Trabajo de Investigación de Maestría

Evaluación del prick test para detección de sensibilización alergénica en perros clínicamente compatibles con dermatitis atópica en la ciudad de Medellín.

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Programa de Maestría en Ciencias Veterinarias

Universidad de Antioquia

Facultad de Ciencias Agrarias

2017

Agradecimientos

La presente tesis es el resumen del reto personal y académico que decidí emprender en pro de mi desarrollo profesional y de la posibilidad de llevar la dermatoinmunología veterinaria a otro nivel, para así ejercer con continua dedicación a la ciencia y la academia. Muchas personas contribuyeron en este proceso de formación profesional de manera directa o indirecta, pero siempre manifestando su apoyo y dándome las fuerzas y elementos necesarios para llegar a este punto.

La realización de este trabajo no hubiera sido posible sin la constante ayuda de mi director de tesis, el Dr. Juan Guillermo Maldonado, quien gracias a su experticia y capacidad para transmitir el conocimiento, logró despertar en mí el amor a la inmunología veterinaria. Gracias por su apoyo y por depositar en mí la confianza para realizar y culminar con éxito este proyecto.

Agradezco de igual manera a los doctores Thierry Olivry y Manon Paradis quienes desinteresadamente me brindaron asesoría y correcciones en los momentos más oportunos. Han sido mis más grandes maestros y gracias a ustedes pude unir los dos temas que más me apasionan, la dermatología y la inmunología veterinaria.

Al doctor Jorge Sánchez quien me abrió las puertas y el camino para poder cambiar la forma de ejercer la alergología veterinaria en Colombia, contando con su apoyo y valioso aporte de conocimiento para la ejecución de esta tesis.

A todos los docentes a cargo del programa por compartir su conocimiento y permitirme aprender de cada una de sus disciplinas. En especial al Dr. Diego Piedrahita, por ser un claro ejemplo de los frutos que da la unión entre la ciencia y la pedagogía y por despertar en mí el deseo de enseñar de una manera diferente.

A mi familia del Hospital Veterinario, infinitas gracias por subir mis ánimos cuando el cansancio tomaba lo mejor de mí con sus sonrisas y abrazos de apoyo. Más que colegas, son grandes amigos y han contribuido a mi crecimiento profesional.

Gracias!

Dedicatoria

Esta tesis va dedicada al hombre que un día tomó la decisión de acompañarme y apoyarme en los proyectos de vida que me hicieran mejor persona y profesional pero ante todo, que me hicieran feliz. Luis Fernando, sin tu comprensión y ayuda en las etapas que hemos vivido juntos, este proyecto no hubiera sido posible. Gracias por ser mi bastón en este y todos los momentos de mi vida.

A mis hijos les dedico los frutos de mi esfuerzo en estos años. Es por ustedes y por el ejemplo que les quiero dar, que sigo el camino de la academia. Gracias por su paciencia durante las largas jornadas de estudio. Ustedes son mi razón de ser y de querer mejorar cada día más.

A la mujer más valiente y fuerte que conozco, mi mamá, le dedico estas horas de trabajo y estudio. Sin ti nada de esto hubiera sido posible. Gracias por apoyarme en todo momento y de todas las formas. Tú eres mi fuente de inspiración. Te admiro y te amo y no hay palabras de agradecimiento que suplan lo que has hecho por mí en todos estos años.

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Resumen General

La dermatitis atópica (DA) canina se define como una enfermedad cutánea crónica, pruriginosa e inflamatoria de la piel, que se presenta en animales genéticamente predispuestos (Hensel, Santoro, Favrot, Hill, & Griffin, 2015a). Factores como el medio ambiente, la genética y la respuesta del sistema inmune del individuo se unen para generar una compleja red de interacciones que desencadenan ~~en~~ los signos clínicos de la enfermedad. Los caninos son una de las pocas especies no-humanas que desarrollan enfermedades cutáneas alérgicas de manera espontánea (Pucheu-Haston, 2016) y se estima que el 10% de la población total de caninos padece de DA. Los primeros reportes de enfermedades alérgicas en caninos datan de hace aproximadamente 70 años, cuando se describió la hipersensibilidad a alimentos y alérgenos ambientales. Gracias a esto y a la similitud en características clínicas entre la especie humana y la canina, se ha utilizado a esta última como modelo animal para la DA (Marsella & Olivry, 2003). La estrecha relación que los humanos y los perros han tenido, se remonta a más de 32000 años, en los cuales han convivido en los mismos espacios, evolucionando juntos en cuanto a cambios ambientales y socioculturales que han trazado la forma de vivir actual (Tsai et al., 2007). Esto implica que ambas especies han sido expuestas de manera simultánea y sincrónica a los mismos alérgenos que encontramos como causales de la DA.

Lo que antes se consideraba una respuesta inmune tipo Th2 en fase aguda y Th1 en fase crónica en la DA canina, es ahora considerada una respuesta predominantemente Th2 en fase aguda y una respuesta mixta entre Th1, Th2, Th17 y Th22 en fase crónica (Pucheu-Haston, Bizikova, Marsella, et al., 2015). Los avances en el conocimiento y la comprensión de los mecanismos involucrados en la patogenia de la DA tanto en humanos como en caninos, han abierto las puertas de una visión más amplia y multimodal de la enfermedad. Comprender los mecanismos genéticos, ambientales, microbiológicos, físicos, bioquímicos, neurológicos y celulares que llevan a la presentación clínica de la DA, permite llegar a un nuevo entendimiento de las interacciones moleculares del sistema inmune y su implicación para el paciente a corto y largo plazo. En esta tesis se pretende describir los mecanismos inmunopatológicos

conocidos a la fecha y las perspectivas a futuro de la DA canina. Adicionalmente, se analiza el uso del prick test como herramienta para la detección de sensibilización alergénica en esta enfermedad.

General Abstract

Canine atopic dermatitis (AD) is defined as chronic, pruritic and inflammatory cutaneous disease that is present in genetically predisposed animals (Hensel et al., 2015a). Factors such as the environment, genetics and the individuals immune response join to generate a complex network of interactions that lead to the clinical manifestations of the disease. Canines are one of the few non-human species that develop cutaneous allergic diseases in a spontaneous manner (Pucheu-Haston, 2016) and it is estimated that 10% of the total population of dogs suffers from AD. The first reports of allergic disease in dogs dates to approximately 70 years, when hypersensitivity towards food and environmental allergens was described. According to those descriptions and to clinical characteristics between human and canine species, dogs have been used as animal models of AD for their human counterpart (Marsella & Olivry, 2003). The tight relationship that dogs and humans have had dates to over 32000 years where they have lived amongst the same spaces and have evolved together due to the environmental and sociocultural changes have shaped our current ways of life (Tsai et al., 2007). This implies that both species have been exposed simultaneously and synchronically to the same allergens that are found as causative agents of AD.

What used to be considered as a Th2 type response in an acute phase and a Th1 response in a chronic phase in canine AD is now considered as a predominantly Th2 response in an acute state and a mixed response between Th1, Th2, Th17 and Th22 in a chronic state (Pucheu-Haston, Bizikova, Marsella, et al., 2015). Advances in knowledge and the comprehension of the pathogenesis of AD in humans as well as dogs has opened the doors of a wider, multimodal approach to the disease. The comprehension of the genetic, environmental, microbiologic, physical, biochemical, neurologic and cellular processes lead to the clinical presentation of AD, allowing a new understanding of the molecular interactions of the immune system and their implications

for the patient in the short and long term. This thesis describes the immunopathological mechanisms known to date and future perspectives of canine AD while analyzing the prick test as a tool in the detection of the allergenic sensitization in this disease.

CAPÍTULO I

Introducción a la dermatitis atópica.

La inmunología es la rama de la biología que estudia y contextualiza el funcionamiento fisiológico del sistema inmune en estado de salud y enfermedad. Una de las enfermedades con mayores avances en investigación y conocimiento del rol del sistema inmune como factor causal es la dermatitis atópica (Manriquez, Andino-Navarrete, Cataldo-Cerda, & Harz-Fresno, 2015). La dermatitis atópica es una patología cutánea presente en humanos (Bieber, 2010) y caninos (Santoro & Rodrigues Hoffmann, 2016) y las similitudes clínicas e inmunológicas que se han descubierto a través de los años han permitido utilizar a los perros con DA como modelo de investigación para el humano (Marsella & Girolomoni, 2009). A su vez, los avances en el conocimiento de la DA en humanos ha permitido extrapolar y comparar los mismos procesos en los caninos con esta enfermedad (Marsella & Olivry, 2003).

La DA es multifactorial y requiere de la combinación del medio ambiente, la genética y el sistema inmune para que se observen las manifestaciones clínicas características de la patología. En conjunto, la comprensión que se tiene sobre la DA ha crecido de manera exponencial en los últimos 10 años, a la luz del entendimiento del rol que tiene el sistema inmune en esta enfermedad (Novak, Bieber, & Leung, 2003). La DA canina es definida como una dermatopatía crónica, genéticamente predispuesta, pruriginosa e inflamatoria (Hensel et al., 2015a). Debido a esto, es necesario abarcar todos los componentes del sistema inmune para comprender la transición entre la predisposición genética a la manifestación clínica aguda y finalmente, a un estado crónico de inflamación en la piel.

Tanto en medicina humana como en medicina veterinaria, es clave comprender los fenómenos inmunológicos que se dan en esta enfermedad. Desde la detección del alérgeno vía percutánea, el inicio de la cascada inflamatoria como parte de la respuesta innata y la estimulación del sistema adaptativo, hasta la respuesta completa del sistema

inmune en los subsecuentes encuentros del huésped con el factor al que está sensibilizado.

Presentación clínica de la DA y comparación entre caninos y humanos

La DA canina ha sido descrita por diferentes autores desde la década de los 60s donde la falta de acceso a la información, la diversidad de criterios y las diferencias geográficas, dificultaban el establecimiento de los criterios básicos más prevalentes en cuanto a raza, edad, sexo, localización y descripción de las lesiones. Muchos años y reportes después se han podido determinar algunos de estos criterios. En humanos con DA, el patrón de lesión inflamatoria comprende la cabeza, la cara, el cuello, las regiones flexurales de las extremidades y las manos (Watson W & Kapur S, 2011). Esto en gran medida es compatible con las lesiones presentes en caninos con DA, que principalmente se observan en la región periocular y perioral, la parte cóncava del pabellón auricular externo, las axilas, la región interdigital de los cuatro miembros y la región inguinal (Thierry Olivry, 2012). Las lesiones en humanos consisten en piel seca y eritematosa que se complica con escoriaciones producidas por autotraumatismo, como consecuencia de la sensación pruriginosa que se genera en los sitios afectados. Cuando la condición se vuelve crónica, la piel se liquenifica y se vuelve más susceptible a infecciones bacterianas, principalmente por *Staphyloccoccus aureus* (Kobayashi et al., 2015) que empeoran el cuadro inflamatorio y pruriginoso de la enfermedad.

En caninos con DA, se observa eritema y alopecia en la presentación aguda. En la forma crónica, se encuentra además liquenificación de las áreas descritas previamente y se presenta el mismo fenómeno de susceptibilidad cutánea a microorganismos oportunistas, principalmente *Staphylococcus pseudintermedius* y levaduras como *Malassezia spp* (Rodrigues Hoffmann, 2017a). La edad de presentación es una de las características que no comparten caninos y humanos. En humanos se reporta que el inicio de la presentación clínica se da principalmente en niños, donde estudios longitudinales han demostrado que hasta un 70% de ellos entra en remisión completa de la enfermedad cuando ha llegado a la edad adulta (Ricklin, Roosje, & Summerfield,

2010). Los adultos con DA por lo general presentan la forma más severa de la patología (Weidinger & Novak, 2016). No hay predilección por sexo en humanos ni en caninos.

En el caso de los caninos, la edad inicial promedio de presentación de la enfermedad es de 1-3 años, pero en esta especie no se da la remisión en ningún momento de su vida (Hensel et al., 2015a). Teniendo en cuenta que la predisposición genética es un componente importante de la DA, se han establecido las razas más susceptibles a presentar esta dermatopatía: west highland white terrier, pastor alemán, golden retriever, labrador retriever, boxer y bulldog francés (Bizikova et al., 2015). El consenso actual en medicina veterinaria concluye que la DA es una condición crónica vitalicia tratable pero no curable.

Relación de la barrera cutánea con el ambiente.

El rol del medio ambiente en el desarrollo de la enfermedad es fundamental para comprender la patogenia de la DA. El ingreso de los alérgenos se da vía percutánea, y el sistema inmune local comienza a desencadenar una serie de respuestas que llevan al estado inflamatorio agudo y luego crónico (Strid & Strobel, 2005). Debido al incremento exponencial de la incidencia de la enfermedad en humanos y en caninos en los últimos 20 años, se ha propuesto la teoría de la higiene como la principal causa de este fenómeno. Esta teoría estipula que la falta de interacción con elementos ambientales con potencial patogénico como animales, bacterias, hongos, parásitos, entre otros, favorecería el desarrollo de una respuesta inmune hacia antígenos que normalmente son inocuos para la población general (Okada, Kuhn, Feillet, & Bach, 2010).

Los programas de prevención sobre enfermedades parasitarias, el cambio en los estilos de vida en humanos y por ende en los caninos, junto con el aumento de productos antibacteriales, contribuyen a una disminución de la exposición patogénica (Okada et al., 2010). Estudios sobre el sistema inmune en infantes y mujeres embarazadas han demostrado que la exposición temprana de la madre y del hijo a diversos antígenos ambientales, propicia una respuesta inmune moderada acorde al antígeno al que

estuvo expuesto y que dicha exposición evitará una respuesta inmune excesiva al encontrarse nuevamente con el mismo antígeno (Flohr & Yeo, 2011).

La piel, aparte de ser el órgano más grande y extenso del cuerpo, tiene la función crítica de ser la primera barrera de defensa que separa a los órganos del ambiente. Esto implica que debe contar con mecanismos precisos para evitar la entrada de patógenos y la salida de elementos fundamentales para la homeostasis del cuerpo (Wong, Geyer, Weninger, Guimberteau, & Wong, 2016). Uno de los grandes interrogantes relacionados con la DA, es si existe una alteración primaria de la piel que facilite el ingreso de los alérgenos para la inducción de la respuesta alergénica cutánea. En personas con DA, se han encontrado alteraciones en la filagrina (Brown & Irwin McLean, 2012), una proteína estructural encargada de producir materiales higroscópicos como aminoácidos y factor de humectación natural que le da al estrato córneo su calidad de humectación (Irvine, McLean, & Leung, 2011). En caninos con DA, dicha alteración no se ha demostrado (Marsella, 2013a).

Los lípidos que hacen parte de la barrera cutánea también han sido extensamente estudiados en la DA, encontrando que los tres principales constituyentes de la matriz lipídica, que son las ceramidas, el colesterol y los ácidos grasos esenciales, se encuentran alterados (Angelbeck-Schulze et al., 2014). Esto desestabiliza al sistema lamelar que permite la impermeabilización de la piel y evita la pérdida de agua transepidérmica (Shimada, Yoon, Yoshihara, Iwasaki, & Nishifuji, 2009). Los estudios en humanos y caninos con AD sobre la matriz lipídica de la piel han demostrado que hay una disrupción y desorganización de las placas lamelares. Esto ocasiona una pérdida aumentada de agua transepidérmica que agrava el cuadro de descamación cutánea y favorece la replicación bacteriana (Marsella, 2013b). Aún se desconoce si las alteraciones encontradas en la barrera cutánea constituyen una verdadera alteración primaria que lleve a la presentación clínica de la DA o si la disrupción de la barrera sea una consecuencia y no la causa de la cascada inflamatoria iniciada por la estimulación alergénica del sistema inmune.

