

# Atopic dermatitis with possible polysensitization and monkey esophagus reactivity

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## Abstract

**Context:** Atopic dermatitis is a chronic inflammatory skin disease resulting from interactions between environmental and genetic factors. Recent studies link atopic dermatitis with asthma and with eosinophilic esophagitis. **Case Report:** Based on this association, we investigated by indirect immunofluorescence the immunoreactivity patterns on monkey esophagus substrate utilizing the serum of a patient with severe atopic dermatitis. We also examined the patient's skin biopsy by H&E histology and immunohistochemistry. We detected strong deposits of albumin, IgE, IgG, IgD, IgA, Complement/C1q and mast cell tryptase in multiples structures of the skin, as well as a broad pattern of intraepithelial staining on monkey esophagus. Strong staining positivity was also detected within the inflammatory infiltrate around the upper dermal vessels, as well as additional positive staining for the human leukocyte antigen system antigens DR DP and DQ. **Conclusion:** Our findings demonstrate that there could be an indication for testing patients with severe atopic dermatitis for autoreactivity to filaggrin (anti-keratin antibodies) utilizing monkey esophagus. Larger studies are needed to clarify any immunologic interaction between the reactivity to albumin and food allergens that may sensitize patients via the esophageal mucosa.

**Keywords:** atopic dermatitis, monkey esophagus, albumin, vessels, HLA DRDPDQ, IgD, eosinophilic esophagitis, filaggrin.

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## Introduction

Atopic dermatitis (AD) is a multifactorial skin disease with a complex etiology including genetic, altered skin structure, immunologic, psychological and environmental components [1]. There are two main subtypes of AD, i.e. the IgE-associated type ("extrinsic atopic eczema") and the non-IgE-associated type ("intrinsic atopic eczema"); these subtypes display different prognoses relative to the development of respiratory diseases [1]. The role of allergens in AD also displays chronologic variation; thus, in early childhood, food allergens often play a significant role, in contradistinction to the relevance of mites and microbial antigens in adolescence and adulthood. Recently, it was demonstrated that filaggrin represents a major associated gene locus for atopic eczema. [1]. Filaggrin antibodies (also known as anti-keratin antibodies) are found in 30-40% of patients with rheumatoid arthritis by

indirect immunofluorescence (IIF) using monkey and/or rat esophagus substrates. These antibodies appear to be predominately intraepithelial. In addition, although presence of the antibody represents a fairly specific prognostic factor, its absence in many patients makes it less useful in many patient evaluations than traditional rheumatoid factor assays.

Eosinophilic esophagitis (EE) has been associated with AD, asthma and allergic rhinitis [2]. All of these disorders have allergen triggers that could be food related [2]. Little is known about how EE may be immunologically linked with atopy. For instance, infants may be sensitized to dietary antigens even during exclusive breast feeding. Thus, food antigen traces in breast milk may have harmful effects on gut barrier function in infants with atopy [3]. Many researchers have tried to find a correlation with atopic dermatitis in the initial years of either breast or

formula feeding (with or without hypoallergenic food and/or hypoallergenic formula) [3]. No study has reported significant immunologic reactivity in the esophagus that correlates with skin findings in patients with recalcitrant atopic dermatitis. Here we report a case of strong immunologic reactivity on monkey esophagus using an AD patient sera and correlating with findings on skin hematoxylin and eosin (H&E) histology review and immunohistochemistry (IHC) evaluation.

