

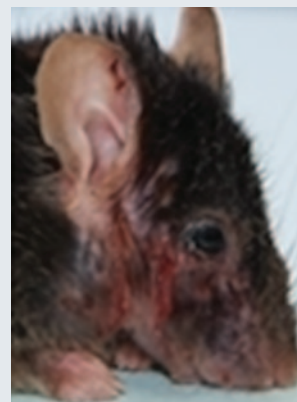
An Itchy Mouse: A Potential Model of Atopic Dermatitis

Christopher Spock¹ and Robert S. Kirsner¹

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Atopic dermatitis (AD) is a common chronic inflammatory skin disorder with both acute and chronic stages (Akdis *et al.*, 2006). Often referred to as “the itch that scratches,” AD is characterized by severe pruritus, which may substantially impact the quality of life in sufferers. Recently, human protease-activated receptor-2 (PAR-2) was found to mediate itch (Reddy *et al.*, 2010), generating considerable interest in compounds that activate this receptor. Among these compounds is cathepsin S (CTSS), a lysosomal cysteine protease that functions in a variety of ways, including as an elastase in alveolar macrophages. This compound may also play a role in the degradation of antigenic proteins into peptides for presentation on major histocompatibility complex class II molecules.

To study the role of CTSS, Kim *et al.* (2012, this issue) utilized a transgenic mouse that overexpresses human CTSS. The investigators hypothesized that overexpression of human CTSS in this model would lead to the AD-like symptoms and signs of itching and scratching and histopathological changes that resemble those in AD. Indeed, the transgenic mice that overexpressed CTSS scratched more, and this increased scratching led to reddening, scaling, and thickening of the skin. PAR-2 expression was also increased as a result of CTSS overexpression, and both CTSS and PAR-2 levels correlated with a thickened epidermis. Although barrier function was not examined, a variety of cytokines, including IL-4, IL-10, tumor necrosis factor- α , IL-2, IFN- γ , IL-1 β , monocyte chemoattractant protein-1, and granulocyte-macrophage colony-stimulating factor, were analyzed. Through the following questions, we examine this paper in greater detail. For brief answers, please refer to the supplementary information online <<http://www.nature.com/jid/journal/v132/n4/supinfo/jid201249s1.html>>.



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QUESTIONS

1. Describe the pathogenesis of atopic dermatitis.
2. What is cathepsin S?
3. How did the investigators carry out their study?
4. What were their results and conclusions?

For answers, visit our website or scan the adjacent tag.



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¹Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, USA

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Topic article:

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1. Describe the pathogenesis of atopic dermatitis.

AD is a chronic pruritic inflammatory skin disorder that most commonly presents during childhood and is characterized by both acute and chronic phases. The acute phase typically presents with pruritic red papules, often with excoriations and serous exudate. Chronic lesions exhibit lichenification, papules, and excoriations. The pathogenic mechanisms of AD appear to be multifactorial, and the exact relationship between stratum corneum dysfunction and altered immune responses remains to be determined (Hanifin, 2009).

Barrier dysfunction of the stratum corneum plays an important role in AD. An association exists with filaggrin null mutations (Palmer *et al.*, 2006; Sandilands *et al.*, 2007), suggesting that a barrier defect may be a primary factor that leads to T helper type 2 (Th2) cell and IgE abnormalities in the acute phase of the disease. Immunologically, the acute phase of AD is characterized by Th2 cell predominance, whereas the chronic phase is characterized by Th1 cell predominance (Grewe *et al.*, 1998). A genetic predisposition probably leads to the initial immune imbalance that favors the differentiation of Th2 cells. This development, in turn, leads to increased IgE levels and peripheral blood eosinophilia, and it is driven by cytokines from activated allergen-specific Th2 cells. Cells such as macrophages and eosinophils invade the skin as a result of Th2 cytokines and produce IL-12, which then leads to activation of allergen-specific and nonspecific Th1 and Th0 cells. The precise mechanism by which sequential activation of Th2 and then Th1 cells occurs is not yet known; however, the predominance of IFN- γ producing T cells contributes to the chronicity of the disease (Grewe *et al.*, 1998).

2. What is cathepsin S?

The 15 human cathepsins comprise 11 cysteine, 2 aspartic, and 2 serine proteases. Initially considered lysosomal proteases, cathepsins are now known to have many functional roles in tissue remodeling, metastasis, and inflammation, such as occur in atherosclerosis and asthma (Felbor *et al.*, 2000; Nakagawa *et al.*, 1999). CTSS is a lysosomal cysteine protease that functions as an elastase in alveolar macrophages and may also play a role in the degradation of antigenic proteins into peptides for presentation on major histocompatibility complex (MHC) class II molecules. CTSS also activates the human protease-activated receptor-2 (PAR-2), which serves to mediate itch (Reddy *et al.*, 2010).

PAR-2 is a member of the G-protein-coupled receptor family. These receptors are activated when serine proteases cleave receptors within their N-termini, thereby exposing a tethered ligand domain that binds and activates the cleaved receptor. The presence of PAR-2 on free nerve endings in the skin, keratinocytes, and dorsal root ganglia links this receptor to itch (Steinhoff *et al.*, 2003).

