Treating insect-bite hypersensitivity in horses with active vaccination against IL-5

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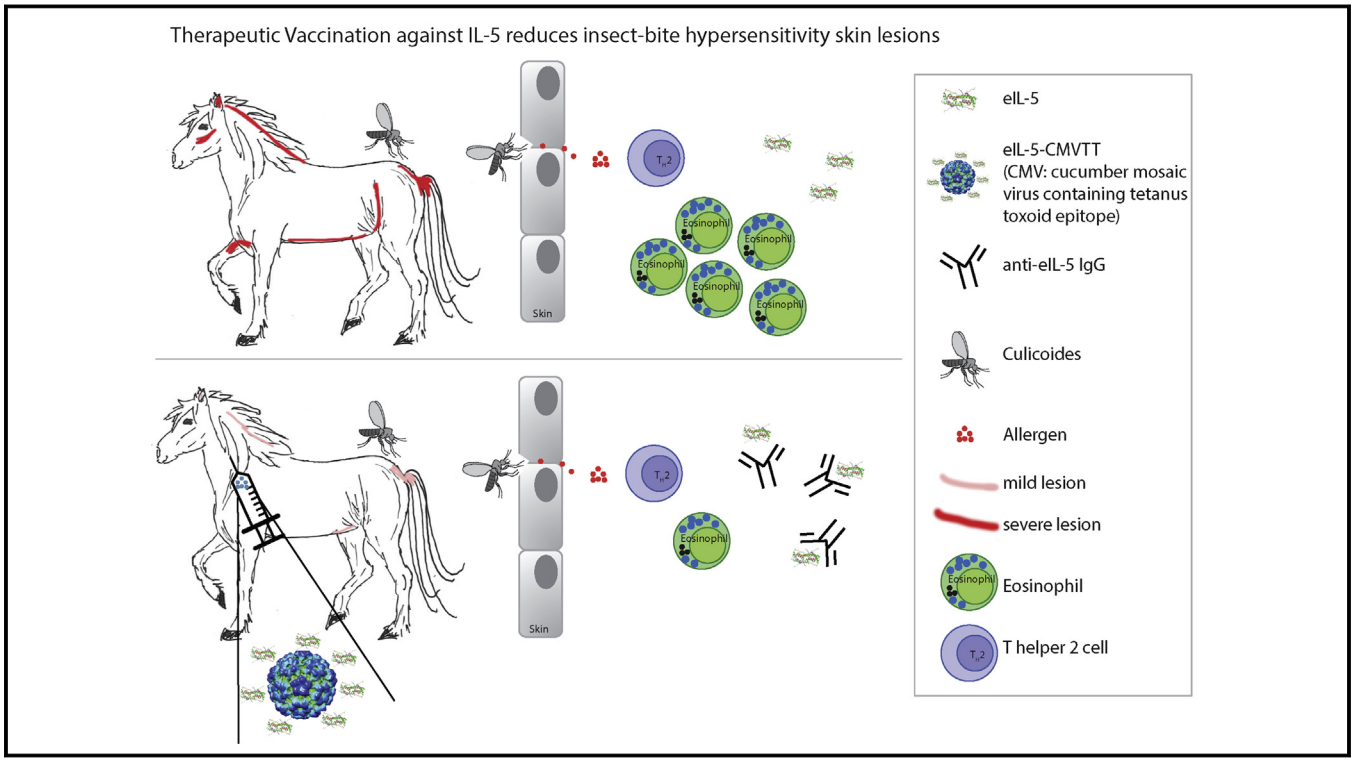
GRAPHICAL ABSTRACT



Background: Insect-bite hypersensitivity is the most common allergic dermatitis in horses. Excoriated skin lesions are typical symptoms of this seasonal and refractory chronic disease. On a cellular level, the skin lesions are characterized by massive eosinophil infiltration caused by an underlying allergic response.

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Objective: To target these cells and treat disease, we developed a therapeutic vaccine against equine IL-5 (eIL-5), the master regulator of eosinophils.
Methods: The vaccine consisted of eIL-5 covalently linked to a virus-like particle derived from cucumber mosaic virus containing the tetanus toxoid universal T-cell epitope tt830-843 (CMV_{TT}). Thirty-four Icelandic horses were recruited and immunized with 400 μg of eIL-5–CMV_{TT} formulated in PBS without adjuvant (19 horses) or PBS alone (15 horses).
Results: The vaccine was well tolerated and did not reveal any safety concerns but was able to induce anti–eIL-5 autoantibody titers in 17 of 19 horses. This resulted in a statistically significant reduction in clinical lesion scores when compared with previous season levels, as well as levels in placebo-treated horses. Protection required a minimal threshold of anti–eIL-5 antibodies. Clinical improvement by disease scoring showed that 47% and 21% of vaccinated horses reached 50% and 75% improvement, respectively. In the placebo group no horse reached 75% improvement, and only 13% reached 50% improvement.
Conclusion: Our therapeutic vaccine inducing autoantibodies against self IL-5 brings biologics to horses, is the first successful immunotherapeutic approach targeting a chronic disease in horses, and might facilitate development of a similar vaccine against IL-5 in human subjects. (J Allergy Clin Immunol 2018;■■■:■■■-■■■.)