## Case Report

A 30 year old Hispanic female from Venezuela was diagnosed with severe atopic dermatitis. The patient had a history of atopic rhinitis, ocular conjunctivitis and asthma since early childhood. As a child, intolerance to lactose was discovered early, and her food was supplemented with soy milk. Frequent hospitalizations were part of her early life. Most of the hospitalizations episodes resulted in systemic and topical corticosteroid therapy. In addition, multiple antibiotics were given due to impetiginization of the lesions. Radioallergosorbent (RAST) tests were performed on the patient's blood to determine specific allergenic substances. Of note, the RAST test is different from a skin allergy test, which determines allergen sensitivity due to the reaction of the patient's skin to specific test substances; and a skin allergy test was also performed. These tests revealed that the patient was reactive to polyester, wool, tomatoes, chocolate, milk, eggs, banana, cheese, fish, crab, shrimp, butter, *Dermatophagoides pteronissinus* and *farinae*, and dog and cat hair. The serum levels of immunoglobulin E (IgE), and eosinophils were also significantly elevated. The patient always had very dry skin, with scaly patches, plaques, and eczematous plaques around the ears. Upon relocation to a warmer geographic area, her skin lesions exacerbated. The patient was properly treated with skin emollients, corticosteroids, and antihistamines for almost 30 years. Although the patient denied any severe heartburn, difficulty swallowing, esophageal food impaction, nausea, vomiting or weight loss, we decided to test her serum against monkey esophagus (ME), as is routinely performed during IIF testing for patients with autoimmune blistering diseases. It was recommended that the patient not take or apply any immunosuppressant agents for at least 15 days prior to collecting our relevant skin and serum samples. All immunological tests were performed in the USA.

## Methods

### *Hematoxylin and eosin*

All tissue samples were processed in an automated Tissue Tek processing system, and subsequently cut at five micron thickness. Staining of tissue sections was performed in a Shandon XY automated staining system, with Gill 3 hematoxylin and Eosin Y per manufacturer's instructions.

### *Indirect immunofluorescence (IIF)*

Monkey esophagus was obtained from Oregon National

Primate Research Center, (Beaverton, Oregon). Cryosections of 4 micron thickness were prepared and partially fixed for 10 minutes with paraformaldehyde, 4% in phosphate buffer. A further partial solubilization and blockage of nonspecific background was performed with Triton X-100 0.01%, bovine serum albumin 3% and normal goat serum for 15 minutes. The samples were then washed and incubated with the patients sera at 1:100 dilution for one hour at room temperature, and washed twice with the phosphate buffer. A further incubation with multiple fluorescein isothiocyanate (FITC)-conjugated secondary antibodies was then conducted. The secondary antibodies were of rabbit origin and included: a) anti-human IgG ( $\gamma$  chain), b) anti-human IgA ( $\alpha$  chains), c) anti-human IgM ( $\mu$ -chain), d) anti-human fibrinogen, e) anti-human albumin, and f) anti-complement C3 (all utilized at 1:20 to 1:60 dilutions and obtained from Dako (Carpinteria, California, USA). We also utilized secondary antibodies of goat origin, including: a) anti-human IgE antiserum (Vector Laboratories, Bridgeport, New Jersey, USA) and b) anti-human C1q (Southern Biotech, Birmingham, Alabama, USA). Finally, monoclonal FITC conjugated anti-human IgG4 antibody was used to test for possible intercellular staining between keratinocytes (Sigma-Aldrich, Saint Louis, Missouri, USA). We also utilized Alexa Fluor<sup>®</sup>597 conjugated antibodies directed against human IgG (Invitrogen, Carlsbad, California). The slides were counterstained with 4',6-diamidino-2-phenylindole (Dapi) (Pierce, Rockford, Illinois, USA) or with TO-PRO<sup>®</sup>-3/DNA (Invitrogen). Slides were washed, coverslipped, and dried overnight at 4°C. In order to co-localize any reactivity of the vessels with the patient serum, we also utilized antibodies against anti-intercellular adhesion molecule 1 (ICAM-1)/CD54 (Lab Vision Corporation, Thermo Fisher, Fremont, California, USA). As a correlating ICAM/CD54 secondary antibody, we also utilized donkey anti-mouse IgG heavy and light (H and L) chains antisera, conjugated with Alexa Fluor<sup>®</sup>555 (Invitrogen).

