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TITLE: Development of a Novel Treatment for Food Allergy Using a New Genetically Defined Mouse Model of the Disease

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14. ABSTRACT Loeys Dietz Syndrome (LDS) is an autosomal dominant disorder caused by mutations in the receptor for TGFbeta, a multifunctional cytokine that plays a key role in the development of mucosal tolerance. LDS patients exhibit an increased propensity to develop nearly all forms of allergic disease. The goals of this proposal are to examine whether LDS mice are more susceptible to developing peanut allergy, and whether treatment with losartan, an angiotensin II (ATII) receptor blocker that inhibits TGFbeta signaling, reduces the development and/or severity of allergic disease in this mouse model. Our experiments thus far suggest that LDS mice do exhibit more severe symptoms of anaphylaxis, using both a passive systemic anaphylaxis model as well as a murine model of peanut allergy. These data support a role for dysregulated TGFbeta signaling in the development of food-induced anaphylaxis, suggesting that targeting this pathway with pharmacologic agents may have therapeutic benefit. Over the next year, experiments will be focused on determining whether LDS mutations lead to increased susceptibility to food allergen sensitization, increased effector responses, or both. We will also examine how treatment with losartan modifies the allergic phenotype in LDS mice.					
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INTRODUCTION

Both food allergy (FA) and eosinophilic esophagitis (EE), a related condition characterized by eosinophilic inflammation in the esophagus and epithelial cell hyperplasia, have strong familial associations suggesting a relatively large genetic component. However, few candidate genes have been conclusively linked to the development of these disorders, frustrating efforts to develop mechanism-based therapies. Loeys-Dietz Syndrome (LDS), a recently described autosomal dominant disorder caused by mutations in either of the two genes encoding subunits of the TGFbeta receptor (TGFB1 or TGFB2), is the first Mendelian disorder to be specifically associated with the development of FA and EE. A mouse model of LDS strongly recapitulates the human phenotype, suggesting that altered TGFbeta signaling is sufficient to predispose to FA and EE, and that this pathway is therefore an attractive therapeutic target. In this proposal, we will test whether LDS mice are more susceptible to developing peanut allergy, and whether allergic disease in LDS mice can be prevented or improved by treatment with losartan. Losartan is an FDA-approved drug that inhibits the angiotensin II type I (AT-1) receptor, decreases TGFbeta signaling, and thereby targets the basic pathophysiologic defect caused by LDS mutations.

BODY

TASK 1: Demonstrate a critical role for altered TGFbeta signaling in the pathogenesis of food allergy by investigating the propensity of mice harboring LDS mutations to develop food allergy.

We have begun our experiments to determine whether LDS mice are more susceptible to developing peanut allergy. We did an initial experiment to demonstrate that SV129 mice (the strain of mice the LDS alleles are carried on) were susceptible to developing peanut allergy. The methods for this experiment have previously been described.¹ We first compared the severity of anaphylaxis (as measured by a drop in body temperature) when wild type (WT) mice were sensitized with 1mg peanut protein and 10ug of cholera toxin by oral gavage two, three, or four times (one week apart). Mice were challenged intraperitoneally with peanut protein two weeks after the last sensitization dose was administered. The goal was to induce relatively mild anaphylaxis in WT mice, as we hypothesized the same treatment would result in more severe symptoms in LDS mice. As shown in Figure 1, all treatment strategies induced anaphylaxis in a subset of WT mice, and the differences associated with the number of sensitization doses used were modest. These data suggested we could successfully induce anaphylaxis in SV129 WT mice.