The sensation of itch is mediated by two distinct populations of cutaneous nerve fibers: mechano-insensitive fibers, which are responsive to histamine, and mechano-sensitive fibers, which are responsive to cowhage (Namer *et al.*, 2008). Cowhage (*Mucuna pruriens*) is a tropical legume that contains the active component mucunain, which is a cysteine protease ligand for PAR-2 and PAR-4 and serves as a mediator of non-histamine-mediated itch (Reddy *et al.*, 2008). Interestingly, CTSS shares active site sequence homology with mucunain and binds to PAR-2, leading to stimulation of C fibers and the sensation of itch (Namer *et al.*, 2008). Given that AD is a chronic pruritic skin disorder, CTSS has been hypothesized to be responsible for these chronic symptoms.

3. How did the investigators carry out their study?

To address the hypothesis that overexpression of CTSS leads to activation of PAR-2 and the subsequent symptoms of AD, the investigators used transgenic (TG) mice that developed a chronic and pruritic inflammatory disorder of the skin thought to resemble chronic AD (Kim *et al.*, 2012). To generate these mice, the human CTSS gene was amplified from placental mRNA. Next, complementary DNA (cDNA) was cloned into a vector using the cytomegalovirus promoter to ensure overexpression

of the CTSS gene. This transgene construct was then injected into mouse embryos that were transferred to pseudopregnant female mice. TG founder mice that inherited the overexpressed CTSS gene were identified by PCR screening. Founder mice were mated to wild-type (WT) mice. Offspring of this mating were genotyped, and those that did not inherit the transgene were used as controls. At the start of the experiment, 20 WT and 20 TG male mice aged 16 weeks were used.

The investigators hypothesized that overexpression of human CTSS in this model would result in AD-like symptoms and signs of itching and scratching, leading to skin changes that histologically resembled AD. To test this hypothesis, they analyzed the scratching behavior of mice over a 60-minute interval. Histological sections from dorsal skin samples from 16-week-old WT and TG mice were also analyzed.

To verify overexpression of CTSS and subsequent PAR-2 protein levels, western blot analysis was used. The investigators also analyzed the patterns of overexpression of CTSS in other tissues. Total RNA was isolated from 6-week-old WT and TG mice, and expression levels of CTSS, as well as other cytokines implicated in the pathogenesis of AD (IL-4, IL-10, TNF- α , IL-2, IFN- γ , IL-1 β , monocyte chemotactic protein-1, and granulocyte-macrophage colony-stimulating factor), were analyzed using RT-PCR.

4. What were the results and conclusions?

The results of this study demonstrated that the human CTSS gene was overexpressed in TG mice in the skin as well as in other tissues. PAR-2 expression was also increased as a result of CTSS overexpression. Notably, expression of both CTSS and PAR-2 was strongly increased in thickened epidermis. Increased expression of the Th2-type cytokines IL-4 and IL-10 and the Th1-type cytokines TNF- α , IL-2, IFN- γ , and IL-1 β in TG mice was noted. ELISA analysis demonstrated greater increases in Th1-type cytokines, specifically IFN- γ , as compared with Th2-type cytokines. Serum IgE levels were also higher in TG mice than in WT controls.

Behavioral and clinical analyses revealed that TG mice that overexpress CTSS developed reddening and scaling of dorsal skin and the neck region and exhibited frequent face rubbing and scratching. Total scratch counts in TG mice were significantly higher than in WT mice.

Histologic analysis revealed that TG mice had increased epidermal proliferation and increased numbers of mast cells, a marker of chronic inflammation. The investigators also found high levels of CD11c (a marker of mature dendritic cells) and dendritic cell markers, such as CD4 and MHC class II, in the epidermis and dermis of the TG mice.

Although human CTSS is similar to mouse CTSS and the proteins share 74% overall homology, there remains a remote possibility that the inflammation observed resulted from expression of a “foreign,” immunogenic protein.

In the mouse, Th cells are divided into subclasses based on secreted cytokines. The Th1 subtype is characterized by IFN- γ and IL-2, whereas the Th2 subtype is characterized by IL-4, -5, and -10 (Mosmann *et al.*, 1986). Human Th cells are divided in a similar fashion; however, the cytokine profiles of these Th cell subtypes are more heterogeneous, as IL-2 and IL-10 are not restricted to a specific Th-cell subtype (Del Prete *et al.*, 1993).

While the investigators conclude that overexpression of CTSS in mice leads to a chronic inflammatory skin disorder characterized as chronic AD, this model fails to address the role of barrier function in AD. Importantly, stratum corneum dysfunction is known to play an important role (Elias, 2005; Elias and Steinhoff, 2008).

The investigators also conclude that PAR-2 expression on the surface of dendritic cells induces stimulation of CD4⁺ T cells to produce cytokines, particularly IFN- γ , consistent with the Th1 phenotype. They believe that this cascade of events leads to the chronic inflammatory state of AD.

Understanding the role of CTSS and PAR-2 in AD may ultimately yield new therapeutic agents for the disease. Downregulation of CTSS or blockade of PAR-2 may result in substantial improvement of patients with AD, especially their itching and scratching. Thus, these findings may lead to a new therapeutic agents that go beyond antihistamines, which, according to this study, seem destined to fail on a mechanistic basis.

In the future, it will be important to validate this model by determining whether patients exhibit the abnormalities described in the mice. If such abnormalities do exist, this model will become even more useful.

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¹Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, USA