### *Immunohistochemical (IHC) stains*

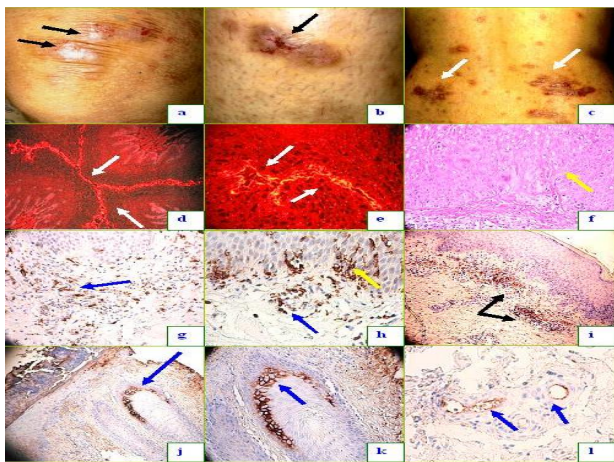
Formalin fixed, paraffin-embedded skin sections were cut and stained. We performed IHC by utilizing a dual endogenous peroxidase blockage according to the Dako insert, with the addition of an Envision dual link. Further, we applied 3, 3 diaminobenzidine(DAB) and counterstained with hematoxylin. The samples were run in a Dako Autostainer Universal Staining System. We tested for rabbit anti-human IgA, IgM, IgG, IgE, IgD, albumin, fibrinogen, Complement/C1q, Complement/C3, HLA DP, DR, DQ and mast cell tryptase (MCT) antibodies. Finally, the nuclei of the cells were counterstained with hematoxylin.

## Results

The figure legends summarize the main findings. H&E stained slides of the skin demonstrated moderate epidermal acanthosis with spongiosis, and some exocytosis of predominately eosinophils (Figs. 1-3). The dermis displayed a moderately florid, superficial,

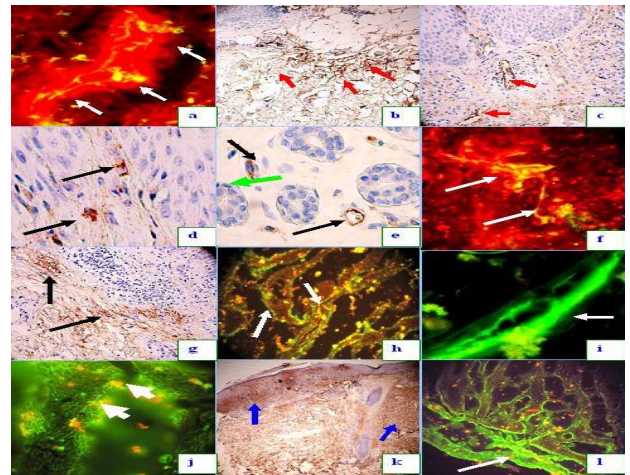
perivascular infiltrate of lymphocytes, histiocytes and occasional eosinophils. Evidence of a vascular allergic component was noted, ie, focal perivascular leukocytoclastic debris was present without frank vasculitis. The leukocytoclasia was confirmed by our IIF and IHC findings. The H&E diagnosis was consistent with a spongiotic dermatitis, with further specific findings of an atopic dermatitis. However, because of the previously described vascular findings, a concomitant diagnosis of a vascular allergic component was suggested. The primary findings on skin IHC staining were the strong presence of IgE at multiple anatomic levels, in addition to strong staining with MCT in these areas. In addition, strong expression of HLA DP, DR, and DQ was observed in the inflammatory infiltrate around the upper dermal vessels. Of interest, the presence of positive staining with IgD and IgA was seen in several small vessels, in addition to deposits of IgG. Strong positivity to albumin was also present in these areas. The albumin staining could not be attributed to background staining, based upon our final antibody dilution of 1:30.000. The presence of this molecule was also seen by IIF on the ME (Figs. 1-3).

On ME review, we detected strong positivity to IgG in multiple layers of the epithelium in a pattern that was both pericytoplasmic and perinuclear. In addition, very strong reactivity against albumin was observed, especially around the outer muscularis layer of the esophagus. Several immunoglobulins were detected within the intimal layer of the esophagus, adjacent to the lumen (Figs. 1-3).

