

Letter to the Editor

Vaccination against IL-31 for the treatment of atopic dermatitis in dogs

To the Editor:

Atopic dermatitis (AD) is the most common allergic skin disease in dogs. Extensive itching causes scratching, which results in loss of fur and secondary infections of the skin, accelerating the symptoms. AD not only affects the well-being of dogs but also impacts the quality of life of their owners. IL-31 is a key cytokine driving itching, and an mAb against IL-31 has been licensed for use in dogs for the treatment of AD.¹ Here, we describe the development of a virus-like particle-based vaccine against canine IL-31, and demonstrate that immunized dogs mount a robust IgG response that essentially abrogates symptoms of itching in house dust mite (HDM)-sensitized and -challenged dogs. Hence, the here-presented breakthrough therapy of vaccination against IL-31 is not only a promising mode to treat AD in dogs but may also facilitate the development of a similar vaccine in humans.

AD is the most common allergy in dogs, affecting approximately 10% of the dog population, resulting in 15 million to 20 million dogs suffering from the disease in Europe and the United States alone.² Itching caused by this allergic skin disease is usually recurrent or chronic and deeply impacts the quality of life for both the dog and its owner unless symptoms are controlled with effective management. There are a number of treatment options that target the inflammation or itching. Apoquel from Zoetis, the best current standard for the treatment of AD-induced pruritus in dogs, targets Janus kinase 1, a downstream molecule of a number of cytokines including those pivotal for the allergic reaction, including IL-31.³ In animals and humans, IL-4 and IL-13 are known to be critical for the inflammatory process in AD, while IL-31 is pivotal for the itching.¹ Furthermore, an mAb against IL-31 has recently been approved for the treatment of AD in dogs.⁴ Both Apoquel as well as the mAb against IL-31 have to be given relatively frequently (twice daily for Apoquel and monthly for the antibody). Therefore, vaccination against IL-31, which should induce a more sustained antibody response, may be a valuable addition or alternative to existing therapies.