We then compared the severity of anaphylaxis between LDS mice and WT mice when they were challenged with peanut protein either two or three weeks after the last sensitization dose was administered. A total of three sensitization doses were administered in both experiments. As shown in Figure 2, LDS mice showed a trend towards more severe anaphylaxis (drop in body temperature) under both experimental settings compared to WT littermates. These results support our hypothesis that LDS mice are more susceptible to developing food allergic reactions, but led us to question whether LDS mice are more likely to become sensitized to allergen, or whether they have increased effector responses independent of the allergen sensitization phase, or both. To address this question, we used a previously described model of passive systemic anaphylaxis, in which mice are administered allergen-specific IgE through the tail vein, followed by allergen challenge 24 hours later.² This approach allows us to essentially bypass the sensitization phase, so we can evaluate effector responses independent of sensitization. As shown in Figure 3, LDS mice showed a greater anaphylactic response than their WT littermates in this model, suggesting they possess more robust effector responses in anaphylactic reactions. While clinical scores were ascertained for each of these experiments, measurements of rectal temperature proved to be a more sensitive and reproducible measure of anaphylactic reactions. We are now in the process of developing ELISAs to measure peanut-specific IgE and IgG1, to assess the propensity for LDS mice to become sensitized to peanut in our model of peanut allergy, compared to WT littermates.

TASK 2: Determine the efficacy of losartan in reducing the development and/or severity of allergic disease (eosinophilic esophagitis and susceptibility to peanut allergy) in LDS mice.

Unfortunately, our progress on this task was hampered by several factors, which have led us to request a no cost extension for this award. Over the last year, we have discovered that LDS mice refuse to drink losartan in their drinking water (and die from dehydration). We have worked with our veterinary staff and tried multiple additives to make the water more palatable but with no success. Post-mortem examination of the

mice has been consistent with dehydration. We believe this behavior may be due to the fact that these mice develop severe eosinophilic esophagitis and esophageal dilatation. Indeed, this problem appears to be specific for our LDS mice, as we have not encountered this issue with any of our other strains of mice that have been treated with losartan. Therefore, we are actively investigating alternate routes of delivering the drug to the LDS mice, possibly by gavage or in the animal's food. The progress of our research was also disrupted earlier this year when animals in our facility tested positive for pinworms. While our animals tested negative, we were limited in our abilities to breed and manipulate the mice until this testing was complete. We plan to continue with the aims of this task over the coming year.

KEY RESEARCH ACCOMPLISHMENTS

- We have successfully induced peanut allergy in SV129 line of mice
- We have demonstrated that LDS mice manifest more severe anaphylactic reactions in a model of passive systemic anaphylaxis
- We have demonstrated that LDS mice spontaneously develop eosinophilic esophagitis
- We have preliminary data suggesting LDS mice manifest more severe anaphylactic reactions in a murine model of peanut allergy

REPORTABLE OUTCOMES

None to report

CONCLUSIONS

Mutations in the receptor for TGFbeta that lead to Loeys Dietz Syndrome confer an increased susceptibility to develop IgE-mediated anaphylaxis to food proteins. While LDS mice manifest exaggerated effector responses in a model of passive systemic anaphylaxis, additional experiments will be done to examine whether these mice are more susceptible to allergen sensitization as well. These data support a prominent role for TGFbeta in the clinical manifestations of food allergy. LDS mice also spontaneously develop eosinophilic esophagitis (EE), a condition characterized by eosinophilic inflammation in the esophagus that is often caused by an abnormal immune response to food proteins, suggesting these mutations predispose to the development of allergic disease.

The monogenic nature of LDS suggests that mutations in a single gene can influence susceptibility to food-induced anaphylaxis and eosinophilic esophagitis (EE), suggesting this pathway may be an attractive therapeutic target. Since no treatment for food allergy is currently available, the identification of specific molecular targets that drive the development and/or severity of this disease are critically needed. In the next phase of experiments, we will address whether losartan, which reduces TGFbeta signaling and therefore targets the basic pathophysiologic defect associated with LDS, can prevent or reduce the severity of allergic reactions (both food-induced anaphylaxis and EE) in mice with LDS. Losartan is already FDA-approved, including for use in children. If losartan proves to be efficacious in our mouse model, then human trials could follow quickly.

