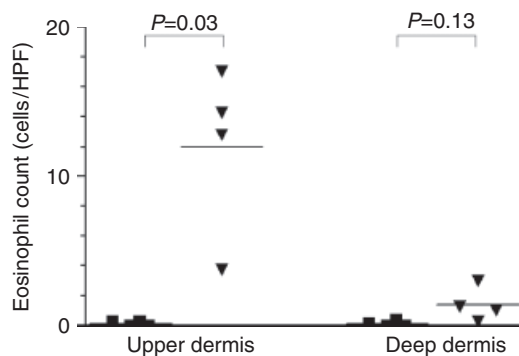


Fig. 3. Dermal eosinophilia was increased 20 h post-allergen (*Ascaris suum* extract, 6:100, i.d.) challenge. PBS (■), *A. suum* extract (▼). Giemsa stains were applied to skin biopsy sections to highlight eosinophils, and the numbers of eosinophils were quantified per high-power field for both superficial and deep dermis ($P=0.03$ and 0.13 , respectively). Data shown as mean, Student's *t*-test ($n=4$ /group).

respectively, at the 20 h LPR time-point (Fig. 4). Similarly, dermal eosinophilia was significantly reduced by dexamethasone when compared with vehicle (citrate buffer) in both upper dermis by 97% and deep dermis by 70% ($P<0.001$ and $P=0.02$, $n=5-6$) (Fig. 5).

Discussion

Preclinical assessment of protein therapeutics is often limited because of poor species cross-reactivity. Thus, new animal models with greater homology to humans, allowing evaluation of candidate protein therapeutics, are of considerable value. The present study was initiated to evaluate the cutaneous response of cynomolgus monkeys with a natural sensitivity to a protein extract of the parasite *A. suum*. Intradermal administration of *A. suum* allergen induced a significant weal and flare EPR within 15 min of allergen injection, and is consistent with previous reports of the *Ascaris*-induced cutaneous weal and flare response in *Ascaris*-sensitive rhesus monkeys [10]. In the present study, the response to allergen was dose dependent, with a no effect level at an allergen dilution of 1:1 000 000, and producing a maximal response at a 1:100 dilution. The observed dose dependency of the allergen-induced EPR is consistent with previously reported clinical studies, and in the form of the allergen skin prick test, is used to help select the starting concentration for clinical allergen inhalation studies [3, 6, 7, 29]. It is generally accepted that allergen-specific IgE plays a primary role in the acute EPR. Indeed, a positive cutaneous or lung response to *Ascaris* allergen in the monkey is associated with elevated IgE levels [10]. Cross-linking of cell-bound IgE on the surface of mast cells with the specific allergen causes degranulation of the cells and the release of several proinflammatory mediators (e.g. histamine, leukotrienes, prostaglandins, tryptase) and cytokines that result, within minutes, in the oedema and erythema of the weal and flare EPR [21, 30]. Although not clearly defined for the cutaneous response, the early-phase airway obstruction following *Ascaris* allergen inhalation in the monkey is associated with the release of histamine, LTC₄ and PGD₂ into the airways, consistent with mast cell activation [25].

In a proportion (~50–60%) of atopic individuals, the allergen-induced cutaneous EPR develops into a more sustained (6–24 h) local LPR [6, 31–33]. Likewise, following allergen inhalation in asthmatics, a similar proportion of patients develop a late response following the early asthmatic response, although the dual asthmatic response is generally more biphasic in nature than the EPR/LPR skin response [3, 7, 16].

