Selectin Dependence of Allergic Skin Inflammation Is Diminished by Maternal Atopy

Ibrahim M. Khan, Benjamin J. Ulrich, Andrew S. Nelson, Sarita Sehra, Geoffrey S. Kansas and Mark H. Kaplan

ImmunoHorizons 2021, 5 (8) 703-710
doi: https://doi.org/10.4049/immunohorizons.2100052
http://www.immunohorizons.org/content/5/8/703

This information is current as of August 31, 2021.

References
This article cites 47 articles, 16 of which you can access for free at:
http://www.immunohorizons.org/content/5/8/703.full#ref-list-1

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://www.immunohorizons.org/alerts
Selectin Dependence of Allergic Skin Inflammation Is Diminished by Maternal Atopy

Ibrahim M. Khan,*† Benjamin J. Ulrich,*† Andrew S. Nelson,*† Sarita Sehra,‡ Geoffrey S. Kansas,° and Mark H. Kaplan*†

*Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN; †Herman B. Wells Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN; and ‡Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL

ABSTRACT

Allergic skin inflammation requires the influx of inflammatory cells into the skin. Extravasation of leukocytes into the skin requires interactions between endothelial selectins and their glycan ligands on the surface of leukocytes. Selectin-ligand formation requires the activity of several glycosyltransferases, including Fut7. In this report, we tested the importance of Fut7 for the development of allergic skin inflammation in the Stat6VT transgenic mouse model. We observed that Fut7 deficiency was protective but did not eliminate disease. Segregation of the data by gender of the parent that transmitted the Stat6VT transgene, but not by gender of the pups, which were analyzed for disease, revealed that the protective effects of Fut7 deficiency were significantly greater when dams were Stat6VT negative. In contrast, in mice from litters of Stat6VT+ dams, Fut7 deficiency resulted in only modest protection. These findings indicate that pups from atopic dams exhibit a greater propensity for allergic disease, similar to observations in humans, and that the effect of maternal atopy is due to enhanced selectin-independent mechanisms of leukocyte recruitment in their offspring. Together, these results demonstrate that Fut7 deficiency can be protective in a model of atopic dermatitis but that maternal atopy diminishes these protective effects, suggesting alternative pathways for leukocyte recruitment in the absence of Fut7 enzyme activity. These observations have implications for understanding how the environment in utero predisposes for the development of allergic disease. ImmunoHorizons, 2021, 5: 703–710.

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease presenting with a variety of clinical manifestations, including blepharitis and eczema (1, 2). Asthma, allergic rhinitis, and food allergy are common comorbidities associated with AD. Children from parents with a history of allergic disease exhibit an increased risk of allergies, asthma, and AD (3), demonstrating the significance of the early life environment and genetic risk factors in developing AD. The predisposition to allergic disease is even more pronounced in offspring of allergic mothers (4, 5).

This role for the early life environment has been recapitulated in mouse models of allergic disease. Dams sensitized to egg albumin prior to mating produce mice that are prone to develop allergic inflammation. Inhibition of allergic responses in the mother by anti–IL-4 prior to conception reduces the risk of allergic responses in offspring (6), demonstrating the importance of the Th2 response to maternal transmission of allergic potential. However, the effects of the maternal environment...
The Stat6VT transgenic mouse was developed to define the effects of a hyperactive Th2 response on allergic inflammation (7). These mice show a strong predisposition to developing allergic inflammation in lungs, skin, and eyes (8–10), suggesting that this constitutively active Stat6 mutant is sufficient to induce a pathologic Th2 environment, including production of the cytokine IL-4, which is implicated in AD pathogenesis. Stat6VT mice respond to aggravating stimuli in an exaggerated manner and exhibit scratching behaviors similar to patients (10–12). Together, these findings suggest that the Stat6VT mouse model is a useful tool for exploration of AD-like allergic skin inflammation.