El sistema inmune y la dermatitis atópica

La DA canina se define como una hipersensibilidad tipo I donde la IgE es la mediadora de la respuesta alérgica. No obstante, no se puede incluir a la IgE dentro de la definición general de la enfermedad, puesto que perros sanos que no presentan ningún signo clínico de DA pueden tener niveles séricos de IgE o presentar reactividad cutánea con pruebas intradérmicas y algunos perros con todos los signos clínicos de DA no presentan reactividad en pruebas serológicas o cutáneas (DeBoer & Hillier, 2001). Esta condición se ha denominado “dermatitis atópica-simil” (Hoffmann et al., 2014). Igualmente, en la contraparte humana, se ha descrito una variedad de DA denominada “intrínseca”, donde los pacientes presentan todos los signos clásicos de DA, pero no presentan niveles detectables de IgE en suero ni reactividad en pruebas cutáneas (Tokura, 2010).

El conocimiento que tenemos actualmente sobre la DA es que es una enfermedad multifactorial que involucra una gran diversidad de respuestas inmunes, físicas e incluso neurogénicas que llevan al paciente al estatus inflamatorio y pruriginoso que la caracteriza (Bizikova et al., 2015). La compleja interacción que se presenta entre los queratinocitos, las células de la respuesta innata y adaptativa del sistema inmune, sumada a la alteración de la barrera cutánea (M Leung et al., 2013), hacen de la DA una de las patologías cutáneas más difíciles de definir y caracterizar. Esto a su vez, dificulta el hallazgo de herramientas farmacéuticas o elementos de prevención que eviten la presentación de los signos clínicos en la enfermedad.

En el primer contacto del alérgeno con la piel, es capturado y presentado por las células centinelas de la epidermis; estas son células dendríticas localizadas en la primera capa externa de la piel, encargadas de vigilar el ambiente circundante cutáneo en busca de cualquier material foráneo potencialmente patogénico. Dichas células, denominadas células de Langerhans, migran hacia el linfonodo más cercano luego de haber procesado el alérgeno y de expresar sus principales epitopes proteicos en las moléculas de su complejo mayor de histocompatibilidad (CMH) tipo II (CMH-II), para presentarlo a los linfocitos T CD4+ (T ayudador o Th) (Dubrac, Schmuth, & Ebner,

2010). Posteriormente se presenta la sinapsis inmunológica, donde la citoquina IL-4 es la principal mediadora encargada de generar el cambio de Th virgen (no expuesto al antígeno) a Th2 (estimulado por el antígeno) (Thierry Olivry et al., 2016). El objetivo de la estimulación del sistema adaptativo es generar memoria frente al antígeno de modo que, cuando ocurra una segunda exposición, el cuerpo esté preparado para reaccionar más rápidamente frente a este.

Una vez la célula Th2 se diferencia, comienza a liberar las citoquinas IL-3, IL-4, IL-5, IL-9, IL-13 e IL-31 (T. J. Nuttall, Knight, McAleese, Lamb, & Hill, 2002b). Dentro de éstas, la que más efecto ejerce en la primera exposición es la IL-4, que se encarga de inducir la diferenciación de los linfocitos B en células plasmáticas productoras de anticuerpos específicos para el antígeno presentado (Hill & Olivry, 2001). En este caso, es la IgE el anticuerpo circulante que estará listo para neutralizar el patógeno. Simultáneamente, las demás células con receptores de alta afinidad para la IgE, que poseen su receptor específico (denominado FcεRI), aumentarán la cantidad de receptores de superficie para poder amplificar la respuesta inmune encargada de contrarrestar el antígeno (Brazís, De Mora, Ferrer, & Puigdemont, 2002). Células como los queratinocitos, células dendríticas, mastocitos y basófilos se sensibilizan frente a un subsecuente encuentro (Mu, Zhao, Liu, Chang, & Zhang, 2014).

En la fase aguda de la dermatitis atópica canina, los queratinocitos no solo cumplen la función protectora, sino que tienen un rol activo dentro de la liberación de citoquinas y quimiocinas importantes dentro de la respuesta innata del sistema inmune frente a un alérgeno (Suter et al., 2009). Cuando se estimula el queratinocito con el alérgeno, comienza a liberar citoquinas como IL-1, IL-6, IL-8 y TNF-α, que a su vez reclutará células como neutrófilos y eosinófilos al sitio de estimulación e inducirá la degranulación de los mastocitos (Asahina & Maeda, 2017). Simultáneamente, el queratinocito libera las quimiocinas TARC (Thymus activation- regulated chemokine, o CCL17), TSLP (Thymic Stromal Lymphopoietin), GM-CSF (granulocyte and monocyte colony stimulating factor) y CCL5 (Gröne, 2002a). La TARC es una quimiocina que atrae a los

linfocitos T que poseen el receptor CCR4 al sitio efector, para reclutar a más linfocitos T de memoria (Maeda, Fujiwara, et al., 2002a).

La quimiocina TSLP ha sido encontrada tanto en DA canina como humana (Y.-J. Liu, 2006; Sakamoto, Asahina, Kamishina, & Maeda, 2016) y su primera función es la maduración de las células dendríticas para reclutar linfocitos T de memoria a la lesión; su segunda función es la estimulación de neuronas sensitivas en estrecha aposición con los queratinocitos para la inducción de la sensación pruriginosa en la piel (Turner & Zhou, 2014). El GM-CSF es una quimiocina encargada de estimular la maduración y reclutamiento de los macrófagos de manera paracrina (Shi et al., 2006). Otras citoquinas y quimiocinas descritas en los estudios, que son producidas por los queratinocitos en el contexto de la DA canina son TGF-β, IL-12, IL-18, IL-17A, IL-33 y CXCL-8, CCL-27, CCL-28 (Asahina & Maeda, 2017). En general, cumplen la función de reclutar células pro-inflamatorias, linfocitos T reguladores (T-reg) y linfocitos Th2 al sitio de presentación antigénica para la inducción de la respuesta inflamatoria.

Como componentes característicos de la respuesta inflamatoria aguda, encontramos a los mastocitos, los eosinófilos y los basófilos que son reclutados y estimulados por la cascada de citoquinas descritas previamente, para estimular la degranulación de sus componentes: vía IgE dependiente por el receptor FcεRI; y vía IgE independiente por otros receptores inespecíficos (Hammerberg, 2009; F. T. Liu, Goodarzi, & Chen, 2011). Algunas de las citoquinas y quimiocinas liberadas son: histamina, TNFα, prostaglandina (PG) D2, PGE2, leukotrieno (LT) B4 y C4, IL-3, IL-4, IL-6, IL-13, GM-CSF, CCL5, CCL2 y CCL3 (Brazís et al., 2002; Thierry Olivry et al., 2016; Pucheu-Haston et al., 2006; Ständer et al., 2011). Los macrófagos son otras células reclutadas durante el proceso de presentación y estimulación antigénica y su principal función es la presentación de antígeno y la estimulación de factores quimiotácticos para ayudar a establecer la respuesta adaptativa (Czogala, Marycz, Kuryszko, & Zawadzki, 2011).

Los componentes anteriormente descritos favorecen una respuesta inflamatoria adaptativa dominada por células tipo Th2, en la presentación aguda de la DA canina. Al

parecer, los mecanismos de regulación en la DA canina están alterados, dado que se ha encontrado un aumento en el conteo de los linfocitos T reguladores en la piel de caninos con DA activa con respecto a controles, aunque se sospecha que éstos pueden presentar algún tipo de disfunción en cuanto a las principales citoquinas reguladoras que son la IL-10, IL-35 y TGF-β (Auriemma, Vianale, Amerio, & Reale, 2013; Campbell, 2015; Keppel et al., 2008).

La alteración ocasionada por la inflamación continua de la piel y el predominio inicial de la respuesta tipo Th2, hace que los mecanismos cutáneos y sistémicos de control antimicrobiano dominados por una respuesta tipo Th1 estén bloqueados por las citoquinas producidas en la fase aguda (Romagnani, 2000). Esta situación, adicional a la disrupción de la barrera cutánea por autotraumatismo secundario al prurito o por la misma inflamación persistente, facilita la proliferación microbiana, que agrava aún más el estado pruriginoso y complica el cuadro inflamatorio (Murota & Katayama, 2017).

Una vez la respuesta inflamatoria se vuelve crónica, se presenta un cambio en el tipo de infiltrado de células inmunes. En la fase crónica de DA canina se observan cambios físicos sobre la piel del paciente, donde hay replicación anómala de queratinocitos generando liquenificación de las zonas afectadas (Mu et al., 2014). Este hecho, sumado a las infecciones cutáneas recurrentes, hace que células en el tejido afectado liberen citoquinas como IFN-γ, lL-12, IL-17A, IL-18, IL-22 y GM-CSF (T. J. Nuttall et al., 2002b; Ong & Leung, 2016; Santoro et al., 2015). El IFN-γ es una citoquina que induce la apoptosis en los queratinocitos, contribuyendo al desorden en la queratinización. Adicionalmente, estimula a las células NK en la respuesta innata y a los LT CD8+ en la adaptativa para ejercer efectos apoptóticos y líticos sobre las células o tejido afectado (T. J. Nuttall et al., 2002b; Schindler, Levy, & Decker, 2007).

En la fase crónica de la DA también se encuentra una respuesta de los linfocitos Th22, que al ser estimulados por la citoquina IL-6 y TNF-α, producen lL-22. Esta citoquina induce la remodelación y replicación cutánea por medio del factor de crecimiento de fibroblastos implicado en procesos de angiogénesis y fibrosis y podría ser el factor

causal de la liquenificación en la fase crónica de la DA canina (Eyerich et al., 2009). La IL-6 y el TGF-β son citoquinas que inducen la diferenciación de células Th17. Estas células producen las citoquinas IL-17A, IL-17F, IL-21 e IL-22 que son pro-inflamatorias y se encargan de inducir hiperproliferación epidérmica (Schmidt-Weber, Akdis, & Akdis, 2007). También estimulan la producción de citoquinas pro-inflamatorias de los queratinocitos perpetuando el cuadro.

OBJETIVOS

Objetivo General

Comprender la dermatitis atópica canina desde la inmunopatogenia de la enfermedad para el desarrollo de nuevos conocimientos en dermatología veterinaria.

Objetivos Específicos

1. Realizar una revisión bibliográfica de la inmunopatología de la dermatitis atópica canina como modelo animal de la dermatitis atópica humana.
2. Hacer un análisis comparativo entre los elementos conocidos de la inmunopatología, tanto de la dermatitis atópica humana como de la dermatitis atópica canina
3. Identificar los componentes inmunológicos involucrados en la dermatitis atópica canina que requieren mayor investigación
4. Analizar la utilidad del prick test como herramienta para identificar los principales alérgenos que afectan a caninos con dermatitis atópica en la ciudad de Medellín, Colombia.

CAPITULO II

Recent Advances in the Immunopathogenesis of Canine Atopic Dermatitis: A Review.

Abstract

Canine atopic dermatitis (AD) is the most prevalent allergic skin disease in dogs. The dog is the only animal species that shares marked resemblance of the disease compared to human AD. A combination of environmental, genetic and immunological factors leads to chronic barrier dysfunction, inflammatory and pruritic manifestations on the skin that are worsened by opportunistic secondary infections. The immunopathological networks in canine AD are complex and have led to the understanding that there is not a single common trigger for the disease but a summary of causal factors in the individual that lead to the same clinical presentation in dogs with AD. The purpose of this review is to illustrate key concepts of the immunopathogenesis of canine AD. The latest findings on each of the cells, cytokines, chemokines and cofactors involved in the immunopathogenesis of AD will be summarized and discussed. Knowledge on pathogenic factors involving skin barrier dysfunction and the role of microbiome and genetics is summarized. Furthermore, we will elucidate the role of innate and adaptive immune responses in AD and how antigen specific interaction between T and B cells are involved in the skin during different stages of the disease. This article reviews all of these immune components implicated in AD and how they may become a pivotal part of therapeutic targets and preventative measures in the treatment of canine AD.

Key words: Canine allergy, Cytokines, Food-borne Allergy, Reactive Skin, Skin Diseases.

Methods

Citation databases, abstracts and proceedings from international meetings from human and veterinary research published between 2000 and 2017 were reviewed. When

necessary, older articles were included for background information. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Introduction

Canine atopic dermatitis (AD) is a multifactorial pruritic skin disease present in genetically predisposed dogs. It shares many characteristics of human atopic dermatitis, and affected dogs have often been used as research models for human medicine (Marsella & Olivry, 2003). This is possibly due to the close interaction and bond created between man and dog in the last 32,000 years, where being exposed to the same environment and stressors may have led to common pathways of genetic selection that are evidenced in many diseases we see today, being one of them AD (Meury et al., 2011; Tsai et al., 2007). However, there are slight differences between human AD and canine AD. The prevalence of the age of onset of the disease differs (Ricklin et al., 2010). In dogs, AD ranges from one to three years of age, which would be the equivalent of adolescence or early adulthood in humans (Looringh van Beeck., 2014). In the latter, it is more common to find the beginning of the disease at childhood or even infancy (Kim et al., 2016). Canine AD is currently a treatable yet not curable chronic skin disease characterized by pruritus which varies from a mild to a severe presentation from one patient to another or on the same patient depending on the flares (Thierry Olivry, Mueller, Nuttall, Favrot, & Prélaud, 2008). This disease affects approximately 15% of the overall population and does not exhibit spontaneous remission contrary to what has been described in human literature (Hillier and Griffin, 2001; Ricklin et al., 2010). Its diagnosis and severity is defined according to standardized clinical criteria and scoring systems proposed by the International Committee on Allergic Diseases for Animals (ICADA). Due to the multifactorial component of AD there is no specific diagnostic test currently available for dogs, and this is true for humans as well. There are several allergen tests that can be carried out in skin and in blood, however the results in serum testing do not always correlate to the results in skin testing and neither of these tests prove that the dog is suffering from the disease (Hensel, Santoro, Favrot, Hill, & Griffin,

2015b). The only conclusion that can be drawn from the results from serum or intradermal testing is that the dog has been in contact with certain allergens and has mounted an immune response, but that does not translate into an active disease unless it has been clinically proven (DeBoer & Hillier, 2001; Hillier & DeBoer, 2001).

Histopathology of dogs affected with AD describe a spongiotic dermatitis with an inflammatory infiltrate composed mainly of mononuclear cells described as T cells and dendritic cells (DC) (Bizikova et al., 2015). Dogs with AD have clinical signs and distribution of lesions similar to their human counterpart (Pucheu-Haston, 2016; Santoro & Rodrigues Hoffmann, 2016). The main areas affected are periocular, perioral, pinnae, interdigital spaces, axillae and groin (Bizikova et al., 2015; Griffin & DeBoer, 2001). Canine AD also favors the colonization of the skin of bacteria, mainly *Staphylococcus pseudintermedius* and yeasts such as *Malassezia spp*. which take advantage of the altered skin environment to proliferate and worsen the clinical signs (Santoro et al., 2015). The pruritus caused whether by the allergic response or by the secondary infections induce the affected dog to scratch, often causing self-trauma to the skin, thus creating a vicious cycle between all of the contributing factors and the inflamed skin.

At first it was believed that the primary cause of canine AD consisted of an alteration in the immune system that led to abnormal production of allergen specific IgE which would clinically be observed as an inflammatory and pruritic condition of the skin (Thierry Olivry & Hill, 2001a). However, complex immune interactions that develop directly on the skin of dogs with AD due to alterations in different components of the skin barrier have been recently deciphered (Marsella, Olivry, & Carlotti, 2011). These abnormal conditions in the skin facilitate allergens and microbes to easily penetrate and initiate an onsite cascade overstimulating the innate and adaptive immune response (Boguniewicz & Leung, 2011; Kobayashi et al., 2015; J. D. Sinke, Rutten, & Willemse, 2002). This new insight has led researchers to consider a multimodal approach into the understanding of this disease where there is a combination between an altered skin barrier, environmental factors and abnormal immune reactivity (Hill & Olivry, 2001; Marsella et al., 2011; Meury et al., 2011).

It is still unclear whether the reported skin barrier abnormalities in canine AD are primary or secondary to inflammation (Thierry Olivry, 2011). Another area that is being deeply studied are the mechanisms that lead to the chronicity of the disease. At this time, and due to the amount of variables found from one patient to another, AD is beginning to be considered a syndrome instead of a disease *per se* that is caused by alterations in one or several genetic pathways that would lead to common manifestations in the skin (Wüthrich B, 2003). This article summarizes and updates the immunopathogenesis of canine atopic dermatitis covering the epidermal barrier alteration, the onset of the innate and adaptive immune response in the skin and the activation of the systemic immunological dysregulation that leads to the chronic presentation of the disease.