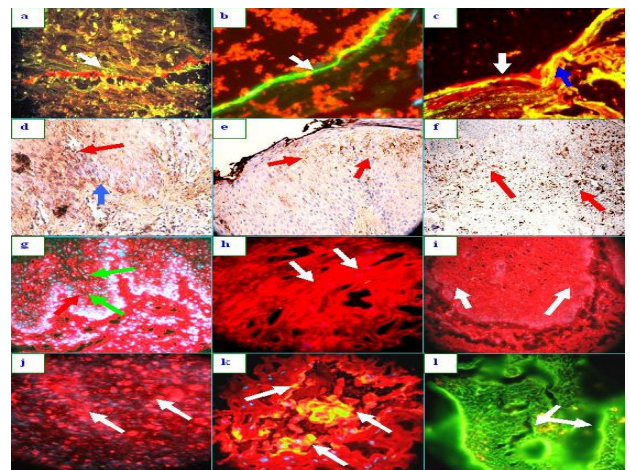


**Fig. 1** Figure 1a through 1c, clinical lesions of excoriated plaques, papules, and plaques in the knees, thighs and lower back, some of them hyperpigmented and some manifesting crusts (black arrows). 1d and e, we tested our patient's serum utilizing monkey esophagus for any reactivity. An IIF using FITC conjugated anti human IgE showed strong positivity against the monkey esophagus ductal epithelium (white arrows). 1f shows an H & E stained section revealing a spongiiform dermatitis in the epidermis. Note 1g through 1l represent IHC stains. In 1g, 1h and 1i, positive staining is noted against IgE (blue arrows). In 1i, positive staining is also present with HLA DR DP and DQ is present in the upper dermal infiltrate (black arrows). In 1j and 1k, note the positive staining against Complement/C3, at the blue arrows (lower and higher amplification respectively). In 1l, note the positive staining with IgD inside some small vessels in the

upper and intermediate dermis.



**Fig. 2** IIF using ME. In 2a, FITC conjugated IgE positive staining against some vessels and also against some inflammatory cells around the vessels (yellow staining) (white arrows). In 2b and 2c, IHC positive staining of some superficial and deep dermal vessels, respectively, utilizing anti-human IgM. In 2d, positive staining of cells to Complement/C1q (black arrows). In 2e, positive staining against the vessels around the sweat glands utilizing anti-human IgD (black arrows). In 2f, IIF on monkey esophagus displays positive staining using FITC conjugated anti-human IgA (green staining) (white arrow). In 2g through 2i, positive staining of some vessels using anti-human fibrinogen 2g (by IHC), and also in 2h and 2i (by IIF). In 2j through 2l, positive staining of several areas of the epidermis and dermis utilizing anti-human albumin.



**Fig. 3a.** Positive staining to vessels using Alexa Fluor 594<sup>®</sup> conjugated anti-human IgG (red staining, white arrows). The yellowish staining is reactivity to FITC conjugated fibrinogen. In 3b. Positive staining to a long vessel using IIF on ME; however, in this case using FITC conjugated anti-human IgG (green staining, white arrows). The red staining represents the nuclei of adjacent cells, stained with Tropro3/DNA<sup>®</sup>. In 3c. ME, with positive staining of the vessels on the periphery with ICAM-1/CD54 (red staining) (white arrow). The yellowish staining was seen with FITC conjugated anti-human albumin (blue arrow), also colocalizing with the vessels reactivity. In 3d. IHC staining against albumin; note some BMZ staining (blue arrow) and some intraepidermal staining between keratinocytes (red arrow). In 3e, IHC staining against IgM; note some intercellular staining between the keratinocytes (red arrows). In 3f, IHC displaying strong positivity of IgE in cells around the



upper vessels of the skin (red arrows, brown staining). In 3g By IIF, ME positive round epithelial cells reactivity when using Alexa Fluor 597<sup>®</sup> anti-human IgE (green arrows). Of note, the staining pattern resembles the positivity of antibodies to filaggrin. The nuclei of the cells were counterstained with Dapi (blue). In 3h and 3i., again IIF on ME Some perinuclear and pericytoplasmic staining of the epithelial layer utilizing Alexa Fluor 597<sup>®</sup> conjugated anti-human IgG (red staining, white arrows). 3j, Similar to 3i, but at higher magnification. In 3k. Positive staining of ME epithelium intimal layer with FITC conjugated antihuman IgE (green staining). The red staining represents positivity of Alexa Fluor 597<sup>®</sup> conjugated anti-human IgG as intranuclear and pericytoplasmic staining in the epithelium of the esophagus (white arrows). In 3l. IIF on ME, displaying positive staining in an unusual pattern in the muscularis layer of the esophagus (green staining, white arrows).