Analysis of AD skin lesions show increased infiltration by CD4+ T cells, dendritic cells, mast cells, and eosinophils (13). Selectins play an important role in leukocyte traffic into tissues, especially skin, during the inflammatory process, and therefore are a potential target to impair leukocyte infiltration. E- and P-selectin are particularly important for rolling during extravasation as a prelude to leukocyte adhesion and transmigration (14, 15). Selectins recognize glycan ligands on the leukocyte surface that require the α1,3-fucosyltransferase FucT-VII for synthesis (16). Induction of FucT-VII, encoded by Fut7, in activated T cells is repressed by IL-4 (17), but in the context of allergic disease, can be induced by IL-9 or IL-25 through a common p38 MAPK pathway (18). T cells are highly dependent on selectin-dependent migration, and in Fut7-deficient mice, T cell migration into sites of inflammation is severely impaired (16, 19). However, the effects of disrupting selectin-ligand formation, and the role of maternal atopy in determining the relevance of specific pathways of inflammatory cell infiltration, have not been examined in a model of spontaneous AD.

This study focused on defining the role of FucT-VII–dependent fucosylation in the Stat6VT model of AD. Although we observed diminished development of AD-like disease in mice lacking Fut7, the magnitude of the protection conferred by Fut7 deficiency differed as a function of whether the mother of the analyzed mice expressed the Stat6VT transgene (Tg). These studies define a role for Fut7 and selectin ligands in AD-like disease, document a contribution of the fetal microenvironment to the development of AD, and show, for the first time, to our knowledge, that the fetal microenvironment can influence the relative importance of distinct pathways of leukocyte recruitment.

**MATERIALS AND METHODS**

**Generation of Stat6VT and Stat6VT Fut7−/− mice**

The generation of Stat6VT transgenic mouse on a C57BL/6 background was previously described (7). To obtain Stat6VT mice lacking α1,3-fucosyltransferase, Stat6VT mice were mated to Fut7−/− mice. Mice were weaned at 28 d of age. All experiments used littermates as controls. All mice were maintained in specific pathogen–free conditions.

**Quantification of incidence and mortality in Stat6VT and Stat6VT Fut7−/− mice**

Mice with ages ranging from 9 to 20 wk were monitored weekly for the onset and development of characteristic features of AD consistent with the Stat6VT model. Severity of disease was scored using the mouse Eczema Area and Severity Index (EASI) scale. Briefly, scores were generated by evaluating individual regions of the body including the head and neck, extremities, back and abdomen, and tail. The severity index was calculated by evaluating the erythema, infiltration/papulation, excoriations, and lichenification within a single body region on a scale of 0–3. The percentage area affected by one or more of the key signs within a body region was estimated. The severity index was multiplied by the percentage area and multiplied by 10 to make whole numbers. Scores from different body regions were summed for a final disease score for the animal. Mice were photographed from consistent positions using a camera affixed to a stable stand at fixed height. Disease scoring was blinded from genotype to minimize bias.

**Histology**

Ear tissues were preserved in fixative 10% buffered formalin at room temperature. After 1 wk, formalin was replaced with 70% ethanol and stored at room temperature until processing. All samples were submitted and processed with H&E by the Indiana University Histology Core facility.

**Tissue processing**

Cells from inflamed ear tissues were identified using flow cytometry. Briefly, inflamed ear tissues were cut in half along the cartilage to expose the dermis and incubated for 1.5 h at 37°C with the dermis side in contact with serum-free DMEM (Life Technologies) containing 100 µg/ml Liberase (Roche Diagnostics) and 10 µg/ml DNase (Thermo Fisher Scientific). Ears were then cut into small pieces using scissors before being transferred into a C tube where they were processed on the GentleMACS (Miltenyi Biotec). Samples were then filtered through a 100-µm filter screen and cells were purified using a 40% Percoll (Sigma-Aldrich) gradient. Cells were counted and subsequently identified using flow cytometry analyzing for both granulocyte and CD4 T cell cytokine production.

**Flow cytometry analysis**

The frequency of cytokine-producing T cells was determined by intracellular cytokine stimulation. Briefly, 0.2 × 10^6 to 1.0 × 10^6 cells were stimulated in media containing PMA (50 ng/ml) and ionomycin (1 g/ml). After 4 h, monensin (2 µM) was added to stimulated cells, and 2 h later, cells were washed with FACS buffer (PBS with 0.5% BSA). All cell populations were stained for surface markers and a fixable viability dye (eBioscience) and fixed with 4% formaldehyde at room temperature for 10 min.

https://doi.org/10.4049/immunohorizons.2100052
Granulocyte gating strategy is as follows: granulocytes > single cells > viable cells > CD45+CD11b+CD45+CD11b+ was only used when labeled. From either viable or CD45+ cell populations neutrophils were defined as Ly-6G+CD11c−, and eosinophils were defined as Ly-6G−CD11c−Siglec-F+. Lymphocyte gating strategy is as follows: lymphocytes > single cells > viable cells. From the viable population, CD4+ T lymphocytes were defined as CD3+CD4+. Cytokine production was the measure from the CD4+CD4+ population after intracellular cytokine stimulation.