Skin barrier

Filaggrin (FLG) and the stratum corneum (SC)

The skin is a natural anatomical and physical barrier between the environment and the organism and as such, it must provide an important line of defense in order to avoid potential hazardous physical, chemical or biological agents from penetrating it. The main cells that compose the skin are keratinocytes. The outermost region of the epidermis is made up of flattened protein enriched corneocytes and intercellular lipid layers (Nishifuji & Yoon, 2013). The corneocyte has a cornified cell envelope on the inside of the plasma membrane which provides mechanical strength to the cell thanks to the keratin intermediate filaments that compose its cytoskeleton which is aggregated in bundles by the FLG monomers (Brown & Irwin McLean, 2012). This gives the corneocyte its flattened shape. Once the cell has reached the upper SC, these FLG proteins are degraded into natural moisturizing factors (NMF) by proteases in order to maintain skin hydration and pH (Nishifuji & Yoon, 2013).

The skin barrier impairment in the pathogenesis of canine AD is important because it increases the risk of allergic sensitization. Currently, it is still unclear whether there truly exists a primary defect in the skin of dogs with AD leading to the onset of the disease or if the defective barrier is secondary to the inflammatory response once the immune

system initiates its inflammatory response (Thierry Olivry, 2011). A study in West Highland White Terriers (WHWT) with AD demonstrated that there are no alterations in FLG and NMF from a genetic perspective (Salzmann et al., 2011). However, this may be a breed specific result, and cannot be generalized for all dogs with AD. In human AD, a loss of function mutation in the *flg*-gene has been found to be associated to 10% of patients with European ancestry (Irvine et al., 2011). In dogs, there are no current direct correlations between FLG alterations or deficiencies and severity of disease, nor has there been found mutations in the FLG genes that can be associated as the cause of canine AD.

Transepidermal Water Loss and Lipids in the skin barrier

The main adhesive proteins in the SC are the corneodesmosomes. They allow cell to cell binding and have a cytoplasmic site and an extracellular site. The extracellular components of the corneodesmosomes are degraded by kallikreins (KLK) and cathepsins, which leads to desquamation (Nishifuji & Yoon, 2013). When there is an imbalance between serine proteases and protease inhibitors, there is enhanced barrier permeability and inflammation. This phenomenon has been studied in humans but has yet to be found in canine AD. It has been demonstrated that an alteration in the stratum corneum leads to transepidermal water loss (TEWL) and that as the damage on the surface increases so will the associated desquamation (Cornegliani, Vercelli, Sala, & Marsella, 2012). Several methods for measuring TEWL have been proposed, but have not been validated to date. There is no current standardized method to evaluate TEWL. In a study done by Zajac et al (2014) a positive correlation was found between TEWL and CADESI-03 scoring in 5 out of ten body areas tested in atopic beagles, indicating that the severity of the lesions in canine AD correlate with TEWL (Zajac et al., 2014). However, in a study done by Marsella, no correlation was found (Marsella, 2012). Several studies have also evaluated the correlation between abnormalities in the extracellular lipid lamellae as a cause of increased TEWL (Cork et al., 2009; Shimada et al., 2009). The association between altered lipid barrier function and the pathogenesis of canine AD is still not fully understood. The main lipid components of the stratum corneum are ceramides, cholesterols and long chain free fatty acids (Thierry Olivry &

Hill, 2001b). An important cause of TEWL in dogs with AD has been associated with a decreased ceramide content (Zajac et al., 2014). Ceramides are the main constituents of the lipidic part of the stratum corneum, and thus an alteration in their production or structure can facilitate the penetration of allergens into the skin (Nishifuji & Yoon, 2013). Several studies available in veterinary literature have demonstrated by means of electron microscopic analysis of the skin that there is an alteration in the continuity and the thickness of intercellular lipid lamellae when comparing non-lesional canine AD skin to controls (Reiter, Torres, & Wertz, 2009). In one study, free and protein bound glycosylceramides were decreased in the SC of atopic dogs (Yoon et al., 2011). This was found to be due to a lower amount of sphingosine 1 phosphate (S1P) which is the intercellular product of ceramides by the conversion of the sphingosine 1 phosphate lyase (SGPL1) (Wood et al., 2009). S1P is a structural lipid and a signaling molecule. The role of genetic mutations that affect the synthesis and degradation of ceramides still need to be studied in depth in order to establish ceramide alterations as a primary cause of skin barrier alteration instead of a secondary cause due to inflammation.

Tight junctions.

Tight junctions (TJ) are closely associated areas in epithelial cells that create an impermeable fluid barrier between them. They consist of proteins embedded in both plasma membranes with an intracellular portion and an extracellular portion that adheres one cell to another. They mainly consist of claudins, occludin, junctional adhesion molecules and zonula occludens. According to previous studies, TJ protein expression may play a role in the development and progression of human AD (Yuki, Tobiishi, Kusaka-Kikushima, Ota, & Tokura, 2016). Pattern recognition receptors (PRR) can affect tight junction integrity. A defect derived from PRR signaling may lead to chronic inflammation, and alter the epidermal repair process (Bäsler & Brandner, 2017). In a study by Kim et al., (2016) a group of atopic dogs were compared with a group of normal dogs where both groups were sensitized with house dust mites and skin biopsies were taken and analyzed for TJ protein expression by immunohistochemistry. Objective and subjective analysis revealed a significant decrease in the intensity of the Zonula Occludens-1 staining compared to the control group (H.-J. Kim et al., 2016). It is still

unclear whether this defect found in atopic dogs is another factor to take into account in canine AD.

Canine Atopic Dermatitis and the skin microbiota

The skin microbiome is extensively diverse among individuals as well as among anatomical sites (Two et al., 2016). The microbiological diversity in the skin allows it to remain under homeostatic conditions. Many studies have demonstrated the relationship between cutaneous infections and atopic dermatitis (Kong et al., 2012; Ong & Leung, 2016). Recurrent staphylococcal skin colonization is a common presentation in canine AD where long term antibiotic treatment is often needed (Rodrigues Hoffmann, 2017b). In humans the main bacteria that proliferates in AD is *Staphylococcus aureus*, and in dogs it is *Staphylococcus pseudintermedius* (Jun et al., 2017; Pierezan et al., 2016). The conditions that favor the proliferation of these species over others are still under investigation.

Antimicrobial Peptides

Keratinocytes express a number of innate immune receptors collectively referred to as pattern recognition receptors (PRRs) that enable them to respond to microbes or tissue damage by releasing a broad range of inflammatory mediators (eg, cytokines, chemokines, and AMPs) (Hari, Flach, Shi, & Mydlarski, 2010). Several studies have analyzed the role of antimicrobial peptides (AMPs) in canine atopic dermatitis with conflicting results (Bao, Zhang, & Chan, 2013; Leonard, Affolter, & Bevins, 2012; Santoro, Bunick, Graves, & Segre, 2013; Santoro, Marsella, Bunick, Graves, & Campbell, 2011). To date, no immunohistochemical studies have been carried out in dogs to determine if there exists a decrease in keratinocyte derived AMPs that could lead to the proliferation of Staphylococcal bacteria in canine atopic skin. However, it is clear that the presence of pathogenic bacteria such as *Staphylococcus pseudintermedius* worsens the clinical signs associated with canine AD. One study evaluated changes in the skin microbiota in house dust mite sensitized beagles, and found that an allergen challenge leads to bacterial dysbiosis with a prevalence of *S. pseudintermedius* at the site of the induction of the lesion (Pierezan et al., 2016).

Another study found that allergic dogs have lower bacterial species richness than normal dogs in lesional and non-lesional skin (Hoffmann et al., 2014). This dysbiosis persists for at least four weeks after the allergen challenge or until antimicrobial therapy is initiated (Santoro & Rodrigues Hoffmann, 2016). With the sum of these studies it could be inferred that allergic patients, and specifically in this case, dogs with AD have an innate dysbiosis which worsens as the allergic flares begin in favor of the increase of *S. pseudintermedius* species.

Immune reactions in Canine Atopic Dermatitis

Keratinocytes as antigen presenting cells in Canine AD

Keratinocytes are amongst the first cells to come into contact with exogenous antigens and thus are ideally suited to inducing or perpetuating a protective immune response (Suter et al., 2009). Therefore, new research into their role as an immune cell in the pathogenesis of canine AD is ongoing. It has been known that keratinocytes produce a wide range of cytokines and chemokines in response to various stimuli‑ Thanks to their cell surface receptors such as Toll like receptors (TLR) and Nod like receptors (NLR) which are important mediators of the innate immune response in the recognition of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) (Hari et al., 2010). In the study by Mullin et al in 2013 in the canine keratinocyte progenitor cell line (CPEK) in dogs, authors demonstrated the ability of TLRs to induce transcription of TLR-1, TLR-2, TLR-3 that binds double stranded RNA as well as TLR-4 which binds LPS (Mullin, Carter, Williams, McEwan, & Nuttall, 2013). This indicates that canine keratinocytes are able to detect PAMPS in order to produce the signaling pathways that will lead to the necessary cytokines and chemokines to initiate and maintain an innate and later adaptive immune response (Asahina & Maeda, 2017). Black et al. 2007 found that human keratinocytes were able to enhance expression of Intracellular Adhesion Molecule 1 (ICAM-1) as a substitute for interactions between CD80/CD86 and CD28. In the same study, class I and class II MHC expression was also enhanced under inflammatory conditions (Black et al., 2007), providing the necessary conditions for the keratinocytes to present peptides to CD4+ or CD8+ T lymphocytes. However, in that same study, the activated keratinocytes primed memory T cells, not

naive T lymphocytes, suggesting that once the adaptive response initiates, the keratinocytes are able to reinforce the immune response by recalling memory T cells. The prior belief was that dendritic cells (DC) were needed in first instance to be activated in order to present antigen, and current emphasis is on the role of keratinocytes as antigen presenting cells, and how this influences the adaptive immune response in canine AD (Asahina & Maeda, 2017; Black et al., 2007).

Another important cytokine produced by canine keratinocytes is TSLP (Sakamoto et al., 2016). An important function discovered for TSLP is the activation of mast cells which are important in the primary immune response in the skin and the priming of DCs and Langerhans cells (LC) for the release of T-cell attracting chemokines. In vitro studies have suggested that keratinocytes may also directly communicate with sensory neurons through the release of TSLP, highlighting its importance in the induction of itch in AD (Turner & Zhou, 2014; Wilson et al., 2013). A new subset of sensory neurons that required functional TSLP receptors as well as the ion channel TRPA1, was found to promote TSLP mediated itch behaviors, and researchers identified the signaling pathway ORAI1/NFAT as the main regulator of protease activator receptor 2 (PAR2)-mediated TSLP secretion by epithelial cells (Wilson et al., 2013). By in-situ hybridization the authors also found TSLPR-positive DRG neurons, matching the characteristics of sensory neurons that mediate itch and pain. These neurons were found in close apposition to keratinocytes in the epidermis (Elmariah & Lerner, 2013). The role of TSLP in canine atopic dermatitis has been studied from cultures of primary canine keratinocytes finding a significantly higher expression of TSLP in the skin of atopic dogs compared to the control group (Klukowska-Rötzler et al., 2013).

Canine keratinocytes have been proven to produce TARC which is in charge of recruiting T lymphocytes that carry the chemokine receptor CCR4 which in turn will recruit more memory T cells into the skin (Shibata et al., 2011). In one study, TARC mRNA was found to be selectively expressed in lesional skin of dogs with AD, but not in non-lesional skin of the dogs with AD or the normal skin of the healthy dogs (Maeda, Fujiwara, et al., 2002b). Furthermore, the same study found expression levels of IL-1β,

IFN-γ and TNF-α in the lesional skin of dogs with AD to be significantly higher than those in the non-lesional skin of the same animals (Maeda, Fujiwara, et al., 2002b).

Canine keratinocyte cell lines have also been proven to produce a number of pro-inflammatory cytokines and chemokines such as TNFα, IL-12p35, IL-18 and IL-17A which are important in stimulating the production of INF-gamma and the up-regulation of IL-12, GM-CSF (T. J. Nuttall, Knight, McAleese, Lamb, & Hill, 2002a; Shibata et al., 2011). These mediators are a family of glycoproteins that have the ability to increase DC and macrophage maturation and function in a paracrine matter. Other cytokines produced by keratinocytes are TGF- β which is a multifunctional cytokine important in the regulatory T cell response, CXCL-8 (previously known as IL-8) which is an important chemokine attracting neutrophils, CCL27 (cutaneous T-cell-attracting chemokine) and CCL28 by which memory Th2 cells are recruited to the skin (Asahina & Maeda, 2017; Gröne, 2002b).

One of the most recent cytokines found to be produced by canine keratinocytes in the context of AD is IL-33. The receptor for IL-33 has been found to be expressed in humans in the following cells: mast cells, basophils, eosinophils, Th2 cells, macrophages, DC, NK cells, NKT cells, type 2 innate lymphoid cells (ILC2), endothelial cells, epithelial cells and fibroblasts (Schmitz et al., 2005). Its main function has been attributed to the survival of mast cells, eosinophils and the development of Th2 associated inflammation due to the high level of expression of its receptor on Th2 cells and mast cells (Brandt & Sivaprasad, 2011). In humans, IL-33 has been found to stimulate the production of IL-5 and IL-13 by Th2 cells and mast cells, but not IL-4. However, basophils stimulated with IL-33 were able to produce IL-4 in conjunction with IL-5 and IL-13 (Lee, Noh, Lee, Youn, & Rhim, 2012). IL-33 has been found to be produced three times the normal amount in atopic dogs compared to healthy dogs. This suggests that IL-33 may contribute to the immunopathogenesis of canine AD as has been found in human AD (Thierry Olivry et al., 2016).

The role of Granulocytes in Canine Atopic dermatitis

Mast Cells

Mast cells are sentinel cells in the skin that are responsible for promoting a homeostatic tissue environment by releasing substances that affect the function of blood vessels, composition of surrounding extracellular matrix and maintenance of water balance. They also attract a cascade of defense and inflammatory cells by releasing cytokines and chemokines that mobilize innate immune effector cells into the affected tissue. They play an important role in type I hypersensitivity by IgE-bound receptor cross-linking (Oyoshi, He, Kumar, Yoon, & Geha, 2009). The role of Mast cells in canine AD has been studied, and it was found that these cells degranulate via IgE specific pathways linked to FcƐR1 receptor activation (Brazís et al., 2002) and non-IgE dependent mechanisms mediated through proteases activation (chymase, tryptase and matrix metalloproteinase 9) and stem cell factor (SCF) (Pucheu-Haston, Santoro, et al., 2015; Santoro et al., 2015). SCF is a cytokine produced by fibroblasts that influences mast cells on their secretory functions, the mediators produced and their adhesive capacity to the dermis (Hill & Olivry, 2001).

There are conflicting results with regards to the number of mast cells present in the skin of dogs with AD which seems to vary depending on the body site (Hill & Olivry, 2001; Pucheu-Haston, Santoro, et al., 2015). However, one study found increased infiltrate of mast cells of over 150% on the pinnae and ventral interdigital skin of dogs with AD compared to other skin sites (Auxilia & Hill, 2000). At this time it is unclear whether the mast cells detected are being recruited or if they are proliferating in situ due to the surrounding inflammatory environment (Bizikova et al., 2015; Czogala et al., 2011; Olivry et al., 1997; Schlotter et al., 2011).

What has been demonstrated so far, is that the up-regulatory factors derived from the diverse inflammatory mechanisms involved in canine AD prime mast cells to enhance their ability to release inflammatory mediators such as histamine, TNFα, PGD2, PGE2, LTB4 and C4, IL-3, IL-4, IL-6, IL-13, GM-CSF, CCL5, CCL2 and CCL3 (Brazís, Queralt, De Mora, Ferrer, & Puigdemont, 2000; F. T. Liu et al., 2011; Pucheu-Haston, Santoro, et al., 2015). These mediators are key factors in the maintenance of the inflammatory status in the skin of dogs with AD.