In the present study, the EPR to histamine and lower doses of allergen faded within 1–2 h of allergen administration, with no evidence of a LPR. Even at an allergen dilution of 1:100, a dose sufficient to produce a maximal EPR, evidence of an LPR was largely absent, requiring a further increase in allergen dose (6:100) to produce a

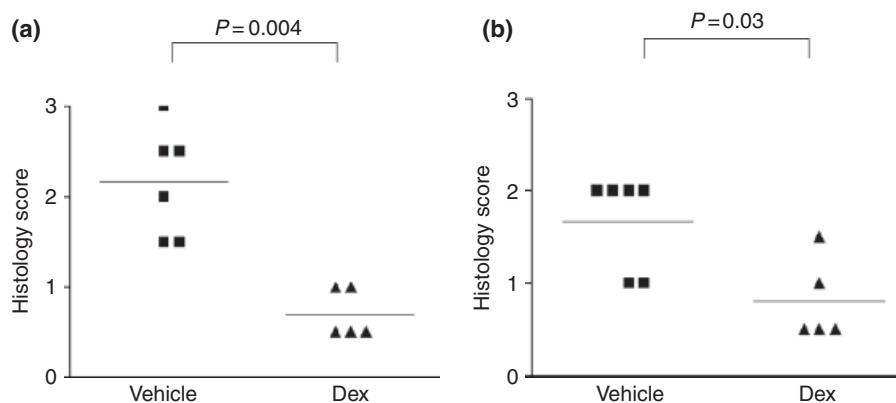


Fig. 4. Dexamethasone (Dex, 1 mg/kg, i.m.) (▲) compared with vehicle (■), significantly inhibited the allergen-induced (*Ascaris suum* extract 6 : 100, i.d.) late-phase (20 h) inflammatory response in the upper (a) and deep (b) dermis ($P = 0.004$ and 0.03 , respectively). Hematoxylin and eosin slides were graded on a 0–4 scale for the severity of the inflammatory response, where 1, minimal; 2, mild; 3, moderate; and 4, defined a severe response. Data shown as median, Mann–Whitney test ($n = 5$ –6/group).

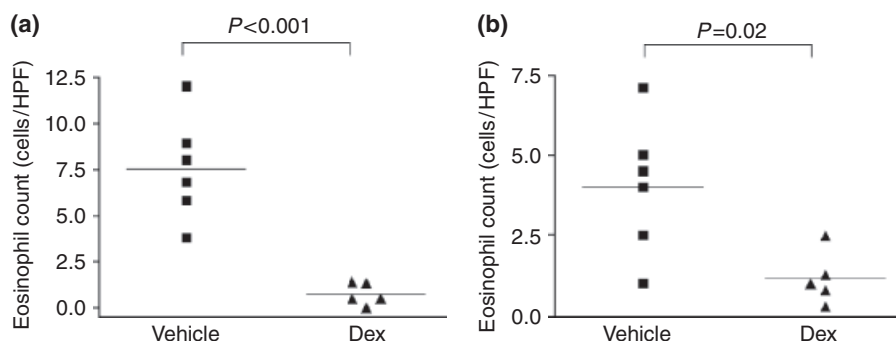


Fig. 5. Dexamethasone (1 mg/kg, i.m.) (▲) compared with vehicle (■) significantly inhibited the allergen-induced (*Ascaris suum* extract 6 : 100, i.d.) late-phase (20 h) eosinophil response in the upper (a) and deep (b) dermis ($P < 0.001$ and $P = 0.02$, respectively). Giemsa stains were applied to skin biopsy sections to highlight eosinophils, and the numbers of eosinophils were quantified per high-power field for both superficial and deep dermis. Data shown as mean, unpaired Student's t -test ($n = 5$ –6/group).

more persistent LPR. Although we are the first, to our knowledge, to report on the cutaneous LPR in the NHP, the late-phase airway obstruction associated with BAL neutrophil infiltrate and eosinophil activation has been reported in the NHP following *Ascaris* allergen challenge [8, 25, 26]. Interestingly, in one study, single EPR responders and dual EPR/LPR responding monkeys were compared, however, it is unclear if the dose of allergen was titrated to achieve animals showing either the EPR alone or both EPR and LPR, and whether increasing the dose of allergen converted the EPR responders to dual responding animals [8].