Abs used included those from BioLegend (anti-CD3–PerCP-Cy5.5, clone 17A2; anti-CD4–PE, clone GK1.5; anti-CD11b–PerCP-Cy5.5, clone ICR44; anti-CD11c–PE-Cy7, clone N418; anti-CD45–FITC, clone 104; anti–IL-4–PE-Cy7, clone 11B11; anti–IL-17A–allophycocyanin, clone eBio17B7; anti–IFNg–FITC, clone XM1G12; and anti–Ly-6G–allophycocyanin, clone 1A8), BD Biosciences (anti–Siglec-F–PE, clone E50-2440), and Invitrogen (eFluor 780 Viability Dye).

Statistics and data analysis
All statistics were done using Prism software version 9 (GraphPad). Welch t test was used for the comparisons between groups. An area under the curve analysis was used to compare disease score time courses. All data are representative of two to three experiments. Flow cytometry data were collected using an Attune flow cytometer (Life Technologies) and was analyzed using FlowJo version 10 (Tree Star).

RESULTS

FucT-VII deficiency reduces Stat6VT-induced dermatitis
To elucidate the role of FucT-VII in AD, we used the Stat6VT Tg model of AD with mice either wild type (Fut7+/−) or deficient for FucT-VII (Fut7−/−). Mice carrying the Stat6VT Tg that were heterozygous for FucT-VII deficiency (Fut7+/−) were crossed to Fut7−/− mice to obtain littermate controls (Fig. 1A). Mean disease scores were analyzed in control or Fut7-deficient Stat6VT transgenic mice every week beginning at 9 wk of age (Fig. 1B). Severity of AD was scored using a modified EASI scoring method (20). This quantitative scoring system incorporates both degree of severity and percentage of total body area affected and was modified for this study to account for anatomical differences between mice and humans.

We observed significantly reduced disease progression in Stat6VT Fut7−/− mice (Fig 1C). At 17 wk of age, mice were euthanized, and the severity of inflammation was assessed as moderate, mild, or lacking disease. Mice lacking the Stat6VT Tg were uniformly free of disease (Fig 1D). Approximately half of the Stat6VT Fut7+/− mice showed moderate disease, with the remaining showing mild disease. In sharp contrast, ~50% of Stat6VT Fut7−/− mice showed no disease, with the remaining showing only mild disease (Fig 1B). These data demonstrate a strong protective effect of Fut7 deletion on development of AD-like disease. Interestingly, Stat6VT+ Fut7+/− mice exhibited an intermediate disease phenotype, with a significant population free of disease and a ~50% reduction in the fraction of mice with moderate disease, suggesting an influence of Fut7 gene dose (Fig 1D). Together, these decreases in disease scores and disease incidence in Fut7−/− Stat6VT mice show that deficiency in Fut7 gene expression leads to significantly decreased Stat6VT-induced allergic skin inflammation.

Maternally expressed Stat6VT decreases the protective effect of Fut7 deficiency
Previous studies stressing the importance of the in utero environment on subsequent development of allergic disease (21, 22) led us to assess disease after grouping mice based on whether dams expressed the Stat6VT Tg. This segregation revealed that Fut7−/− mice born to Stat6VT-negative dams exhibited the lowest disease scores (Fig. 2A). Importantly, there was no difference in disease scores by gender of offspring. This difference in disease incidence and severity as a function of whether the dam expressed the Stat6VT Tg was clearly evident at a representative time point of 15 wk of age (Fig. 2B). To account for variation of scores over time, incidence of moderate disease or greater severity (score of 3 or higher) was examined throughout the lifetime of the mice. Stat6VT Fut7−/− mice that were born to Stat6VT-negative dams had the lowest lifetime incidence of moderate disease (Fig. 2C). Differences in inflammation could also be appreciated visually by blepharitis and skin inflammation on the ear and in histological examination of ear tissues stained with H&E (Fig. 2D, 2E). In the histological examination, the protective effect of Fut7 deficiency can be seen in diminished epidermal thickening and overall inflammation and swelling in the tissue, but only in mice from an Stat6VT-negative dam (Fig. 2D). Thus, the protective effect seen by impairing leukocyte traffic in Stat6VT+ Fut7−/− mice was diminished in mice born to atopic mothers.