Neutrophils in canine AD

Neutrophils are polymorphonuclear cells in charge of the innate response whenever the affected tissue needs a phagocytic and inflammatory defense against an offending agent. They are especially active when the host is facing bacterial infections, which is a common secondary effect in canine AD. Neutrophils have been identified as part of the first infiltrating inflammatory cells in the skin, but are found in less percentage in patients with a chronic presentation of AD (T Olivry et al., 1997; Thierry Olivry et al., 2001). Some of the chemotactic molecules that attract canine neutrophils are LTB4, IL1, CXCL8 and complement fragment C5a (Thomsen, 1991). Total counts of circulating neutrophils in dogs with AD vary between studies, this could be due to methodological differences or to the stage of the disease at which the patient is in (Pucheu-Haston, Santoro, et al., 2015). A study on the inflammatory infiltrate in the skin of dogs with AD showed a low infiltrate of neutrophils in lesional skin compared to the skin of control dogs (T Olivry et al., 1997). The percentage of neutrophils on the skin of patients with AD does not have a direct correlation to the percentage of the same cells in blood, and the functional role of neutrophils in canine AD has not been fully studied (Nestle, Di Meglio, Qin, & Nickoloff, 2009; T Olivry et al., 1997; Thomsen, 1991).

Eosinophils in canine AD

Eosinophils are mainly recognized for their roles in parasitic infestations and in hypersensitivity type 1 reactions. Some of the chemotactic factors for eosinophils are RANTES, histamine, LB4, and C5a (F. T. Liu et al., 2011). They express low affinity receptors for IgE but also have non IgE pathways for activation and degranulation (Mu et al., 2014). The cytokines IL-4, IL-5,IL-13 and GM-CSF play important roles in the survival, recruitment and function of eosinophils (Hill & Olivry, 2001; Oyoshi et al., 2009). Once in the inflamed tissue, eosinophils have low phagocytic activity and high secretory activity where they release their contents slowly into the surrounding environment. Three of the most important inflammatory mediators released in the degranulation of eosinophils are eosinophil-derived neurotoxin, major basic protein (MBP) and eosinophil cationic protein (Novak et al., 2003). Eosinophil-derived neurotoxin induces maturation

and activation of DCs via TLR2-MyD88 pathway, increasing the Th2 response (J. E. Kim et al., 2016). MBP has the ability of downregulating the integrity of lipid bilayers by disrupting and lysing liposomes prepared from acidic lipids (Mu et al., 2014).

Eosinophils constitutively express IL-31RA and upon stimulation with IL-31, they have the ability of prolonging their survival by activating the ERK signaling cascade and also releasing various proinflammatory cytokines and chemokines, such as IL-1β, IL-6, IL-31, CXCL1, CXCL8, CCL2, CCL18 and CCL26 (Hill & Olivry, 2001; J. E. Kim et al., 2016; Mizuno, Kanbayashi, Okawa, Maeda, & Okuda, 2009a; J. D. Sinke et al., 2002). Cutaneous eoshinophilic inflammation is uncommon in the dog compared to other animals (Hill & Olivry, 2001). Several studies have found eosinophil granule contents in the skin of dogs with atopic dermatitis, and one study reported that only 3% of the inflammatory infiltrate in skin biopsies of atopic dogs corresponded to eosinophils (T Olivry et al., 1997; Thierry Olivry et al., 2001; Pucheu-Haston et al., 2006). In blood of dogs with AD, frank eosinophilia is rare, but studies have found elevated numbers of circulating eosinophils compared to normal dogs (Hayashiya et al., 2002). More studies on the functional role of eosinophils in canine AD are needed to have a better understanding of innate immune mechanisms in a chronic inflammatory environment.

Dendritic cells in the skin

Dendritic cells in the skin are classified according to their localization in the anatomical compartments. In the epidermis, DCs are termed Langerhans Cells (LC). These cells are positioned where the skin barrier, the environment and the cutaneous immune system intersect. The main characteristic of dendritic cells is the ability to uptake antigen and present it to naive T cells, creating a crosslink between an innate and adaptive immune response. In a study in human AD, antigen delivered directly through the epidermis of atopic patients versus antigen placed directly in the dermis skewed a Th2 response and a Th1 response, respectively (Strid & Strobel, 2005). This emphasizes the importance of understanding the context in which antigen is presented because this will determine which cell is activated and the subsequent cytokine, chemokine and immune

response that follows. Depending also on the type of antigen, the milieu of cytokines and cells recruited varies (Pucheu-Haston, Santoro, et al., 2015; Said & Weindl, 2015).
Canine DC that were stimulated by TNF-α of keratinocyte or mastocyte origin differentiated naive T cells into Th2 polarized T-cells thanks to cytokines and chemokines such as IL-4, IL-25, IL-13, IL-33, and TSLP (Asahina & Maeda, 2017; Hill & Olivry, 2001; Pucheu-Haston, Santoro, et al., 2015; Ricklin et al., 2010). LC as well as DC exhibit high affinity receptors for specific IgE (FCεRI) (Czogala et al., 2011). However, IgE has been found to be expressed by LCs only in inflamed skin and in DCs in normal and lesional skin of dogs with AD (Ricklin et al., 2010).

Once FCεRI ligates IgE, the cell produces IL-16 and IL-13 and this in turn will recruit macrophages and CD4 T cells to the affected skin (Dubrac et al., 2010). A different type of DC recently described in humans with AD is termed inflammatory dendritic epidermal cell (IDEC) which differentiates itself from LCs because of the expression of the macrophage mannose receptor CD206 on its surface, and the lack of Birbeck granules (Dubrac et al., 2010). The IDEC cells are believed to contribute to the switching of the Th2 response to a Th1 response by the production of IL-12 and IL-18 (Yoshida et al., 2014). They also have the ability to move from the dermis to the epidermis upon antigen challenge. In a study on DCs, cells carrying CD 206 were found only in the dermis of dogs with AD, and were found to co-express receptors for IgE, CD14 and MHC II (Ricklin et al., 2010).

Another receptor found to be expressed by LCs is TSLP-receptor (TSLPR) (Zheng et al., 2010). A study on the gene expression of TSLP produced by keratinocytes in acute and chronic lesions in humans with AD, found that TSLP failed to stimulate memory DCs to produce Th1 polarizing cytokines from the IL-12 family, Type I IFNs, TNF-α, IL-1β or IL6-9 (Y.-J. Liu, 2006). However, TSLP did cause the mDCs to produce great amounts of IL-8, eotaxin-2, TARC and MDC (macrophage derived chemokine), which attract neutrophils, eosinophils and Th2 cells respectively. This in turn, will allow a Th2 permissive environment in the skin (Y.-J. Liu, 2006). In an in vitro study, TSLP-DCs stimulated naïve T cells CD4+ cells to differentiate into a Th2 inflammatory cell thanks to

the expression of the protein OX40L (Ito et al., 2005). The same study found that in the presence of IL-12, OX40L lost the ability to induce the inflammatory Th2 differentiation. The Th2 inflammatory cells produce IL-4, IL-5, IL-13, and TNFα, but little to none IL-10 or IFN-γ (Brandt & Sivaprasad, 2011). This would in part, explain the blockage of the regulatory pathway in allergic inflammation in the skin and the inability to down regulate the Th2 cascade. This could be a molecular support to the hygiene theory which states that microbial infections that skew a Th1 response could decrease the Th2 presentation in atopic patients (Romagnani, 2004). TSLP was also found to induce LC migration and activation *in situ*, which would then move to draining lymph nodes and continue the development of the Th2 type response systemically (Lee et al., 2012). Canine TSLP has been cloned and characterized. A study in dogs with canine AD found increased TSLP expression in lesional and non-lesional skin compared to controls (Klukowska-Rötzler et al., 2013). Canine TSLP shares a 70% nucleotide identity with human TSLP (Klukowska-Rötzler et al., 2013). This supports the idea that TSLP is an important cytokine involved in the pathogenesis of canine AD, and possibly shares the same pathways that induce the inflammatory response and itch in human AD.

Macrophages in canine atopic dermatitis

Macrophages are resident monocytic cells present in the skin that have the dual function of aiding the innate immune system in their phagocytic role and having the ability of uptaking and processing an antigen in order to migrate and present it to T cells in the draining lymph nodes. In canine AD, macrophages help establish an adaptive immune response. A study in dogs with AD, found that putative macrophages type MAC387+ were found in lesional as well as non-lesional skin of atopic dogs, but were absent in the skin of normal dogs(Ricklin et al., 2010). Further tests have been carried out evaluating the presence of circulating monocytes in the context of canine atopic dermatitis finding no correlation between the disease and this circulating cell type (Pucheu-Haston, Santoro, et al., 2015).

T cells in canine atopic dermatitis

Th1 and Th2 cells

Healthy skin contains more than twice the amount of T cells in the blood (Nestle et al., 2009). Epidermal T cells are located in the basal and suprabasal keratinocyte layer where they are in close proximity to LC (Jacqueline D. Sinke, Thepen, Bihari, Rutten, & Willemse, 1997). Olivry et al., 1997 found γδ as well as αβ TCR in the epidermal T cells of dogs with atopic dermatitis, but only αβ T cells in the dermis (T Olivry et al., 1997). These results are in contrast with studies in the human counterpart which may be attributable to a species variation or to a contributing mechanism in the pathogenesis of canine AD (Auriemma et al., 2013). In dermis, T cells are mainly located beneath the dermal-epidermal junction, around post-capillary venules and close to cutaneous appendages (Bizikova et al., 2015). Tissue-tropism is mediated by the association and sequential action of adhesion molecules and chemoattractant receptors that are in charge of controlling the homing of leukocytes to the skin. This intricate process depends on the interaction of multiple molecules, such as selectins, chemokines and integrins with their respective ligands (Andrian & Mackay, 2000; Garrood, Lee, & Pitzalis, 2006).

By a process known as imprinting, DCs interact with and instruct lymphocytes to migrate to specific tissues by means of the peptide-MHC complex (Nestle et al., 2009). The DC also primes the naive T-cell into a CD4+ or CD8+ T cell, differentiating them into memory or effector cells equipped with their own homing properties (Said & Weindl, 2015). Th1-type cytokines produce proinflammatory attack-like responses, in charge of killing intracellular parasites and also accounting for the perpetuation of autoimmune responses. The main cytokine in the Th1 response is INF-γ (T. J. Nuttall et al., 2002b). An excessive proinflammatory response can lead to tissue damage, so there needs to be a balance between Th1/Th2 to prevent an abnormal immune response. Th2 produce IL-4 and IL-13 which are mainly responsible for B-cell activation and differentiation generating a strong antibody production (Romagnani, 2000).

Studies in humans with AD found that these cytokines also inhibit AMP production which could provide a permissive environment for microbial overgrowth and epidermal barrier dysfunction by the reduction of lipid production in the stratum corneum, suppress

keratinocyte differentiation via STAT3 and induce spongiosis (Ogg & Ogg, 2009). Th2 cells also produce IL-5 which is involved in eosinophilic activation, attracting eosinophils into chronic AD lesions (Brandt & Sivaprasad, 2011). IL-10 is another Th2-primed cytokine that has three main functions: the down regulation of several macrophage functions, enhancing B-cell survival and is an important immune regulator by blocking intracellular signal transduction pathways such as NF-κβ, which provides a phagocyte-independent protective response (T. J. Nuttall et al., 2002b). In excess, Th2 responses will affect the microbicidal action mediated by Th1 cells possibly enhancing susceptibility to infectious agents (Romagnani, 2004).

In canine AD there are polarized Th1 or Th2 responses according to the stage at which the disease is in (T. J. Nuttall et al., 2002a). As mentioned in previous paragraphs, keratinocytes have the ability of producing TARC, a chemokine that attracts cells that possess the CCR4+ receptor, which is another form of stimulating the recruitment of T-cells to the affected skin of atopic dogs (Maeda, Okayama, et al., 2002). In an acute or early stage of the disease there seems to be a Th2 skewed response, but in a chronic setting there is a switch to a Th1, Th2, Th17, and Th22 response (Pucheu-Haston, Bizikova, Marsella, et al., 2015). Several mechanisms are appointed to influence the Th cell differentiation, such as the cytokine profile of "natural immunity" evoked by different offending agents, the nature of the peptide ligand, as well as the activity of some co-stimulatory molecules and micro environmentally secreted hormones, in the context of the individual genetic background (Romagnani, 2000).

Many studies on the immune profile in canine AD have found that this disease is not a complete Th2 response, rather a mixed T-cell profile is presented where according to the acute or chronic state of the disease, the immune system will skew towards a Th1 or Th2 response (Schlotter et al., 2011). Other key players such as regulatory cytokines may play a role. Normally, canine skin carries low numbers of T lymphocytes, but several studies have proven an increase in CD4+ and CD8+ T cells in the skin of atopic dogs compared to controls (Czogala et al., 2011). Other studies have also demonstrated

an increase in circulatory T-cell numbers in canine AD at a ratio of CD4 to CD8 of 2.1:1 compared to 1.7:1 in normal dogs (Pucheu-Haston, Bizikova, Marsella, et al., 2015).

The switch from a Th2 to a Th1 profile may be attributed to a change in the DC infiltrating the skin (Yoshida et al., 2014). The Th1 cytokines IFN-γ, IL-12 and GM-CSF are elevated in patients with chronic AD (Breuer, Werfel, & Kapp Nordseeklinik Norderney, 2006). These cytokines have been isolated from peripheral blood mononuclear cells (PBMCs) and lesional skin of dogs with AD (Hayashiya et al., 2002). In canine and human AD, IFN-γ acts on keratinocytes by inducing their apoptosis, GM-CSF prolongs monocyte survival and induces a state of persistent inflammation, and IL-12 is involved in the differentiation of naive T cells into Th1 cells (Oyoshi et al., 2009).

One of the most recent Th2 cytokines discovered to be an important mediator in the immunopathogenesis of canine and human AD is IL-31 (Gonzales et al., 2013; McCandless et al., 2014). In humans, IL-31´s biological activities are correlated to the homeostasis of myeloid progenitor cells (MPGs), and the production of proinflammatory chemokines that recruit T-cells (Zhang, Putheti, Zhou, Liu, & Gao, 2008). Levels of IL-31 in blood and skin correlate with disease severity. In dogs with AD, IL-31 was found present in blood contrary to normal dogs (Mizuno, Kanbayashi, Okawa, Maeda, & Okuda, 2009b). Its main role proven so far has to do with the induction of pruritus in canine AD (Gonzales et al., 2013). IL-31 receptors (IL-31R) have been found on keratinocytes, monocytes and neuronal dorsal root ganglia (DRG) cells (McCandless et al., 2014). These cells are implicated in the transduction of pruritic signals to the spinal cord (Roosterman, Goerge, Schneider, Bunnett, & Steinhoff, 2006). The IL31/IL31A complex activates the Janus Kinase-STAT JAK/STAT signal transduction cascade as well as the phosphatidylinositol 3-kinase (PI3K) pathway (Bao et al., 2013; Lee et al., 2012). This complex has also been proven to stimulate nerve growth and branching of DRG through the STAT3 pathway (Bao et al., 2013). The same pathway in activated keratinocytes through the IL-31A complex induces the production of β-endorphin (Kim et al., 2016). It is an endogenous opioid peptide that modulates the perception of pain.

Low doses of IL-31 promote the antimicrobial barrier, and complete blockage may lead to an increased risk of infections (Hänel et al., 2016).

Studies in humans with AD and a concurrent *Staphylococcus aureus* infection have proven an impaired sensitization to IL-31 via alteration of the TLR-2 activation on keratinocytes (Jun et al., 2017). Whether the same effect is seen in dogs with atopic dermatitis is currently unknown. Recently, a caninized monoclonal antibody against IL-31 has proven to be effective as an antipruritic treatment in a large population of dogs with AD (Michels et al., 2016). However, given the multifactorial pathways of the disease some dogs may not respond as well to the antipruritic effect possibly due to other pruritic stimulus that override the IL-31 path.