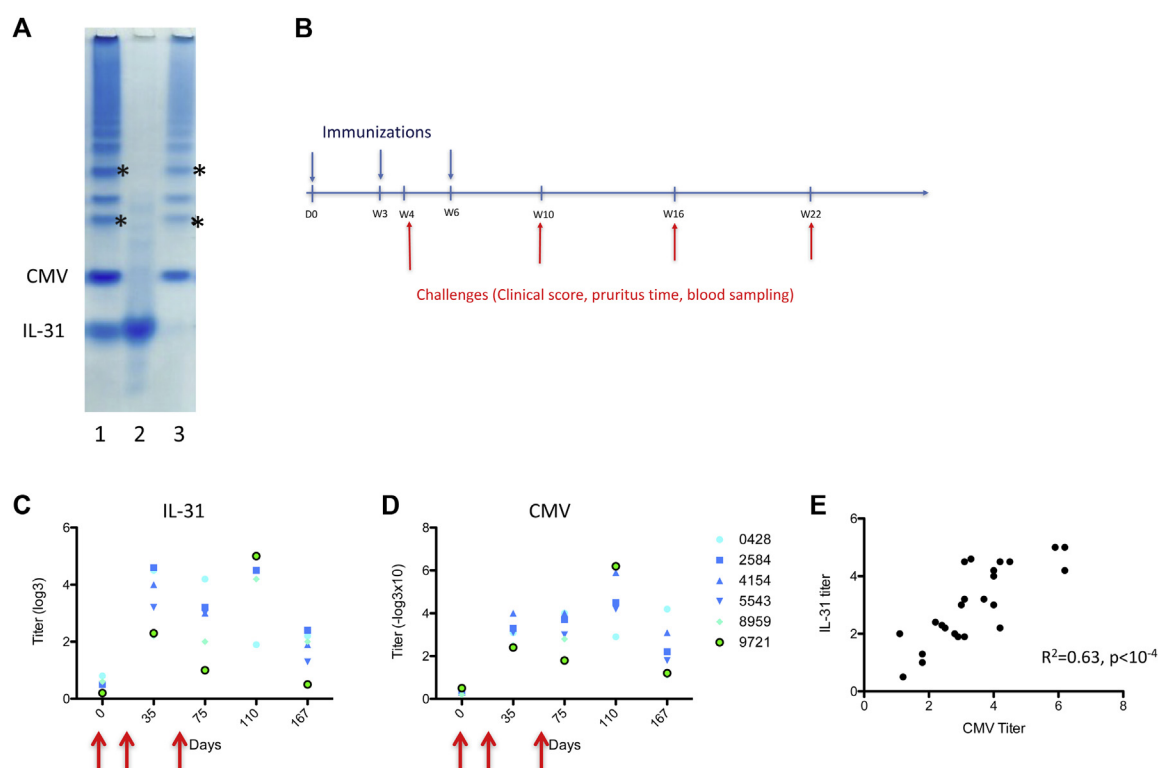


FIG 1. Generating of canine IL-31-CMV_T and antibody responses. **A**, SDS page analysis of coupling reaction before and after removal of free IL-31. Lane 1: coupling reaction of IL-31 and CMV_T before purification. Lane 2: free IL-31; Lane 3: coupling product after purification. *Presence of extra bands due to coupling of canine IL-31 to monomers of CMV_T VLPs. **B**, Outline of the study showing time points of immunization and experimental challenges with HDM. Six dogs were immunized with the vaccine in alum, whereas the other 6 were treated with alum alone. **C** and **D**, Antibody responses were measured in the sera against IL-31 (Fig 1, C) or CMV (Fig 1, D) by ELISA at the indicated time points. Arrows in C and D indicate time points of vaccination. The numbers indicate individual dogs. **E**, A correlation between IL-31 and CMV titers is shown. ELISA titers are expressed as log3 of 10-fold prediluted sera leading to half-maximal absorbance in the ELISA. The graph includes all 4 time points after vaccination and does not take into account the placebo dogs.