Maternal Stat6VT Tg expression promotes increased infiltration and altered CD4 T cell cytokine production in Fut7 deficiency
To further investigate leukocyte infiltration in lesional skin, we isolated cells from inflamed ear tissue and analyzed them using flow cytometry. Both Stat6VT Fut7−/− and Fut7+/− progeny from Stat6VT Fut7+/− dams demonstrated higher eosinophil (defined as Ly-6G+ Siglec-F− CD11c+) infiltration in inflamed ear tissue, compared with Fut7−/− progeny of Stat6VT-negative Fut7+/− transgenic dams (Fig. 3A, 3B). Although in some mice neutrophil frequency was increased in progeny from Stat6VT− Fut7+/− dams, there was variation with some mice having little or no neutrophil infiltrate. There were similar observations when examining the frequency of eosinophils among the CD45+ cellular compartment (Fig. 3C). These data further
document the effects of maternal Tg expression on allergic skin inflammation in the progeny.

Because Stat6VT-mediated AD is defined by a Th2 pathology (9), we next analyzed if there were alterations in CD4$^+$ lymphocytes as well as cytokines produced by CD4$^+$ T cells. Stat6VT$^+$ Fut7$^+$/C0 progeny from Stat6VT$^+$ Fut7$^+$/damns had around a 5-fold increase in CD4$^+$ T cell (defined as CD3$^-$CD4$^+$) infiltration in inflamed ear tissue, compared with Stat6VT$^+$ Fut7$^-$/ progeny from Stat6VT-negative Fut7$^+$/damns (Fig. 4A, 4B). There was not a significant increase in CD3$^-$CD4$^-$ population that are likely CD8 T cells (Fig. 4B). Among CD4$^+$ T cells, we observed no significant difference in expression of the key Th2 cytokine IL-4 in mice from atopic versus nonatopic dams (Fig. 4C). However, because of the higher degree of inflammation and consequent increase in recruited T cells in mice from atopic mothers, there was a significant increase in the fraction of all cells that expressed IL-4 in mice from atopic dams compared with that of mice from nonatopic dams (Fig. 4D). In contrast, there was a striking increase in the proportion of IFN-γ-producing T cells among CD4$^+$ T cells of mice from atopic dams compared with nonatopic dams, and this difference was even greater among total cells (Fig. 4C, 4D). These findings show that mice that develop in atopic mothers have much greater T cell infiltration with greater production of IFN-γ than mice that develop in a nonatopic environment.

**DISCUSSION**

Selectin-ligand expression is critical for the ability of leukocytes to infiltrate tissues, particularly the skin (15, 23–26). Fut7 is critical for selectin-ligand formation, as mice deficient in FucT-VII have negligible L-, E-, or P-selectin–ligand activity (16, 27). In this report, we have defined an important role for FucT-VII in allergic skin inflammation associated with Stat6VT Tg expression. We observed that allergic skin inflammation was significantly diminished in Stat6VT$^+$ Fut7$^+$ mice. Unexpectedly, we observed that this reduction in allergic skin inflammation as a result of Fut7 deficiency was closely linked to whether the Stat6VT Tg was expressed by the pregnant dam or not. Our results document a switch in the importance of distinct leukocyte adhesion pathways induced by an atopic maternal environment.