γδT-Cells

γδT-Cells are considered to be an unconventional type of T cells that have an innate-like effect. They have a low ability to present antigen but have also been found to produce growth factors such as fibroblast growth factor (FGF9), Keratinocyte growth factor (KGF) and insulin-like growth factor 1 (IGF-1) and AMPs such as cathelicidins in humans (Nestle et al., 2009). A study by Olivry et al. in 1997 found an increase in epitheliotropic γδT-Cells in some of the dogs with AD, but made no significant associations to the disease (T Olivry et al., 1997). Very little is currently known about the role of γδT-Cells in the immunopathogenesis of AD in humans as well as in dogs.

Th17 cells

Another subtype of Th cells studied in the immunopathogenesis of atopic dermatitis is Th17. As a first line for bacterial and fungal infections Th17 cells produce potent pro-inflammatory cytokines such as IL-17 A, IL-17F, IL-21 and IL-22 (Cesare, Meglio, & Nestle, 2008). They are also characterized for the chemokine receptor CCR6, which is responsible for the homing of these cells into the skin and mucosa. Keratinocytes have receptors for these cytokines. These cytokines were shown to stimulate keratinocytes to produce GM-CSF, TNF-α, IL-8, CXCL10, and VEGF (vascular endothelial growth factor) which are important chemotactic factors for neutrophils and eosinophils, hence the

importance of its role in innate immunity. Th17 cells have also been shown to stimulate AMP expression, and induce epidermal hyperproliferation (Schmidt-Weber et al., 2007). A study in the pathogenesis of human atopic dermatitis found that the number of Th17 cells is increased in the peripheral blood and acute lesional skin of AD (Cesare et al., 2008). However other studies have found decreased numbers compared to controls. Pathogenic Th17 cells are stimulated by IL-23 and IL-1α and IL-21 (Brandt & Sivaprasad, 2011). These cells are inhibited by the cytokines IFN-γ and IL-4, whereas the regulatory type of Th17 cells are stimulated by TGF-β and IL-6 (Biedermann, Skabytska, Kaesler, & Volz, 2015). By means of the chemokine CXCL 13 produced by Th17 cells, B lymphocytes can be recruited, and this may imply antibody production (Mitsdoerffer et al., 2010).

In humans, IL-17 levels in chronic atopic dermatitis are decreased as Th2 cytokines inhibit the production of this cytokine. Whether it is due to the suppression of the regulatory effect of IL-17 or the pathogenic form is still to be elucidated. Cells other than Th17 such as cytotoxic CD8+ cells and γδT-cells also have the ability to produce IL-17 and their role in inflammatory disease is currently being studied. A study on the gene transcription of pro-inflammatory cytokines and chemokines induced by IL-17A in canine keratinocytes found that the transcription of GM-CSF, S100A8, IL-8 and IL-19 in cultured keratinocytes was significantly enhanced after stimulation with recombinant canine IL-17A (Asahina, Kamishina, Kamishina, & Maeda, 2015). This suggests a role for the Th2 derived response with Th17 mediated inflammation in canine AD, however this line of T-cells has been poorly studied.

Th22 cells

Th22 cells are one of the most recently discovered subtypes of T helper lymphocytes. Their role in adaptive immune responses is characterized by the production of IL-22. This cytokine has a synergistic function with IL-17 and TNF-α, and triggers cells that carry the IL-22R (receptor), which are mainly tissue cells (Mu et al., 2014). In human skin, IL-22 has been proven to induce AMPs, inhibit keratinocyte differentiation by the enhancement of IL-6 production and promote keratinocyte proliferation by the production

of matrix metalloproteinases (MMPs), suggesting its role in skin barrier impairment and in innate defense mechanisms (Eyerich et al., 2009). A study in canine AD, found that acute skin lesions from dogs with atopic dermatitis had significant up-regulation of genes encoding IL-22 derived from Th22 cell types (Thierry Olivry et al., 2016). Another study evaluated the skin associated T-lymphocytes in canine AD found significant levels of IL-22, but could not exclude the possibility of cells other than Th22 producing this cytokine (Jassies-Van Der Lee, Rutten, Bruijn, Willemse, & Broere, 2014). Further studies on the role of Th22 cells in canine atopic dermatitis are needed to evaluate its role in the inflammatory response of the disease.

B-cells and antibody response in canine atopic dermatitis

The ability of the immune system to create memory for antigen is key in the adaptive immune system. Apart from memory T CD4+ cells, B-lymphocytes are the cells responsible for the humoral response in the adaptive immune system and once stimulated, create plasma cells which are memory B-cells in charge of the production of antibodies to counteract against the offending antigen. Another form of activation of T-cells is by stimulation from B-cells which present the processed antigen to T helper cells and initiate a response via a MHCII/TCR interaction along with the co-stimulatory molecules and ligands needed to induce signal transcription. B-cells can also attract T-cells to lesional atopic skin by producing chemokines like IL-16, CCL22 and CCL17 (Peng & Novak, 2015). The Th2 cytokine IL-4 induces B cell replication which will lead to antibody synthesis (Romagnani, 2000).

The main antibody implied in humoral responses in allergies is IgE. B-lymphocytes play a significant role in canine atopic dermatitis (Halliwell & DeBoer, 2001). B-cells as well as IgE has been found in the dermis of dogs with AD(Hill & Olivry, 2001). The cells that express the receptor FcεRI on their surface, such as mast cells, basophils and LCs are stimulated by IgE to degranulate and enhance the inflammatory response in the skin(F. T. Liu et al., 2011). Even though high levels of IgE may be found in dogs with AD, this information does not correlate to the clinical signs present in the patient(DeBoer & Hillier, 2001).

Any dog may have elevated serum levels of IgE and show no signs of inflammatory skin disease. Total serum IgE differs from allergen-specific IgE. A study by Lauber et al. in 2012 done in Golden and Labrador retrievers with AD was unable to demonstrate a higher chance of allergen specific IgE reactivity compared to normal dogs (Lauber et al., 2012). In several studies in canine AD, a number of patients with clinical signs resembling canine AD have showed to be void of allergen-specific IgE in serum and skin testing (Looringh van Beeck., 2014). The proposed name for the disease in these patients in human and veterinary medicine is termed intrinsic atopic dermatitis and Atopic-like dermatitis (ALD) respectively (J. E. Kim et al., 2016). Many theories have been proposed for this condition, but one of the main theories is that these patients may not have the typical allergen specific IgE profile, and may be reacting to an allergen that has not been recognized or is uncommon for other humans or dogs with AD.

Studies in dogs treated with allergen specific immunotherapy have evaluated the clinical response to serum IgE with conflicting results. These studies concurred in finding an increase of serum IgE in the lowest clinical responses to immunotherapy (DeBoer & Hillier, 2001; Halliwell & DeBoer, 2001; Shida et al., 2004). Keeping in mind that atopic dermatitis is a disease described as a chronic inflammatory and pruritic disease present in genetically predisposed animals, whether IgE is present or not, would not change the clinical outcome of these patients unless IgE was found to be the cause of the inflammatory and/or pruritic response in atopic dogs. Unveiling the role of IgE in atopic dermatitis will help in the search for a more targeted approach in the treatments for canine AD. Until standardized criteria and regulations are established for laboratories testing for IgE and for the ones that produce the allergen extracts used for intradermal testing, no universally applicable results on the pathogenesis of canine AD can be drawn from these studies.

IgG has been proposed as another antibody that may contribute to the immunopathogenesis of atopic dermatitis. IgG is the most common type of antibody found in the circulatory system. It binds to many kinds of pathogens making it the main

humoral response in the body that controls infections. Several sub classes have been identified, and studies in dogs with atopic dermatitis suggest the subclasses IgG1 and IgG4 may be the ones involved (Pucheu-Haston, Bizikova, Eisenschenk, et al., 2015; Shida et al., 2004). In humans, IgG4 has been found to bind to allergen epitopes which affects the binding of the allergen specific IgE, generating a blockage in the IgE mediated response (Novak et al., 2003). Due to IgG´s ability to act upon many different types of antigen challenge, making it an unspecific form of a humoral response, more research needs to be undertaken to evaluate its effect upon environmental allergens as the main antigens that mediate canine AD.

Another type of allergens implicated in the immunopathogenesis of canine atopic dermatitis are food allergens. This is termed food induced atopic dermatitis or *sensu lato* whereas environmentally induced atopic dermatitis is termed *sensu stricto* (Griffin & DeBoer, 2001; Pucheu-Haston, Bizikova, Eisenschenk, et al., 2015). In this situation, the sensitization to food allergens induces clinical signs identical to canine atopic dermatitis with other variable clinical signs such as perianal pruritus, a very early or very late age of onset, and abnormal gastrointestinal signs. Because of the non-immunological variables present in adverse food reactions (AFR) compared to food induced AD, these two entities are not considered the same, and need to be evaluated accordingly (Pucheu-Haston, Bizikova, Eisenschenk, et al., 2015).

Most dogs with an initial diagnosis of food induced AD also develop environmentally mediated atopic dermatitis (Pucheu-Haston, 2016). However, dogs with AFR do not have the same probability depending on the cause of the AFR. The immune mediated form of AFR has been studied in dogs and proven to produce most of the inflammatory mediators found in canine AD (Pucheu-Haston, Bizikova, Eisenschenk, et al., 2015). The clinical signs present in patients with AFR resemble those present in canine AD with histopathology studies of the skin proving to share the main characteristics such as spongiosis, and occasional acanthosis with superficial perivascular inflammation consisting of a mixed inflammatory infiltrate with a low percentage of neutrophils and eosinophils (Czogala et al., 2011).

Regulatory T cells

Regulatory T cells otherwise known as Tregs are responsible for the control of the immune response once it is activated. Tregs have an immune modulating and anti-inflammatory activity by direct cell to cell contact or by secreting cytokines such as TGF-β and IL-10a (Auriemma et al., 2013). Treg cells have been found in humans and in dogs. Its phenotype is characterized by exhibiting the forkhead/winged helix transcription factor FOXP3+ in Treg lymphocytes (Campbell, 2015). Cells that express FOXP3+ have an important role in peripheral immune tolerance (Rosenblum, Way, & Abbas, 2015). A study measuring the percentage of circulatory Tregs in dogs with AD found no statistical difference between affected dogs and the control group (Majewska et al., 2016). Another study in dogs with AD found no significant differences between atopic dogs and dogs in the control group, but found a significant increase in the percentage of circulating Treg cells in the group of dogs with AD that had received immunotherapy treatment for a period of 12 months (Keppel et al., 2008).

An evaluation of lymphocytic, cytokine and transcriptomic profiles in peripheral blood of dogs with atopic dermatitis found significantly increased levels of FoxP3+ cells compared to the control group and decreased levels of IL-10 and TGF-β1 (Majewska et al., 2016). They speculated that there could be a functional insufficiency of Tregs. Studies in humans with AD have found that Treg cells percentage, Foxp3 and TGF-β mRNA levels were reduced in AD patients compared with healthy controls (Boguniewicz & Leung, 2011). It is speculated that Tregs are down-regulated during the active phase of atopic dermatitis, thereby affecting the dog's ability to keep its inflammatory response under control, however this hasn´t been studied to date.

Genetics and canine atopic dermatitis

A variety of diseases in veterinary medicine are associated to an altered immune response in the skin. The disease that has the most extensive research common to veterinary dermatology and veterinary immunology is canine AD. This could owe to the fact that there are many common factors associated to AD that have also been found in

human medicine (Marsella & Olivry, 2003). Every piece of information gained in one species is valuable for the other. In spite of the ongoing knowledge on the disease, it is far from clear as to a single common factor that leads a dog or a human to the manifestation of the disease. The canine genome has been fully sequenced, and this allows researchers to look deep into the genetic component of the disease (Schoenebeck & Ostrander, 2014). Single nucleotide polymorphism (SNP) has been studied in different breeds and in a study by Wood et al. in 2010 where 8 breeds of dogs with AD were analyzed, found only one SNP common to the 8 breeds associated to the TSLP receptor, but lost significance when analyzed in each individual breed (Wood, Ollier, Nuttall, McEwan, & Carter, 2010). They attributed these results to the sample sizes of the individual breed analysis. In humans, various studies have reported the genetic influence of a family history and the probability of developing allergic disease, and found it to be ranged between 40 and 80% (Weidinger & Novak, 2016).

Quantitative estimation of the heritability is done by estimation of the proportion of the variance of the disease attributable to genetic background (Shaw, Wood, Freeman, Littlewood, & Hannant, 2004). Several studies have attempted to evaluate the heritability of canine AD with conflicting results. A selective control on the breeding of dogs affected with AD yielded that approximately 18% of the offspring would ultimately develop the disease. These results concurred with another study done in West Highland White Terriers with AD, but differed with the results in a study carried out in Beagles with AD (Hillier & Griffin, 2001; T. Nuttall, 2013). Another study by Shaw et al. in 2004 estimated the heritability of a population of atopic Golden Retrievers, Labrador Retrievers and a mix between the two breeds finding a heritability score of 0.47, suggesting that there is a 50% contribution of genetics to the disease (Shaw et al., 2004). The main difficulty in being able to standardize results not only resides on factors associated to the disease, but also on the difference in allergen exposure and age exposure, highlighting the importance of environmental factors as a pivotal component of the disease.

In spite of geographical differences in the breed prevalence, certain breeds have widely characterized the disease (Hillier & Griffin, 2001). Breeds such as WHWT, beagles,

cocker spaniels, German shepherds, and French bulldogs seem to predominate. A study measuring gene expression in canine AD using real time quantitative PCR found that 11 genes demonstrated statistically significant altered mRNA expression between atopic and healthy skin; dipeptidyl-peptidase-4 (DPP4), phosphatidylinositol-3,4,5-trisphosphate-5-phosphatase-2 (INPPL1), serine protease inhibitor kazal type-5 (SPINK5), sphingosine-1- phosphate lyase-1 (SGPL1), peroxisome proliferator-activated receptor gamma (PPARg), S100 calcium binding protein A8 (S100A8), Plakophilin-2 (PKP2), Periostin (POSTN), Cullin4A, TNF-a and metalloproteinase inhibitor-1 (TIMP-1) (Hiedi Wood et al., 2009). The information we currently have on the relationship between canine AD and genetics has shown that there are marked differences amongst breeds, and between genetic pools within the breeds which would have to be analyzed in the context of the environment each group planning on being studied has been in in order to obtain more reliable results. This reinstates the multimodal pathway and the idea that AD is not a disease itself, but a manifestation of a complex altered immune system.

Conclusions

Our understanding of canine AD is rapidly evolving allowing veterinary medicine as well as human medicine to work together towards an integrative approach to this pathology. This in turn leads to better ways of offering therapeutic strategies that will benefit those who suffer from the disease. Due to the multiple pathways involved in the immunopathogenesis of AD, understanding the components that trigger and/or lead to the clinical manifestations of the disease are key to the development of biomedical research. It is clear that an interaction between the environment, genetics and the immune system are key in the development of AD. A disease that bears so many components that branch out into their own multiple amounts of elements requires an in-depth knowledge of two factors that are common to all AD patients, canine and human alike, which are pruritus and inflammation. By enhancing our comprehension in the involvement of the immune system and what leads to these two parts of the puzzle will enable researchers and clinicians to pursue a more specific approach to a very unspecific disease.

CAPITULO III

An analysis of skin prick test reactions to detect allergic sensitization in dogs clinically compatible with atopic dermatitis in Medellín, Colombia.

Background

Skin prick testing is the test of choice for the detection of type I hypersensitivity in human atopic dermatitis. Canine atopic dermatitis resembles the disease in the human counterpart. In canine atopic dermatitis intradermal testing is the test of choice and there are few reports on the use of prick testing in dogs.

Objective

The purpose of this study was to evaluate skin prick test reactions in atopic dogs and a healthy control group to 11 environmental allergens.

Methods

Eleven glycerinated allergens were applied on the left lateral thorax of 9 atopic dogs and 9 controls. The skin was pricked with a feather lancet and evaluated for positive percutaneous reaction at 5, 10, 15 and 20 minutes after application of the allergens.

Results

Six out of 9 atopic dogs tested positive to at least one of the allergens tested. None of the dogs in the control group had a positive reaction to the allergens included in the test. *Blomia tropicalis*, *Dermatophagoides farine* and *Dermatophagoides pteronyssinus* exhibited the highest reaction rate among the group of atopic dogs. No statistical difference was found in histamine reaction between both groups. In this set of dogs, the test exhibited a 100% specificity and a sensitivity of 66%.