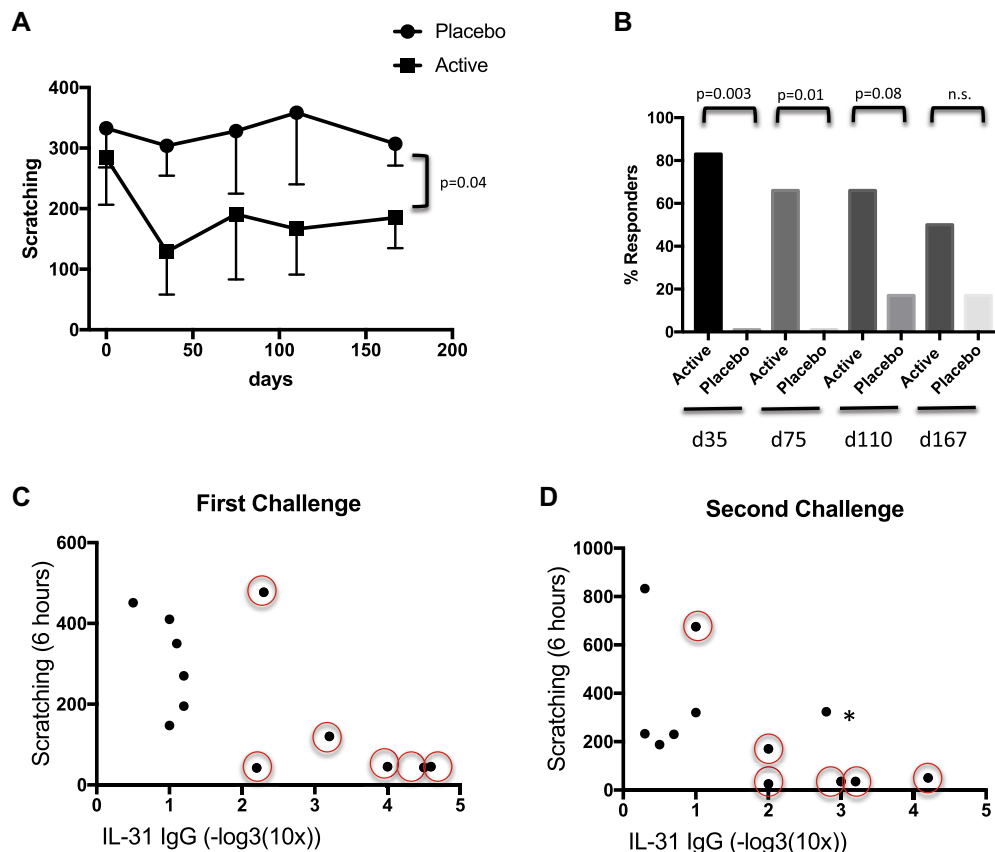


FIG 2. Pruritus in vaccinated and placebo dogs and correlation with antibody responses. **A** and **B**, Dogs allergic to HDM were immunized with placebo (placebo) or vaccine (active) and challenged at the indicated time points with HDM extract. Pruritus was quantified by counting the number of scratches over 6 hours. Day 0 depicts baseline before immunization. Fig 2, **A**, Average number of scratching and SEM. Fig 2, **B**, The number of vaccine responders. Dogs showing more than 50% reduced scratching compared with the average of the placebo dogs were counted as responders. A 1-way ANOVA was performed in Fig 2, **A**, and chi-square tests in Fig 2, **B**. **C** and **D**, Scratching is correlated to antibody titers in immunized and placebo dogs. Antibody levels and scratching were correlated 35 days (Fig 2, **C**) and 75 days (Fig 2, **D**) after initial vaccination. ELISA titers are expressed as log3 of 10-fold prediluted sera leading to half-maximal absorbance in the ELISA. Circled dogs are on active. *A placebo dog that was likely injected because it also has anti-CMV titers at this time point.

There are various platforms to induce high antibody titers by vaccination but virus-like particles (VLPs) have proven best to overcome immunological unresponsiveness and induce self-specific antibodies.⁵ VLPs have the appearance of viruses but lack genetic information and generally have a good safety profile. The highly repetitive surface of the particle (a geometric pathogen-associated structural pattern) together with the ability of some VLPs to activate pathogen-associated molecular patterns due to packed bacterial RNA is responsible for induction of fulminant B- and T-cell responses.⁵

To induce autoantibodies against canine IL-31, we coupled canine IL-31 to VLPs, allowing repetitive display of IL-31 and intermolecular T help through the VLP. The used VLP is derived from cucumber mosaic virus and containing a universal T-cell epitope (CMV_{TT}).⁶ To allow for repetitive display of native IL-31 on the CMV_{TT} surface, we expressed both components separately and chemically conjugated them together. To this end, we introduced free SH groups on IL-31 using N-succinimidyl S-

acetylthioacetate and chemically coupled the SH groups to surface Lys of the VLP using succinimidyl 6-((beta-maleimidopropionamido)hexanoate) (Fig 1, **A**; see Fig E1 in this article's Online Repository at www.jacionline.org).

Before inclusion in the present study, all dogs were sensitized to HDM and challenged to ensure that they all develop clinical signs and typical AD-skin lesions. In addition, all dogs were positive when tested intradermally with HDM extracts, confirming that the clinical signs are associated with HDM sensitization and do not result from an irritative reaction. Furthermore, histological aspects of the lesions were similar to the features observed in natural AD in dogs.

Dogs were immunized with 100 µg CMV_{TT} formulated in alum on day 0. Because the vaccine was well tolerated and caused no itching, the dose was increased to 300 µg on weeks 3 and 6 (see Fig 1, **B**, for study outline). Placebo animals received alum only in a blinded fashion. Blood was taken on day 0 and weeks 4, 10, 16, and 22, namely, after each challenge, and antibody titers were

measured against IL-31 (Fig 1, C) and CMV_{TT} (Fig 1, D). All dogs immunized with the vaccine mounted robust antibody responses of the IgG isotype. Titers in most animals peaked after the third injection and declined thereafter with modest kinetics. As seen previously in humans immunized against IL-1 β or angiotensin II,⁷ responses against the antigen (IL-31) correlated with the response against the carrier (CMV_{TT}) (Fig 1, E).

Dogs were topically challenged with HDM extract and symptoms of itching were monitored by videotaping for 6 hours on the third day after the challenge and counting the time the dogs spent scratching.

While active and placebo dogs showed similar itching before immunization, immunized dogs showed clearly reduced symptoms of itching, in particular early after immunization, when antibody titers were high (Fig 2, A). Assuming that dogs showing more than 50% reduced scratching compared with the average of the placebo dogs, 4 to 5 out of 6 dogs were responders whereas 0 to 1 dog was a responder in the placebo group (Fig 2, B). Reduced scratching correlated well with antibody titers induced (Fig 2, C and D, for the first 2 time points), supporting the mechanism of action. At a later time point, when antibody responses declined, reduced scratching was less pronounced (Fig 2, B).