Key words: *Allergic dermatitis, eosinophils, vaccination*

Abbreviations used
CMV: Cucumber mosaic virus
CMV_{TT}: Cucumber mosaic virus containing the tetanus toxoid universal T-cell epitope tt830-843
eIL-5: Equine IL-5
HRP: Horseradish peroxidase
IBH: Insect-bite hypersensitivity
ISI: Insect-bite hypersensitivity severity index
MALDI: Matrix-assisted laser desorption ionization
MS/MS: Tandem mass spectrometry
PBST: PBS-Tween 0.1% (vol/vol)
VLP: Virus-like particle

Insect-bite hypersensitivity (IBH) of horses, also known as sweet itch, summer eczema, kasen, or Queensland itch, manifests as chronic relapsing seasonal allergic dermatitis caused by the biting of insects of the genus *Culicoides*.[1-5]

Culicoides species midges are found in various areas of the world.[6-8] The incidence of IBH correlates strongly with the geographic distribution of *Culicoides* species, showing the highest incidence in some parts of Australia (60%).[9-11] Overall, approximately 10% of all horses worldwide are affected by IBH,[12-15] and in principle, all breeds can succumb to the allergic disease. During warmer months of the year, IBH-affected horses experience hairless, weeping, and sometimes even ulcerative lesions caused by inflammation and severe itching. Lesions are characterized by hyperkeratosis, lichenification of the skin, bleeding, swelling, scales, and crust formation. Histologic hallmarks of IBH lesions are thickening of the stratum corneum, epidermis, and dermis, with abundant fibrosis in the latter.[9] Commonly, secondary infections with bacteria, mites, and fungi can cause further local irritation, enhancing lesion formation. Although IBH was first described in 1840 and is currently the best characterized allergic disease in horses, treatment options are still poor, and currently, no satisfactory treatment of IBH is available.[9,16]

IBH is classified as IgE-dependent type I allergy,[17] with a strong involvement of type IV allergic hypersensitivity reactions.[4,18] In addition to IgE-mediated cross-linking of FcεRI on mast cells and basophils with subsequent histamine release, type I allergies also consist of a late-phase reaction with eosinophilia and recruitment of eosinophils into the allergic site. Intradermal injection of *Culicoides* species extract into IBH-affected horses leads to recruitment of T_H2 cells and eosinophils to the injection site.[19] Moreover, in addition to the role of IgE and type I allergy in the setting of IBH, an involvement of cell-mediated type IV allergic reactions, also called delayed-type hypersensitivity, has been discussed in recent years.[16]

Type IV allergies can be divided into 4 subgroups: (ie, types IVa, IVb, IVc, and IVd) depending on the cell types involved. Type IVb allergy is strongly associated with IL-5–producing T_H2 cells, eosinophilia, and eosinophilic type of inflammation[20] and reflects the clinical manifestations of skin inflammation in IBH, which are characterized by edema and eosinophil accumulation in perivascular clusters in deeper parts of the dermis.[21] These enhanced eosinophils counts represent the predominant inflammatory cell type accumulating in IBH lesions.[21,22] Activated eosinophils release granule enzymes and other effector molecules, such as histamine, eosinophil-derived neurotoxin, cysteinyl leukotrienes, and major basic protein. Major basic protein triggers degranulation of mast cells and basophils, thereby further enhancing allergic symptoms.[23] Cysteinyl leukotrienes derived from arachidonic acid are inflammatory mediators contributing on multiple levels to an allergic reaction, thereby making an eosinophil a very effective cell type to cause strong allergic symptoms during late-phase and delayed-type reactions. Therefore eosinophil degranulation can mediate hyperreactivity, inflammation, and local tissue damage, thus potentially driving the pathology of IBH.[24]

To dampen eosinophil activity, we decided to target the lineage-specific eosinophil master regulator IL-5, which is a T_H2-type cytokine produced mainly by T_H2 cells and also by mast cells. Eosinophil development in the bone marrow is critically dependent on IL-5.[25,26] Moreover, eosinophil release into blood circulation is mediated by IL-5, and the cytokine plays a key role in eosinophil activation.[27] Also, IL-5 increases survival of tissue-resident inflammatory eosinophils.[28] Mice either vaccinated against murine IL-5 or IL-5–deficient knockout mice show strongly reduced levels of blood eosinophils and eosinophil-mediated inflammation.[26,29] Furthermore, mepolizumab, a humanized anti–IL-5 mAb, significantly reduced circulating eosinophil counts in human subjects and has shown clinical efficacy in patients with eosinophil-mediated diseases, such as eosinophil-mediated asthma and hypereosinophilic syndrome.[30-34] Thus blocking IL-5 can lead to effective control of IBH.

Instead of targeting IL-5 with mAbs,[35] which is not a realistic approach because of the weight of a horse, we developed a