## Discussion

Traditionally, monkey esophagus has been utilized for testing reactivity of serum from patients with skin autoimmune diseases, celiac disease, or rheumatoid arthritis. In our case, we tested patient sera against ME based on the recently described association of AD with eosinophilic esophagitis. Although 1) the patient did not display any history of swallowing problems, and 2) nor was it possible to perform a esophagus biopsy, we cannot exclude that she had some degree of disease sensitization within her esophagus. Such a sensitization could occur in AD patients from sensitization to dietary antigens even early in life during exclusive breast-feeding, as food antigen traces in breast milk may have harmful effects on gut barrier function in infants with AD [4]. Some studies attempted to investigate this issue by comparing infants with atopy fed with breast milk versus those fed a hypoallergenic formula [4]. The authors studied fifty-six infants (mean age, 5.0 months) manifesting atopic eczema during exclusive breast-feeding. They were studied after weaning to a tolerated hypoallergenic formula. The authors reported that in breast-fed infants with atopy, gut barrier function is improved after cessation of breast-feeding and starting of hypoallergenic formula feeding [4].

Another study reported the results of lesional skin biopsies evaluated with immunofluorescent studies from patients with contact dermatitis (positive patch tests), atopic dermatitis and allergic vasculitis compared with both normal-appearing skin from the same patients, and from healthy controls [5]. A variety of deposits of immunoglobulins, complement components and fibrinogen were demonstrated in 6 out of 20 patients with contact dermatitis, 7 out of 10 with atopic dermatitis, 8 out of 10 with allergic vasculitis, and in 4 out of 20 control individuals [5]. No discrete diagnostic pattern of the deposits was found. Elevated serum IgE and eosinophil counts were found in patients with atopic dermatitis, and high serum IgA and fibrinogen levels were found in the allergic vasculitis group [5].

Other authors reported the presence, distribution pattern, and numbers of immunoglobulin and complement-positive lymphocytes in twenty nine biopsies from patients with AD

[6]. In all cases IgE-positive lymphocytes were present in both clinically uninvolved and involved skin. IgG, IgM and IgE-positive lymphocytes displayed a characteristic distribution pattern in the dermis [6]. As shown in our study, earlier authors have also described the association between severe AD and both 1) HLA class II alleles and 2) high serum IgE levels and had documented disease state increases in these molecules [7].

We suggest that our findings (when testing reactivity of the patient's sera against monkey esophagus) might demonstrate immunologic features of a polysensitization; specifically, these findings might explain the patient's long history of "food allergy" associated with her atopic dermatitis. Based on the fact that an esophagus biopsy was not available, a diagnosis of eosinophilic esophagitis (EE) could not be confirmed nor excluded. EE is a clinicopathological disorder with distinctive features. It is etiologically associated with hypersensitivity to airborne allergens and/or dietary components [8]. However, immediate hypersensitivity to foods has rarely been proven as the etiologic cause of the disorder [8]. EE usually presents concomitantly with rhinoconjunctivitis, allergic asthma, atopic dermatitis and food allergies; patients develop acute dysphagia and vomiting immediately after ingesting some foods [8]. Patients suffering from EE also usually display signs of blood hypereosinophilia, while esophageal manometry reveals a motor disorder characterized by peristalsis and non-propulsive simultaneous waves affecting the lower two-thirds (smooth muscle portion) of the organ [8].

In summary, our case likely represents an overlap of more than one of the types of immunity originally described by Coombs. We speculate that albumin could have become an allergen in this patient, based on her history of food allergies. Alternatively, albumin may serve as a transporter or immune regulator for other molecules of the immune system involved in the complex immune processes manifested in this patient. The pattern of reactivity demonstrated by albumin in the outer muscular layer of the esophagus may also represent a harbinger lesion for patients with EE who later develop fibrotic strictures in the esophagus. Additional, larger studies may result in the clinical possibility of routinely testing sera from patients with severe atopic dermatitis for monkey esophagus reactivity.

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