The results of the current study demonstrate the dose dependence of the LPR and indicate the dose of allergen required to induce the LPR is quite different to that required for the EPR. As discussed previously, several clinical studies report that only a proportion (~60%) of atopic patients go on to develop a LPR following the EPR, and this is true for both cutaneous and airway responses

[3, 6, 7, 16, 31–33]. However, consistent with the findings of the current study, in atopic individuals previously only showing a cutaneous or asthmatic EPR, increasing the allergen dose can induce a LPR response [6, 34, 35]. The difference in allergen dose threshold for the EPR and LPR observed suggest that the mechanism of the two reactions to be somewhat independent, whereby exceeding a threshold dose of allergen may activate other cell types, notably antigen presenting cells (APCs) and $CD4^+$ T cells, driving the LPR. Thus, although the LPR typically follow the IgE–mast cell-mediated EPR, the requirement for IgE and mast cells to establish the LPR is less apparent. Certainly, through the release of lipid mediators and cytokines, particularly $TNF-\alpha$, mast cells may contribute to the full expression of the LPR, and the role of IgE is supported by the presence of IgE bound APCs in late-phase cutaneous biopsies [36, 37]. However, isolated late asthmatic responses may be induced in a T cell-dependent, IgE-independent manner [38]. In addition, mast cell stabilizing compounds,

while inhibiting the EPR have little or no effect on the LPR, while corticosteroids, as discussed below, inhibit the LPR without affecting the EPR [39].

Preliminary studies with a limited sample size (data not shown), indicated the presence of a minimal to mild dermal inflammatory infiltrate consisting of a few neutrophils, and an occasional eosinophil and monocyte at the earliest time-points (8–12 h) investigated following allergen injection in the monkey. At later time-points (16–20 h) post-allergen injection, a more moderate perivascular to diffuse inflammation was observed. The cellular infiltrate consisted of more mixed eosinophils, neutrophils, and mononuclear cells. The inflammation was further characterized by the presence of CD4⁺ T cells paralleled by an increase in CD25⁺ cells, suggesting activation of the infiltrating CD4⁺ T cells. These observations are consistent with the cutaneous LPR of atopic individuals, typified by an inflammatory cell infiltrate at the allergic reaction site within 6 h persisting for at least 48 h, with granulocytes prominent at 6 h and mononuclear cells after 24 h [4, 6, 31, 33, 40].

The allergen-induced LPR is of particular interest, as the LPR demonstrates many inflammatory features associated with chronic allergic diseases such as atopic dermatitis and asthma. Indeed, the LPR in both the skin and lung has been used to evaluate the pathogenesis of allergic diseases and, with some success, to predict the potential benefit of various therapeutic approaches [3]. Topical corticosteroids are a mainstay therapy for atopic dermatitis and asthma, while systemic corticosteroids are used for more severe disease exacerbations [41–44]. In the present study, we evaluated the effect of the corticosteroid, dexamethasone, on the monkey cutaneous EPR and LPR. A single i.m. dose of dexamethasone, administered 1 h before allergen, significantly reduced the LPR inflammatory response, but was without effect on the weal and flare EPR. These observations are consistent with several clinical studies which have shown a single dose of corticosteroid, administered before allergen provocation, effectively reduces the cutaneous LPR and associated inflammatory components, without affecting the EPR [12, 45–48]. In the *Ascaris*-induced dual EPR/LPR airway obstruction in monkeys, multiple treatment with dexamethasone effectively inhibited the LPR, consistent with our observations in the skin [8]. However, in contrast to the current study, the EPR airway obstruction was also reduced. This could reflect the difference in the corticosteroid treatment regimen. Based on existing literature, effects of steroid on the EPR may not be expected, albeit they have been observed in some studies [49, 50]. Taken together, these results indicate the potential value of the monkey cutaneous LPR model to evaluate therapeutic candidates for the treatment of allergic diseases.

In summary, the present study characterizes the allergen-induced cutaneous ERP and, for the first time, LPR in the

cynomolgus monkey. The data demonstrate the dose dependency of the EPR and reveal the difference in allergen dose threshold for the EPR and LPR, confirming independent mechanisms driving the different responses. The LPR response was characterized by inflammatory changes similar to those observed in the LPR of individuals with atopic dermatitis, and the response could be modulated by corticosteroids. Collectively, these results indicate that the cutaneous LPR response in the cynomolgus monkey may be a useful model to evaluate new therapeutics for allergic diseases particularly atopic dermatitis.

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