Allergic dermatitis is a major health issue in dogs. Most current treatment options are either ineffective or may be associated with serious side effects. Allergen-specific immunotherapy is safe but not effective in all dogs and compliance is low. Here, we describe a novel treatment option consisting of active vaccination against IL-31, a major cause of itching in dogs and other mammals, such as humans and mice.

IL-31 is currently targeted by 2 different drugs, namely, Apoquel and an mAb directed against IL-31.¹ In contrast to these 2 existing modalities to block IL-31, which require frequent administration, vaccination offers the advantage of high specificity combined with infrequent administration of small amounts of antigen; in the present study, a 100- μ g dose followed by two 300- μ g doses were tested. Two important points emerged, namely, that the vaccine was (1) well tolerated and (2) induced protective antibody levels in most dogs.

In summary, the present study lays the foundation of a novel breakthrough therapeutic modality for the treatment of AD in dogs. Next studies will be performed to increase and prolong efficacy by studying dose responses and the role of adjuvants in more detail. The insights we gain here for the treatment of dogs may be harnessed for the development of a homologous vaccine for use in humans.

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REFERENCES

1. Furue M, Yamamura K, Kido-Nakahara M, Nakahara T, Fukui Y. Emerging role of interleukin-31 and interleukin-31 receptor in pruritus in atopic dermatitis. *Allergy* 2018;73:29-36.
2. Hillier A, Griffin CE. The ACVD task force on canine atopic dermatitis (I): incidence and prevalence. *Vet Immunol Immunopathol* 2001;81:147-51.
3. Cosgrove SB, Wren JA, Cleaver DM, Walsh KF, Follis SI, King VI, et al. A blinded, randomized, placebo-controlled trial of the efficacy and safety of the Janus kinase inhibitor oclacitinib (Apoquel(R)) in client-owned dogs with atopic dermatitis. *Vet Dermatol* 2013;24:587-97, e141-2.
4. Zoetis.com. Zoetis receives USDA license for CYTOPOINT. Available at: <http://news.zoetis.com/press-release/companion-animals/zoetis-receives-usda-license-cytopoint>. Accessed 2016.
5. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol* 2010;10:787-96.
6. Zeltins A, West J, ZabelF AEI, Turabi A, Balke I, Haas S, et al. A robust modular technology opening up the use of vaccine approaches to block signalling and treat chronic diseases even under limiting conditions. *Nat PG Vaccines* 2017;2:30.
7. Cavelti-Weder C, Timper K, Seelig E, Keller C, Osranek M, Lässig U, et al. Development of an interleukin-1 β vaccine in patients with type 2 diabetes. *Mol Ther* 2016;24:1003-12.

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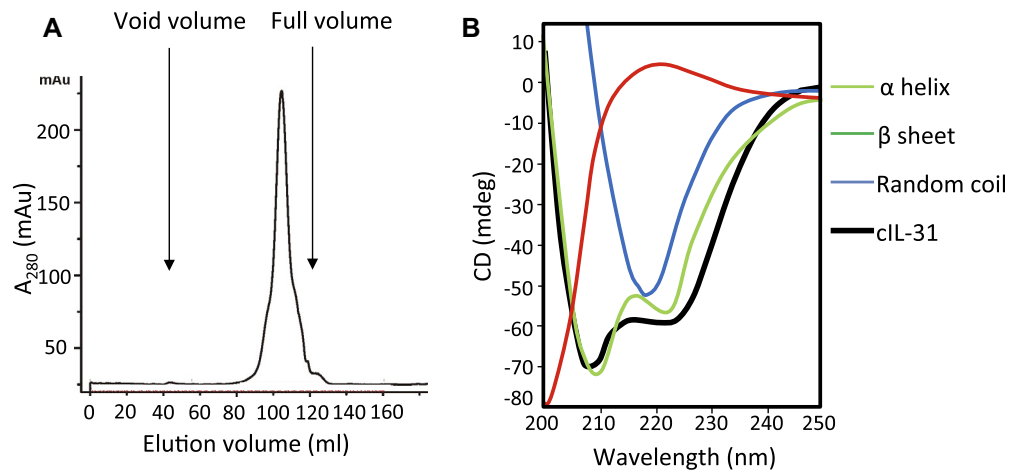


FIG E1. Properties of refolded canine IL-31. **A**, Elution profile from gel-filtration chromatography on HiLoad 16/600 Superdex 200 PG column. **B**, Circular dichroism analysis of refolded canine IL-31. Theoretical curves for helix, sheet, and random coil are shown as well.