Conclusions

Skin prick testing in the detection of causative allergens of human atopic dermatitis has proved to be a sensitive and specific tool used frequently by human allergists. Due to the amount of similarities in canine and human atopic dermatitis, this could be a valuable tool that needs to be researched further in veterinary medicine. The published research so far correlates to the results obtained in this investigation, however future

investigations evaluating the concordance between in vitro specific IgE antibody assays and SPT must be carried out simultaneously to validate the test.

Key Words: Canine atopic dermatitis, skin prick test, serum IgE, intradermal test, allergens

Introduction

Skin prick testing (SPT) is a method used in human medicine to diagnose allergic diseases that are mediated by IgE. SPT can identify a type I hypersensitivity reaction in the skin to environmental or food allergens. The presence and degree of cutaneous reactivity will provide the interpretation grounds for SPT. When an allergen that the patient has been sensitized to is introduced in the skin, mast cells degranulate thanks to the binding of specific IgE on their surface receptors. This will release innate immunity inflammatory mediators that produce a wheal and flare response that is observed and quantitated directly. The clinical history and previous allergen exposure of the patient is relevant for the selection of the allergens utilized in the test. The reaction to each allergen is localized to the immediate area of the SPT allowing many different allergens to be tested at the same time. Results are obtained within 15 minutes after taking the exam and are available for immediate interpretation. In humans, SPT is recommended as the primary diagnostic test for IgE mediated allergic diseases in Europe, US and South America. The other diagnostic tests available for the screening of the allergens mediating an IgE disease are serum testing for specific IgE and intradermal testing (IDT). The advantages that SPT has over these two other tests are the costs, a rapid interpretation of results, safety, and higher specificity (Bousquet et al., 2012).

One of the main diseases in humans that allergists utilize SPT as a screening method is atopic dermatitis (AD) (Bousquet et al., 2012). Atopic dermatitis is also present in dogs, as one of the most prevalent skin diseases in the species with up to 10% of the general population of dogs being affected by the disease (Hillier & Griffin, 2001). Due to the similarities found in canine AD, it has been suggested that dogs are a suitable model for human AD (Marsella & Girolomoni, 2009). Canine atopic dermatitis is characterized as a genetically predisposed chronic and pruritic skin disease, and as the human counterpart, presents a Th2 skewed response in the acute phase and a mixed Th1/Th2/Th17/Th22

response in the chronic phase of the disease (Czogala et al., 2011). It is also predominantly IgE mediated and has similar lesional distribution patterns predominantly on the face, paws and flexural folds (Griffin & DeBoer, 2001).

SPT is currently not being used as a complementary and confirmatory tool in the diagnosis of canine atopic dermatitis. The test of choice are the specific serum IgE or the intradermal test (DeBoer & Hillier, 2001; Hillier & DeBoer, 2001). The earliest report in the literature on SPT in dogs dates to 1991 (Ballauf, 1991). In this article, a comparison was made between SPT and IDT in 5 normal dogs and 18 dogs with a reported history of skin or respiratory allergy. Their findings were that IDT test reactions were identifiable and that the SPT tests did not give easily interpretable reactions. No further studies were published afterwards perhaps due to the promising results at the time with IDT in dogs. Currently, prick testing is only beginning to be explored in veterinary dermatology and only one recent published study on prick testing in dogs with AD was found. The unpublished data available through abstracts is only recently exploring the concentration of the allergenic extracts to be utilized in veterinary medicine, however more studies are warranted to evaluate the usefulness of SPT in the causative allergen detection of canine atopic dermatitis together with the correlation of the seric levels of specific IgE to each allergen tested (Rossi, Messinger, Olivry, & Hoontrakoon, 2013). To date, IDT continues to be the test of choice seconded by serum specific IgE. The objective of this study was to evaluate the reaction with the use of SPT in dogs with atopic dermatitis.

Materials and Methods

Dogs

Nine client owned dogs with a clinical diagnosis of atopic dermatitis and nine non-atopic dogs were used as controls (Table 1). All dog owners agreed to place their dogs in the study and gave full informed consent. The atopic dogs had to fulfill the following inclusion criteria: 1) Patients with chronic history of pruritic skin disease that fulfill at least 5 of Favrot´s criteria; 2) Onset of disease from 1-5 years of age; 3) have ruled out food

allergies through a strict 6 week food trial with 3 week rechallenge; 4) Up to date flea and tick prevention; 5) Ruled out secondary staphylococcal or yeast infections through skin cytology; and 6) have no oral, topical or injectable glucocorticoids, cyclosporine or oclacitinib for a minimum of 3 weeks prior to the study. Control dogs were 1-10 years of age; had no history of allergic diseases; had no topical, oral or injectable requirements 3 weeks prior to the testing; have a normal current physical exam and an unremarkable previous medical history.

Table 1. Breed and age of Dogs with AD included in the study

AD Group		Control group	
Breed	**Age (years)**	**Breed**	**Age (years)**
English Bulldog	1	Mixed breed	5
French Bulldog	3	Mixed breed	8
Beagle	9	Mixed breed	8
West Highland White Terrier	3	Mixed breed	9
Springer Spaniel	1	Afghan	2
Maltese	4	Pittbull	3
Cocker Spaniel	2	Standard Schnauzer	7
Labrador Retriever	7	Standard Schnauzer	4
Yorkshire Terrier	8	Bull terrier	2

Prick Test

None of the dogs required sedation during the procedure and were held down manually. All of the patients were required to be bathed with a 2% Clorhexidine solution at the most two days prior to the test. The lateral thorax was clipped with a 40 blade. The skin surface was then cleaned with 70% isopropyl alcohol prior to the application of the allergens. The test sites were marked using an indelible marker with a 2cm separation between sites and a 5 cm separation between the histamine and control solution. Allergens were selected according to referenced data from standard prick test in

humans in Medellín, Colombia (Sánchez-caraballo, Diez-zuluaga, & Ricardo, 2012). The concentrations of each allergen were also taken from human studies due to the lack of references in veterinary medicine with regards to SPT (Bousquet et al., 2012). The 11 allergens were placed on each designated site and the skin was pricked at a 45-degree angle. A feather lancet (Allmed metallic lancet, Cali Colombia) was used for each allergen and discarded afterwards. A 10mg/ml dihydrochloride glycerinated histamine base was applied as a positive control and a 50% glycerosaline solution was used as negative control. After pricking the skin, the drops were removed simultaneously with a paper towel. Wheals were evaluated at 5, 10, 15 and 20 minutes after the test. Each wheal was evaluated for erythema and the average wheal diameter (mean vertical and horizontal diameters) was measured with a metric ruler. The allergens tested with their respective concentrations are listed in table 2. A reaction was considered positive when the diameter of the wheal was equivalent or greater than 3 mm which is the minimum average between the diameter of the wheals of the positive and negative controls.



Figure 1

Photograph showing the patient being held down manually. The area of the lateral thorax clipped where the PT (prick test) was performed is shown.

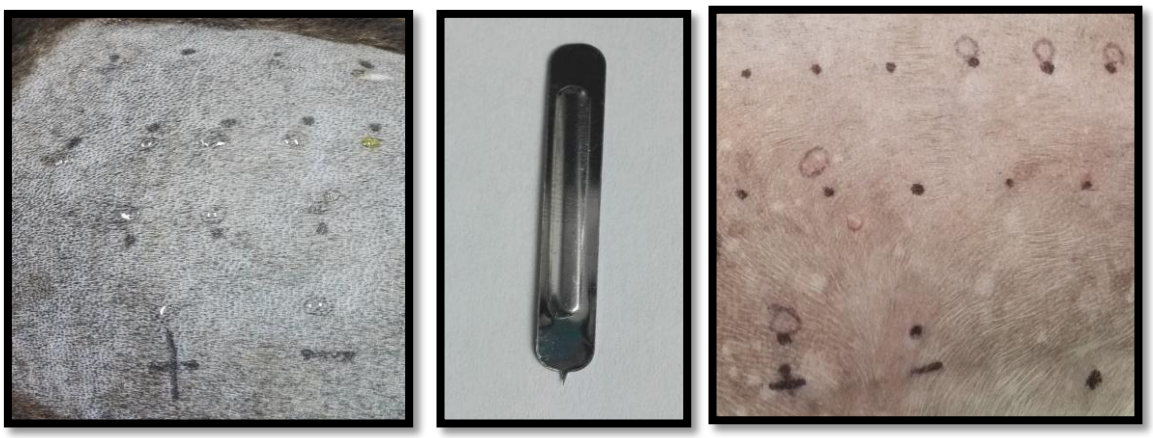

Figure 2.A The skin of a negative control patient with the drops of each allergen applied prior to pricking the skin. **2B** Prick lancet used in the test. **2C** Patient with a positive SPT reaction.

Table 2. Allergens and respective concentration used in the study

Allergen	Concentration
Alternaria (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	3 □g/ml
Aspergillus (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	25 □g/ml
Artemisia vulgaris (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	50 mg/ml
Cupressus Arizona (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	10 mg/ml
Grass mix (Poa pratensis, Dactilis glomerata, Lolium perenne, Phleum pratense, Festuca pratensis, Helictotrichon pretense) (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	50 mg/ml
Cynodon dactylon (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	50 mg/ml

Dermatophagoides farina (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	100 mg/ml
Dermatophagoides pteronyssinus (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	100 mg/ml
Blomia tropicalis (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	150 mg/ml
Fire ant (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	1:100 w/v
Cat epithelium (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark)	10.000 BAU/ml
Histamine (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark) (Positive Control)	10 mg/ml
Diluted glycerosaline solution (Negative Control)	1:20 w/v

Data analysis

The sensitivity and specificity of the test was analyzed under the following formulas: Sensitivity= true positive/ (true positive + false negative) x 100 ; Specificity = True negative / (True negative + False positive) x 100.

Histamine reactions were compared between AD and control group. The Shapiro-Wilk test was performed for the evaluation of normality. Score data was ordinal and not normally distributed. The one sided Wilcoxon ranked sum test was used with a p-value of 0.05.

Results

Dogs with atopic dermatitis

Six out of nine dogs with a clinical diagnosis of canine AD were positive to at least one of the allergens tested. The remainder three dogs did not react to any of the allergens tested, but had a positive reaction to the histamine control, validating the test. None of the AD dogs reacted to Alternaria, Aspergillus, Artemisia vulgaris or Cat epithelium. The allergens Cupressus Arizona, Grass mix, Cynodon dactylon, and Fire ant had two dogs reacting positive for each allergen. Dermatophagoides farinae and Dermatophagoides pteronyssinus had 4 dogs reacting positive to each allergen. Blomia tropicalis had 5 dogs with a positive reaction (Table 3). Positive reactions were obtained withing the first five minutes after pricking the skin with the corresponding allergen, and there were no differences in the wheal diameter between the 5,10,15 and 20 minute measurements.

Table 3. AD Dogs Positive to SPT

Breed	**D.farinae**	**D.pteronyssinus**	**Blomia tropicalis**	**Fire ant**	**Cynodon dactylon**	**Cupressus Arizona**	**Grass mix**
French bulldog	X	X	3mm	X	X	X	X
English bulldog	8mm	7mm	5mm	3mm	X	X	X
Beagle	5mm	3mm	3mm	X	3mm	3mm	4mm
West Highland White Terrier	X	X	X	6mm	X	X	X
Cocker spaniel	6mm	5mm	5mm	X	X	X	X
Yorkshir	4mm	3mm	3mm	X	3mm	3mm	3mm

e terrier							

Negative Controls

Nine out of nine negative control dogs tested negative to all of the allergens, but had a positive reaction to the histamine control, validating the test.



Figure 3. Photograph showing the PT in one of the AD patients with a negative reaction to all of the allergens tested and with a double positive reaction to the histamine control solution. Abbreviations: AD, atopic dermatitis; PT, prick test.

Table 4. Distribution of positive AD and Control dogs according to Allergen

Allergen	**Concentration**	**Positive AD (n)**	**Positive control dogs (n)**
Alternaria	3 □g/ml	0	0
Aspergillus	25 □g/ml	0	0
Artemisia vulgaris	50 mg/ml	0	0
Cupressus Arizona	10 mg/ml	2	0
Grass mix (Poa pratensis, Dactilis glomerata, Lolium perenne, Phleum pratense, Festuca pratensis,	50 mg/ml	2	0

Helictotrichon pretense)			
Cynodon dactylon	50 mg/ml	2	0
Dermatophagoides farinae	100 mg/ml	4	0
Dermatophagoides pteronyssinus	100 mg/ml	4	0
Blomia tropicalis	150 mg/ml	5	0
Fire ant	1:100 w/v	2	0
Cat epithelium	10.000 BAU/ml	0	0
Histamine (Positive Control)	10 mg/ml	9	9
Dilute saline solution Negative Control	1:20 w/v	0	0

Sensitivity and specificity of the PT in this population

For this group of animals, the sensitivity of the prick test was 66% with a 95% CI of 29,9% - 92,5% and a specificity of 100% with a 95% CI of 66,3% – 100%.

Comparison of mean diameter of histamine reaction between AD dogs and controls

The U-value is 22.5. The critical value of U at $p < .05$ is 21. Therefore, the result is not significant at $p < .05$. This indicates no statistical difference in diameters of histamine wheal reactions between atopic dogs and dogs without AD.

Discussion

Traditionally, IDT has been used as the main screening tool for the implementation of allergen specific immunotherapy (ASIT) in dogs and cats contrary to human medicine where prick testing is the primary test for the diagnosis of IgE mediated allergic diseases (Hillier & DeBoer, 2001). This could be owed to the thought that having a dog or cat

remain still during the test is a difficult task, and also to the lack of previous studies in prick testing in dogs (Hillier & DeBoer, 2001). Whereas IDT has become the standard allergy test due to the ease of performing it once the dog is sedated. In vitro measurement of serum IgE specific antibodies has become an important complementary tool in the diagnosis of type I allergy, however seldom correlation exists between serum and intradermal testing (DeBoer & Hillier, 2001). With this, none of the above can be considered a gold standard for the diagnosis of canine atopic dermatitis, and a positive reaction would infer exposure to the allergen, not a diagnosis of the disease per se. Currently, these tests are recommended solely for the purpose of treating clinically compatible atopic dogs with allergen specific immunotherapy (ASIT), according to the results (Hensel et al., 2015a).

At any given time, a patient could move from positive to negative results and vice versa within any of the allergy tests performed, due to variables in the seasonal concentration of the allergens, variations in the irritant threshold concentrations of each allergen and the level of sensitization of the patient to each agent (Hensel et al., 2015a). Careful interpretation of the results must be taken into account along with the clinical history of the dog to consider ASIT. A negative result to one test does not necessarily mean the animal is not allergic to that allergen or that it will not be allergic to it in the future (Aslund, Thomsen, Mølgaard, Nolte, & Backer, 2008).

This study presented 3 dogs with clinical signs compatible with AD that did not react to any of the allergens tested. This could be attributed to the fact that the allergens causing the disease were not included in the ones used in this test. Another possibility is that the concentration of the allergen extracts may not have been high enough to induce a positive reaction in these dogs. The control group was negative to all of the allergens in this trial. A 66% of sensitivity and a 100% of specificity in this group tested, warrants further research into prick testing as a screening tool for dogs that may be misdiagnosed clinically as having atopic dermatitis. However, threshold concentrations and comparisons to other tests must be performed in a representative number of dogs in order to obtain true positive and negative predictive values for the test. *Blomia tropicalis*

was the allergen with the highest reactivity among the allergens, seconded by *D .farinae* and *D. pteronyssinus*. This is consistent with testing in humans where most of the causative allergens are house dust mites(Justo, Díaz, Gil, & Gastaminza, 2016).