REFERENCES

1. Sun J, et al. *Journal of Immunology*. 2007;179(10):6696-6703.
2. Tilley SL, et al. *The Journal of Clinical Investigation*. 2000;105(3):361-367.

APPENDIX

No documents to report.

Figure 1

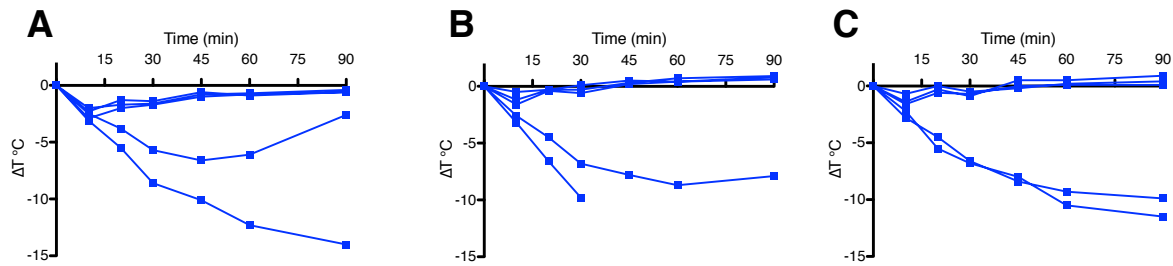


Figure 1. Establishment of peanut allergy in SV129 wild type (WT) mice. (A,B,C) WT mice were sensitized with peanut allergen and cholera toxin by oral gavage weekly for a total of two (A), three (B), or four (C) sensitization doses. Two weeks after the last sensitization dose was administered, mice were challenged intraperitoneally with peanut protein. Changes in rectal temperature (ΔT , °C), relative to baseline, were measured at each of the time points indicated following allergen challenge.

Figure 2

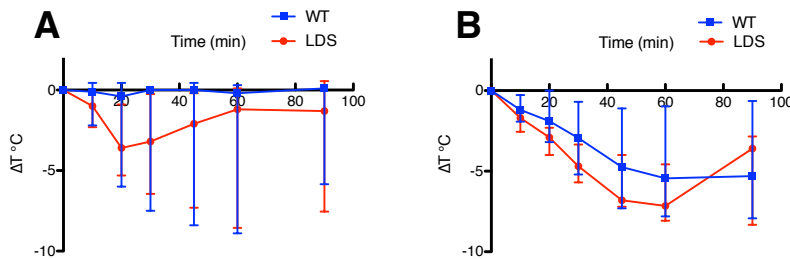


Figure 2. LDS mice exhibit more severe signs of anaphylaxis compared to WT littermates in a model of peanut allergy. (A,B) LDS mice and WT littermates were sensitized with peanut and cholera toxin by oral gavage weekly for a total of three sensitization doses. Two (A) or three (B) weeks after the last sensitization dose was administered, mice were challenged intraperitoneally with peanut protein. Changes in rectal temperature (ΔT , °C), relative to baseline, were measured at each of the time points indicated following allergen challenge.

Figure 3

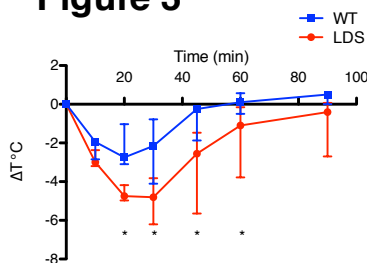


Figure 3. LDS mice exhibit more severe signs of anaphylaxis compared to WT littermates in a model of passive systemic anaphylaxis. LDS mice and WT littermates were administered 20ug of monoclonal anti-DNP IgE via tail vein injection. Twenty-four hours later, mice were challenged with 1mg of DNP-HSA antigen via tail vein injection, and rectal temperatures were recorded as described above. * denotes difference between LDS and WT is significant by Mann-Whitney with $p < 0.05$.