Maternal history of atopic disease is a significant risk factor for the development of atopy in children (5, 28, 29). In mouse models, it has also been documented that sensitized dams will produce pups with a greater propensity for the development of allergic inflammation (6). Consistent with this, when data were segregated according to whether the pregnant dams were atopic because of expression of the Stat6VT Tg, we observed increased disease severity in offspring of both sexes from litters from atopic mothers. Because selectins are essential for leukocyte, especially T cell traffic to skin, we then examined...
the effect of deletion of *Fut7* on development of AD in Stat6VT Tg mice. We found that deletion of *Fut7* largely protected pups of nonatopic mothers from development of AD, whereas this protective effect of *Fut7* deletion was much smaller in pups from atopic mothers. Although we saw increased infiltration of CD4+ lymphocytes and eosinophils in progeny from atopic dams, additional immune populations are most likely playing a role in this process. Indeed, we have observed changes in a number of populations in lesional skin of Stat6VT Tg mice (30). Although we did not see a difference in CD3+CD4+ infiltrate in the progeny from Stat6VT Fut7+/+ dams, more detailed characterization of potential changes in CD8+ or γδ T lymphocytes as well as monocyte/macrophage recruitment are important future directions of investigation. Given the well-documented critical role for *Fut7* in selectin-ligand biosynthesis, this finding clearly implies that an atopic environment leads to the induction of an alternative pathway of leukocyte recruitment.

We have previously established that there are systemic effects of Stat6VT Tg expression in T cells (8–10). Indeed, we previously observed that dendritic cells in neonatal mice from Stat6VT Tg dams exhibited altered cytokine production that was similar to patterns observed in infants from atopic mothers (5, 31). This suggests that although FucT-VII deficiency is protective for allergic skin inflammation in the absence of additional proatopic signals, the effects of an atopic environment on the infant’s immune system can overcome that protection. How cells migrate into tissues in the absence of selectin ligands is still unclear but likely involves compensatory effects of additional adhesion molecules, particularly VLA4/VCAM-1 (32–38).

Consistent with this, VCAM-1 expression on endothelium is...
strongly enhanced by both IL-4 and IFN-γ (39–44), both of which we show in this study are produced at high levels by T cells in offspring of atopic mothers, but not nonatopic mothers. This difference in cytokine profiles could be linked to the development of type I cells in chronic AD lesions (1, 12). Other pathways have been shown to influence leukocyte rolling velocity such as moesin. Lacking this moiety was associated with deficient slow rolling necessary for effective leukocyte recruitment into tissues (38). Other proposed pathways include the concept of “the path of least resistance,” in which strong barrier function and deficient crawling, as is seen in Fut7−/− mice, are associated with increased transcellular migration (37).

FIGURE 3. Progeny from Stat6VT+ dams in the context of Fut7 deficiency is associated with increased eosinophil cell infiltrate. (A) Representative flow cytometry dot plots from cells isolated from inflamed ears. (B) Bar graphs showing frequency of neutrophil and eosinophil populations from total cells. (C) Bar graphs showing frequency of neutrophil and eosinophil populations from CD45+ cells. Data are representative of two independent experiments with three to four mice per group. *p < 0.05, Welch t test was used.

FIGURE 4. Maternal expression of the Stat6VT Tg in the context of Fut7 deficiency is associated with increased CD4+ T cell infiltrate and an inflammatory cytokine profile. (A) Representative flow cytometry dot plots from cells isolated from inflamed ears. (B) Bar graphs showing frequency CD3−CD4− and CD3+CD4− T cell population. (C) Frequency of cytokine-positive cells among CD4+ T cells in the ear. (D) Frequency of cytokine-positive cells from total cells measured via flow cytometry from the ear. Data are representative of two independent experiments with three to four mice per group. *p < 0.05, Welch t test was used.
This would be associated with strengthened adhesion likely via ICAM and invasive podosomes (45, 46). Another mechanism alternative to the selectin pathway is through endothelial-expressed scavenger receptors that may pose a similar purpose and are a field of burgeoning study (47). All these may be the focus of future studies trying to discern this alternative route for leukocyte recruitment into tissues.

Our results highlight the critical importance of selectin ligands in recruitment of Th2 cells and other inflammatory leukocytes that underlie the development of allergic skin inflammation. They further suggest that an atopic in utero environment can alter or induce other potential pathways of leukocyte traffic, which can play a critical role in induction of AD.

ACKNOWLEDGMENTS

We thank Derrick Gray and Drew M. Brown in the Indiana University Histology Core facility and Joan Cook-Mills and Mathew J. Turner for review of the manuscript.

REFERENCES


https://doi.org/10.4049/immunohorizons.2100052