Several factors affecting the results of the SPT must be taken into account. The personnel in charge of applying the SPT on the dogs were previously trained on the technique in humans, however given the fact that there was no training in dogs prior to this trial, there may have been differences in the performance of the test. Another important factor is the utilization of mixed allergens (i.e. grass mix) to identify possible reactivity. It is plausible that any given dog with AD may react to a grass extract not contained in the mix and a negative reaction to the mix may not discard an allergic reaction to all of the extracts along the family of this type of allergen. A third factor taken into account is the objectivity between the time of reading the results and the intensity obtained in the reaction. In this trial, no significant differences were found until the 20 minute mark, however some patients may have had a reaction beyond this point of time. Finally, the allergens were selected based upon the main allergens utilized in human SPT that have positive reactions within the same region as the dogs in the trial, however, there may be a difference between the prevalence of positive SPT reaction to allergens in humans with AD compared to dogs with AD .

Conclusions

Skin prick testing in humans has been the main tool in the diagnosis of type I allergy showing the best positive predictive value to determine clinical allergy (Bousquet et al., 2012). Concordance between in vitro specific IgE antibody assays and SPT of 85% and 95% in humans, depending on the allergen being utilized make it a reliable test with the caveat that SPT provides immediate information versus in vitro testing.

SPT in dogs with atopic dermatitis is only recently being looked into, but further investigation is needed in order to make significant correlations between the results obtained and the causative offending allergens. In order to obtain sensitivity and specificity percentages for the test, a comparison between specific seric IgE levels and

prick results are necessary. More research is needed in order to validate the sensitivity and specificity of the test obtained in the population studied. Irritant thresholds in a significant number of dogs should be looked into in order to have more conclusive results. The lack of information regarding the standardization of allergen concentrations specific to the canine species difficult IDT and SPT interpretation. SPT is a potentially valuable complementary and confirmatory tool in the diagnosis of canine AD. SPT could be a less costly, safer and more specific test compared to IDT for atopic dogs. House dust mites have consistently been the main cause of cutaneous allergic reactions in dogs.

References

Andrian, U., & Mackay, C. (2000). T-cell function and migration Two Sides of the Same Coin. *New England Journal of Medicine*, *343*, 1020–1034.

Angelbeck-Schulze, M., Mischke, R., Rohn, K., Hewicker-Trautwein, M., Naim, H. Y., & Bäumer, W. (2014). Canine epidermal lipid sampling by skin scrub revealed variations between different body sites and normal and atopic dogs. *BMC Veterinary Research*, *10*. https://doi.org/10.1186/1746-6148-10-152

Asahina, R., Kamishina, H., Kamishina, H., & Maeda, S. (2015). Gene transcription of pro-inflammatory cytokines and chemokines induced by IL-17A in canine keratinocytes. *Veterinary Dermatology*, *26*(6), 426–432. https://doi.org/10.1111/vde.12244

Asahina, R., & Maeda, S. (2017). A review of the roles of keratinocyte-derived cytokines and chemokines in the pathogenesis of atopic dermatitis in humans and dogs. *Veterinary Dermatology*, *28*(1), 16-e5. https://doi.org/10.1111/vde.12351

Aslund, N., Thomsen, S. F., Mølgaard, E., Nolte, H., & Backer, V. (2008). Changes in skin test reactivity among adults with atopic disease: a 3-year prospective study. *Annals of Allergy, Asthma & Immunology : Official Publication of the American College of Allergy, Asthma, & Immunology*, *101*(5), 524–8. https://doi.org/10.1016/S1081-1206(10)60292-X

Auriemma, M., Vianale, G., Amerio, P., & Reale, M. (2013). Cytokines and T cells in atopic dermatitis. *European Cytokine Network*.

https://doi.org/10.1684/ecn.2013.0333

Auxilia, S. T., & Hill, P. B. (2000). Mast cell distribution, epidermal thickness and hair follicle density in normal canine skin: possible explanations for the predilection sites of atopic dermatitis? *Veterinary Dermatology*, *11*(4), 247–254. https://doi.org/10.1046/j.1365-3164.2000.00193.x

Ballauf, B. (1991). Comparison of the intradermal and prick tests for diagnosis of allergy in the dog. *Tierarztliche Praxis*, *19*(4), 428–430.

Bao, L., Zhang, H., & Chan, L. S. (2013). The involvement of the JAK-STAT signaling pathway in chronic inflammatory skin disease atopic dermatitis. *JAK-STAT*, *2*(3). https://doi.org/10.4161/jkst.24137

Bäsler, K., & Brandner, J. M. (2017). Tight junctions in skin inflammation. *Pflügers Archiv - European Journal of Physiology*, *469*(1), 3–14. https://doi.org/10.1007/s00424-016-1903-9

Bieber, T. (2010). Atopic dermatitis. *Annals of Dermatology*. https://doi.org/10.5021/ad.2010.22.2.125

Biedermann, T., Skabytska, Y., Kaesler, S., & Volz, T. (2015). Regulation of T cell immunity in atopic dermatitis by microbes: The Yin and Yang of cutaneous inflammation. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2015.00353

Bizikova, P., Santoro, D., Marsella, R., Nuttall, T., Eisenschenk, M. N. C., & Pucheu-Haston, C. M. (2015). Review: Clinical and histological manifestations of canine atopic dermatitis. *Veterinary Dermatology*, *26*(2), 79-e24. https://doi.org/10.1111/vde.12196

Black, A. P. B., Ardern-Jones, M. R., Kasprowicz, V., Bowness, P., Jones, L., Bailey, A. S., & Ogg, G. S. (2007). Human keratinocyte induction of rapid effector function in antigen-specific memory CD4. *European Journal of Immunology*, *37*, 1485–1493. https://doi.org/10.1002/eji.200636915

Boguniewicz, M., & Leung, D. Y. M. (2011). Atopic dermatitis: A disease of altered skin barrier and immune dysregulation. *Immunological Reviews*, *242*(1), 233–246. https://doi.org/10.1111/j.1600-065X.2011.01027.x

Bousquet, J., Heinzerling, L., Bachert, C., Papadopoulos, N. G., Bousquet, P. J., Burney, P. G., … Demoly, P. (2012). Practical guide to skin prick tests in allergy to

aeroallergens. *Allergy: European Journal of Allergy and Clinical Immunology*, *67*(1), 18–24. https://doi.org/10.1111/j.1398-9995.2011.02728.x

Brandt, E. B., & Sivaprasad, U. (2011). Th2 Cytokines and Atopic Dermatitis. *J Clin Cell Inmunol.*, *2*(3). https://doi.org/10.4172/2155-9899.1000110

Brazís, P., De Mora, F., Ferrer, L., & Puigdemont, A. (2002). IgE enhances FcεRI expression and IgE-dependent TNF-α release from canine skin mast cells. *Veterinary Immunology and Immunopathology*, *85*(3–4), 205–212. https://doi.org/10.1016/S0165-2427(01)00428-7

Brazís, P., Queralt, M., De Mora, F., Ferrer, L., & Puigdemont, A. (2000). Stem cell factor enhances IgE-mediated histamine and TNF-α release from dispersed canine cutaneous mast cells. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(00)00188-4

Breuer, K., Werfel, T., & Kapp Nordseeklinik Norderney, A. (2006). Allergic Manifestations of Skin Diseases – Atopic Dermatitis. *Chem Immunol Allergy. Basel, Karger*, *91*, 76–86.

Brown, S. J., & Irwin McLean, W. H. (2012). One Remarkable Molecule: Filaggrin. *Journal of Investigative Dermatology*. https://doi.org/10.1038/jid.2011.393

Campbell, D. J. (2015). Control of regulatory T cell migration, function and homeostasis 1. *Journal of Immunology Research*, *196*(6), 2507–2513. https://doi.org/10.4049/jimmunol.1500801

Cesare, A. Di, Meglio, P. Di, & Nestle, F. O. (2008). A Role for Th17 Cells in the Immunopathogenesis of Atopic Dermatitis? *Journal of Investigative Dermatology*. https://doi.org/10.1038/jid.2008.283

Cork, M. J., Danby, S. G., Vasilopoulos, Y., Hadgraft, J., Lane, M. E., Moustafa, M., … Ward, S. J. (2009). Epidermal Barrier Dysfunction in Atopic Dermatitis. *Journal of Investigative Dermatology*, *129*, 1892–1908. https://doi.org/10.1038/jid.2009.133

Cornegliani, L., Vercelli, A., Sala, E., & Marsella, R. (2012). Transepidermal water loss in healthy and atopic dogs, treated and untreated: A comparative preliminary study. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2011.01003.x

Czogala, J. M., Marycz, K., Kuryszko, J. J., & Zawadzki, M. (2011). Cells of the skin immune system in dogs with atopy. *Acta Veterinaria Brno*.

https://doi.org/10.2754/avb201180010011

DeBoer, D. J., & Hillier, A. (2001). The ACVD task force on canine atopic dermatitis (XVI): Laboratory evaluation of dogs with atopic dermatitis with serum-based “allergy” tests. *Veterinary Immunology and Immunopathology*, *81*(3–4), 277–287. https://doi.org/10.1016/S0165-2427(01)00304-X

Dubrac, S., Schmuth, M., & Ebner, S. (2010). Atopic dermatitis: the role of Langerhans cells in disease pathogenesis. *Immunology and Cell Biology*. https://doi.org/10.1038/icb.2010.33

Elmariah, S. B., & Lerner, E. A. (2013). The missing link between itch and inflammation in atopic dermatitis. *Cell*, *155*(2), 267–269. https://doi.org/10.1016/j.cell.2013.09.038

Eyerich, S., Eyerich, K., Pennino, D., Carbone, T., Nasorri, F., Pallotta, S., … Cavani, A. (2009). Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI40202

Flohr, C., & Yeo, L. (2011). Atopic dermatitis and the hygiene hypothesis revisited. *Curr.Probl.Dermatol.*, *41*(1662–2944 (Electronic)), 1–34.

Garrood, T., Lee, L., & Pitzalis, C. (2006). Molecular mechanisms of cell recruitment to inflammatory sites: General and tissue-specific pathways. *Rheumatology*, *45*(3), 250–260. https://doi.org/10.1093/rheumatology/kei207

Gonzales, A. J., Humphrey, W. R., Messamore, J. E., Fleck, T. J., Fici, G. J., Shelly, J. A., … Mccall, R. B. (2013). Interleukin-31: Its role in canine pruritus and naturally occurring canine atopic dermatitis. *Veterinary Dermatology*, *24*(1), 48-e12. https://doi.org/10.1111/j.1365-3164.2012.01098.x

Griffin, C. ., & DeBoer, D. . (2001). The ACVD task force on canine atopic dermatitis (XIV): clinical manifestations of canine atopic dermatitis. *Veterinary Immunology and Immunopathology*, *81*(3–4), 255–269. https://doi.org/10.1016/S0165-2427(01)00346-4

Gröne, A. (2002a). Keratinocytes and cytokines. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(02)00136-8

Gröne, A. (2002b). Keratinocytes and cytokines. *Veterinary Immunology and Immunopathology*, *88*(1–2), 1–12. https://doi.org/10.1016/S0165-2427(02)00136-8

Halliwell, R. E. W., & DeBoer, D. J. (2001). The ACVD task force on canine atopic dermatitis (III): The role of antibodies in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*, *81*(3–4), 159–167. https://doi.org/10.1016/S0165-2427(01)00309-9

Hammerberg, B. (2009). Canine immunoglobulin E. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2009.09.009

Hänel, K. H., Pfaff, C. M., Cornelissen, C., Amann, P. M., Marquardt, Y., Czaja, K., … Baron, J. M. (2016). Control of the Physical and Antimicrobial Skin Barrier by an IL-31–IL-1 Signaling Network. *The Journal of Immunology*, *196*(8), 3233–3244. https://doi.org/10.4049/jimmunol.1402943

Hari, A., Flach, T. L., Shi, Y., & Mydlarski, P. R. (2010). Toll-like receptors: Role in dermatological disease. *Mediators of Inflammation*. https://doi.org/10.1155/2010/437246

Hayashiya, S., Tani, K., Morimoto, M., Hayashi, T., Hayasaki, M., Nomura, T., … Taura, Y. (2002). Expression of T Helper 1 and T Helper 2 Cytokine mRNAs in Freshly Isolated Peripheral Blood Mononuclear Cells from Dogs with Atopic Dermatitis. *Journal of Veterinary Medicine Series A*, *49*(1), 27–31. https://doi.org/10.1046/j.1439-0442.2002.00413.x

Hensel, P., Santoro, D., Favrot, C., Hill, P., & Griffin, C. (2015a). Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Veterinary Research*, *11*(1), 196. https://doi.org/10.1186/s12917-015-0515-5

Hensel, P., Santoro, D., Favrot, C., Hill, P., & Griffin, C. (2015b). Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Veterinary Research*, *11*, 196. https://doi.org/10.1186/s12917-015-0515-5

Hiedi Wood, S., Ke, X., Nuttall, T., McEwan, N., Ollier, W. E., & Carter, S. D. (2009). Genome-wide association analysis of canine atopic dermatitis and identification of disease related SNPs. *Immunogenetics*, *61*, 765–772.

Hill, P. B., & Olivry, T. (2001). The ACVD task force on canine atopic dermatitis (V): biology and role of in¯ammatory cells in cutaneous allergic reactions. *Veterinary Immunology and Immunopathology*, *81*(3–4), 187–98.

Hillier, A., & DeBoer, D. J. (2001). The ACVD task force on canine atopic dermatitis

(XVII): Intradermal testing. *Veterinary Immunology and Immunopathology*, *81*(3–4), 289–304. https://doi.org/10.1016/S0165-2427(01)00313-0

Hillier, A., & Griffin, C. E. (2001). The ACVD task force on canine atopic dermatitis (I): Incidence and prevalence. *Veterinary Immunology and Immunopathology*, *81*(3–4), 147–151. https://doi.org/10.1016/S0165-2427(01)00296-3

Hoffmann, A. R., Patterson, A. P., Diesel, A., Lawhon, S. D., Ly, H. J., Stephenson, C. E., … Suchodolski, J. S. (2014). The skin microbiome in healthy and allergic dogs. *PLoS ONE*. https://doi.org/10.1371/journal.pone.003197

Irvine, A. D., McLean, W. H. I., & Leung, D. Y. M. (2011). Filaggrin Mutations Associated with Skin and Allergic Diseases. *New England Journal of Medicine*, *365*(14), 1315–1327. https://doi.org/10.1056/NEJMra1011040

Ito, T., Wang, Y.-H., Duramad, O., Hori, T., Delespesse, G. J., Watanabe, N., … Liu, Y.-J. (2005). TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *The Journal of Experimental Medicine*, *202*(9), 1213–1223. https://doi.org/10.1084/jem.20051135

Jassies-Van Der Lee, A., Rutten, V. P. M. G., Bruijn, J., Willemse, T., & Broere, F. (2014). CD4+ and CD8+ skin-associated T lymphocytes in canine atopic dermatitis produce interleukin-13, interleukin-22 and interferon-?? and contain a CD25+FoxP3+ subset. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12140

Jun, S. H., Lee, J. H., Kim, S. I., Choi, C. W., Park, T. I., Jung, H. R., … Lee, J. C. (2017). *Staphylococcus aureus* -derived membrane vesicles exacerbate skin inflammation in atopic dermatitis. *Clinical & Experimental Allergy*. https://doi.org/10.1111/cea.12851

Justo, X., Díaz, I., Gil, J. J., & Gastaminza, G. (2016). Prick test: evolution towards automated reading. *Allergy: European Journal of Allergy and Clinical Immunology*, *71*(8), 1095–1102. https://doi.org/10.1111/all.12921

Keppel, K. E., Campbell, K. L., Zuckermann, F. A., Greeley, E. A., Schaeffer, D. J., & Husmann, R. J. (2008). Quantitation of canine regulatory T cell populations, serum interleukin-10 and allergen-specific IgE concentrations in healthy control dogs and canine atopic dermatitis patients receiving allergen-specific immunotherapy. *Veterinary Immunology and Immunopathology*.

https://doi.org/10.1016/j.vetimm.2008.02.008

Kim, H.-J., Cronin, M., Ahrens, K., Papastavros, V., Santoro, D., & Marsella, R. (2016). A comparative study of epidermal tight junction proteins in a dog model of atopic dermatitis. *Veterinary Dermatology*, *27*(1), 40-e11. https://doi.org/10.1111/vde.12276

Kim, J. E., Kim, J. S., Cho, D. H., & Park, H. J. (2016). Molecular Mechanisms of Cutaneous Inflammatory Disorder: Atopic Dermatitis. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms17081234

Klukowska-Rötzler, J., Chervet, L., Müller, E. J., Roosje, P., Marti, E., & Janda, J. (2013). Expression of thymic stromal lymphopoietin in canine atopic dermatitis. *Veterinary Dermatology*, *24*(1). https://doi.org/10.1111/j.1365-3164.2012.01096.x

Kobayashi, T., Glatz, M., Horiuchi, K., Kawasaki, H., Akiyama, H., Kaplan, D. H., … Nagao, K. (2015). Dysbiosis and Staphylococcus aureus colonization drives inflammation in atopic dermatitis HHS Public Access microbiota-host immunity axis as a possible target for future therapeutics in eczematous dermatitis. *Immunity. April*, *21*(424), 756–766. https://doi.org/10.1016/j.immuni.2015.03.014

Kong, H. H., Oh, J., Deming, C., Conlan, S., Grice, E. A., Beatson, M. A., … Segre, J. A. (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Research*. https://doi.org/10.1101/gr.131029.111

Lauber, B., Molitor, V., Meury, S., Doherr, M. G., Favrot, C., Tengvall, K., … Marti, E. (2012). Total IgE and allergen-specific IgE and IgG antibody levels in sera of atopic dermatitis affected and non-affected Labrador- and Golden retrievers. *Veterinary Immunology and Immunopathology*, *149*(1–2), 112–118. https://doi.org/10.1016/j.vetimm.2012.05.018

Lee, J., Noh, G., Lee, S., Youn, Y., & Rhim, J. (2012). Atopic Dermatitis and Cytokines: Recent Patents in Immunoregulatory and Therapeutic Implications of Cytokines in Atopic Dermatitis -Part I: Cytokines in Atopic Dermatitis. *Recent Patents on Inflammation & Allergy Drug Discovery*, *6*, 222–247.

Leonard, B. C., Affolter, V. K., & Bevins, C. L. (2012). Antimicrobial peptides: Agents of border protection for companion animals. *Veterinary Dermatology*.

https://doi.org/10.1111/j.1365-3164.2012.01037.x

Liu, F. T., Goodarzi, H., & Chen, H. Y. (2011). IgE, mast cells, and eosinophils in atopic dermatitis. *Clinical Reviews in Allergy and Immunology*, *41*(3), 298–310. https://doi.org/10.1007/s12016-011-8252-4

Liu, Y.-J. (2006). Thymic stromal lymphopoietin: master switch for allergic inflammation. *The Journal of Experimental Medicine*. https://doi.org/10.1084/jem.20051745

Looringh van Beeck., F. (2014). Host and environmental contribution to atopic dermatitis in dogs. *Doctoral Dissertation , Utrecht University*.

Maeda, S., Fujiwara, S., Omori, K., Kawano, K., Kurata, K., Masuda, K., … Tsujimoto, H. (2002a). Lesional expression of thymus and activation-regulated chemokine in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(02)00140-X

Maeda, S., Fujiwara, S., Omori, K., Kawano, K., Kurata, K., Masuda, K., … Tsujimoto, H. (2002b). Lesional expression of thymus and activation-regulated chemokine in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*, *88*(1–2), 79–87. https://doi.org/10.1016/S0165-2427(02)00140-X

Maeda, S., Okayama, T., Omori, K., Masuda, K., Sakaguchi, M., Ohno, K., & Tsujimoto, H. (2002). Expression of CC chemokine receptor 4 (CCR4) mRNA in canine atopic skin lesion. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(02)00232-5

Majewska, A., Gajewska, M., Dembele, K., Maciejewski, H., Prostek, A., & Jank, M. (2016). Lymphocytic, cytokine and transcriptomic profiles in peripheral blood of dogs with atopic dermatitis. *BMC Veterinary Research*, *12*(174). https://doi.org/10.1186/s12917-016-0805-6

M Leung, D. Y., Ledford, D. K., Kuo, I.-H., Yoshida, T., De Benedetto, A., & Beck, L. A. (2013). Clinical reviews in allergy and immunology The cutaneous innate immune response in patients with atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *131*, 266–278. https://doi.org/10.1016/j.jaci.2012.12.1563

Manriquez, J., Andino-Navarrete, R., Cataldo-Cerda, K., & Harz-Fresno, I. (2015). Bibliometric characteristics of systematic reviews in dermatology: A cross-sectional study through Web of Science and Scopus. *Dermatologica Sinica*, *33*(3), 154–156.

https://doi.org/10.1016/j.dsi.2014.12.007

Marsella, R. (2012). Are transepidermal water loss and clinical signs correlated in canine atopic dermatitis? A compilation of studies. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01055.x

Marsella, R. (2013a). Does filaggrin expression correlate with severity of clinical signs in dogs with atopic dermatitis? *Veterinary Dermatology*. https://doi.org/10.1111/vde.12007

Marsella, R. (2013b). Fixing the skin barrier: Past, present and future - man and dog compared. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01073.x

Marsella, R., & Girolomoni, G. (2009). Canine Models of Atopic Dermatitis: A Useful Tool with Untapped Potential. *Journal of Investigative Dermatology*, *129*(10), 2351–2357. https://doi.org/10.1038/jid.2009.98

Marsella, R., & Olivry, T. (2003). Animal models of atopic dermatitis. *Clinics in Dermatology*. https://doi.org/10.1016/S0738-081X(02)00369-3

Marsella, R., Olivry, T., & Carlotti, D.-N. (2011). Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2011.00967.x

McCandless, E. E., Rugg, C. A., Fici, G. J., Messamore, J. E., Aleo, M. M., & Gonzales, A. J. (2014). Allergen-induced production of IL-31 by canine Th2 cells and identification of immune, skin, and neuronal target cells. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2013.10.017

Meury, S., Molitor, V., Doherr, M. G., Roosje, P., Leeb, T., Hobi, S., … Favrot, C. (2011). Role of the environment in the development of canine atopic dermatitis in Labrador and golden retrievers. *Veterinary Dermatology*, *22*(4), 327–334. https://doi.org/10.1111/j.1365-3164.2010.00950.x

Michels, G. M., Ramsey, D. S., Walsh, K. F., Martinon, O. M., Mahabir, S. P., Hoevers, J. D., … Dunham, S. A. (2016). A blinded, randomized, placebo-controlled, dose determination trial of lokivetmab (ZTS-00103289), a caninized, anti-canine IL-31 monoclonal antibody in client owned dogs with atopic dermatitis. *Veterinary Dermatology*, *27*(6), 478-e129. https://doi.org/10.1111/vde.12376

Mitsdoerffer, M., Lee, Y., Jager, A., Kim, H.-J., Korn, T., Kolls, J. K., … Kuchroo, V. K. (2010). Proinflammatory T helper type 17 cells are effective B-cell helpers. *Proceedings of the National Academy of Sciences*, *107*(32), 14292–14297. https://doi.org/10.1073/pnas.1009234107

Mizuno, T., Kanbayashi, S., Okawa, T., Maeda, S., & Okuda, M. (2009a). Molecular cloning of canine interleukin-31 and its expression in various tissues. *Veterinary Immunology and Immunopathology*, *131*(1–2), 140–143. https://doi.org/10.1016/j.vetimm.2009.03.014

Mizuno, T., Kanbayashi, S., Okawa, T., Maeda, S., & Okuda, M. (2009b). Molecular cloning of canine interleukin-31 and its expression in various tissues. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2009.03.014

Mu, Z., Zhao, Y., Liu, X., Chang, C., & Zhang, J. (2014). Molecular biology of atopic dermatitis. *Clinical Reviews in Allergy & Immunology*. https://doi.org/10.1007/s12016-014-8415-1

Mullin, J., Carter, S., Williams, N., McEwan, N., & Nuttall, T. (2013). Transcription of canine toll-like receptor 2, ??-defensin 1 and ??-defensin 103 in infected atopic skin, non-infected atopic skin, healthy skin and the CPEK cell line. *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2012.09.017

Murota, H., & Katayama, I. (2017). Exacerbating factors of itch in atopic dermatitis. *Allergology International*. https://doi.org/10.1016/j.alit.2016.10.005

Nestle, F. O., Di Meglio, P., Qin, J.-Z., & Nickoloff, B. J. (2009). Skin immune sentinels in health and disease. *Nature Reviews Immunology*. https://doi.org/10.1038/nri2622

Nishifuji, K., & Yoon, J. S. (2013). The stratum corneum: The rampart of the mammalian body. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01090.x

Novak, N., Bieber, T., & Leung, D. Y. M. (2003). Immune mechanisms leading to atopic dermatitis. *Journal of Allergy and Clinical Immunology*. https://doi.org/10.1016/j.jaci.2003.09.032

Nuttall, T. (2013). The genomics revolution: Will canine atopic dermatitis be predictable and preventable? *Veterinary Dermatology*, *24*(1). https://doi.org/10.1111/j.1365-3164.2012.01094.x

Nuttall, T. J., Knight, P. A., McAleese, S. M., Lamb, J. R., & Hill, P. B. (2002a).

Expression of Th1, Th2 and immunosuppressive cytokine gene transcripts in canine atopic dermatitis. *Clinical and Experimental Allergy*, *32*(5), 789–795. https://doi.org/10.1046/j.1365-2222.2002.01356.x

Nuttall, T. J., Knight, P. A., McAleese, S. M., Lamb, J. R., & Hill, P. B. (2002b). T-helper 1, T-helper 2 and immunosuppressive cytokines in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*, *87*(3–4), 379–384. https://doi.org/10.1016/S0165-2427(02)00076-4

Ogg, G., & Ogg, G. (2009). Role of T cells in the pathogenesis of atopic dermatitis Clinical and Experimental Allergy. *Clinical and Experimental Allergy*, (39), 310–316. https://doi.org/10.1111/j.1365-2222.2008.03146.x

Okada, H., Kuhn, C., Feillet, H., & Bach, J. F. (2010). The “hygiene hypothesis” for autoimmune and allergic diseases: An update. *Clinical and Experimental Immunology*, *160*(1), 1–9. https://doi.org/10.1111/j.1365-2249.2010.04139.x

Olivry, T. (2011). Is the skin barrier abnormal in dogs with atopic dermatitis? *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2011.07.014

Olivry, T. (2012). What can dogs bring to atopic dermatitis research? *Chemical Immunology and Allergy*. https://doi.org/10.1159/000331884

Olivry, T., Dunston, S. M., Murphy, K. M., & Moore, P. F. (2001). Characterization of the inflammatory infiltrate during IgE-mediated late phase reactions in the skin of normal and atopic dogs. *Veterinary Dermatology*, *12*(1), 49–58. https://doi.org/10.1046/j.1365-3164.2001.00230.x

Olivry, T., & Hill. (2001a). The ACVD task force on canine atopic dermatitis (VIII): is the epidermal lipid barrier defective?, *81*, 159–167.

Olivry, T., & Hill, P. B. (2001b). The ACVD task force on canine atopic dermatitis (XVIII): Histopathology of skin lesions. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(01)00305-1

Olivry, T., Mayhew, D., Paps, J. S., Linder, K. E., Peredo, C., Rajpal, D., … Cote-Sierra, J. (2016). Early Activation of Th2/Th22 Inflammatory and Pruritogenic Pathways in Acute Canine Atopic Dermatitis Skin Lesions. *Journal of Investigative Dermatology*, *136*(10), 1961–1969. https://doi.org/10.1016/j.jid.2016.05.117

Olivry, T., Mueller, R., Nuttall, T., Favrot, C., & Prélaud, P. (2008). Determination of

CADESI-03 thresholds for increasing severity levels of canine atopic dermatitis. *Veterinary Dermatology*, *19*(3), 115–119. https://doi.org/10.1111/j.1365-3164.2008.00668.x

Olivry, T., Naydan, D., & Moore, P. (1997). Characterization of the cutaneous inflammatory infiltrate in canine atopic dermatitis. *The American Journal of Dermatopathology*, *9*(5), 477–486.

Ong, P. Y., & Leung, D. Y. M. (2016). Bacterial and Viral Infections in Atopic Dermatitis: a Comprehensive Review. *Clinical Reviews in Allergy & Immunology*. https://doi.org/10.1007/s12016-016-8548-5

Oyoshi, M. K., He, R., Kumar, L., Yoon, J., & Geha, R. S. (2009). Chapter 3 Cellular and Molecular Mechanisms in Atopic Dermatitis. *Advances in Immunology*. https://doi.org/10.1016/S0065-2776(09)01203-6

Peng, W., & Novak, N. (2015). Pathogenesis of atopic dermatitis. *Clinical and Experimental Allergy*, *45*, 566–574. https://doi.org/10.1111/cea.12495

Pierezan, F., Olivry, T., Paps, J. S., Lawhon, S. D., Wu, J., Org, J., … Hoffmann, A. R. (2016). The skin microbiome in allergen-induced canine atopic dermatitis. *Veterinary Dermatology*, *27*, 332-e82. https://doi.org/10.1111/vde.12366

Pucheu-Haston, C. M. (2016). Atopic dermatitis in the domestic dog. *Clinics in Dermatology*. https://doi.org/10.1016/j.clindermatol.2015.10.010

Pucheu-Haston, C. M., Bizikova, P., Eisenschenk, M. N. C., Santoro, D., Nuttall, T., & Marsella, R. (2015). Review: The role of antibodies, autoantigens and food allergens in canine atopic dermatitis. *Veterinary Dermatology*, *26*(2), 115-e30. https://doi.org/10.1111/vde.12201

Pucheu-Haston, C. M., Bizikova, P., Marsella, R., Santoro, D., Nuttall, T., & Eisenschenk, M. N. C. (2015). Review: Lymphocytes, cytokines, chemokines and the T-helper 1-T-helper 2 balance in canine atopic dermatitis. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12205

Pucheu-Haston, C. M., Santoro, D., Bizikova, P., Eisenschenk, M. N. C., Marsella, R., & Nuttall, T. (2015). Review: Innate immunity, lipid metabolism and nutrition in canine atopic dermatitis. *Veterinary Dermatology*, *26*(2), 104-e28. https://doi.org/10.1111/vde.12199

Pucheu-Haston, C. M., Shuster, D., Olivry, T., Brianceau, P., Lockwood, P., McClanahan, T., … Hammerberg, B. (2006). A canine model of cutaneous late-phase reactions: Prednisolone inhibition of cellular and cytokine responses. *Immunology*. https://doi.org/10.1111/j.1365-2567.2005.02276.x

Reiter, L. V., Torres, S. M. F., & Wertz, P. W. (2009). Characterization and quantification of ceramides in the nonlesional skin of canine patients with atopic dermatitis compared with controls. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2009.00759.x

Ricklin, M. E., Roosje, P., & Summerfield, A. (2010). Characterization of canine dendritic cells in healthy, atopic, and non-allergic inflamed skin. *Journal of Clinical Immunology*. https://doi.org/10.1007/s10875-010-9447-9

Rodrigues Hoffmann, A. (2017a). The cutaneous ecosystem: the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12408

Rodrigues Hoffmann, A. (2017b). The cutaneous ecosystem: the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. *Veterinary Dermatology*, *28*(1), 60-e15. https://doi.org/10.1111/vde.12408

Romagnani, S. (2000). T-cell subsets (Th1 versus Th2). *Annals of Allergy, Asthma & Immunology*. https://doi.org/10.1016/S1081-1206(10)62426-X

Romagnani, S. (2004). Immunologic influences on allergy and the TH1/TH2 balance. *Journal of Allergy and Clinical Immunology*. https://doi.org/10.1016/j.jaci.2003.11.025

Roosterman, D., Goerge, T., Schneider, S. W., Bunnett, N. W., & Steinhoff, M. (2006). Neuronal Control of Skin Function: The Skin as a Neuroimmunoendocrine Organ. *Physiological Reviews*. https://doi.org/10.1152/physrev.00026.2005

Rosenblum, M. D., Way, S. S., & Abbas, A. K. (2015). Regulatory T cell memory. *Nature Reviews Immunology*, *16*(2), 90–101. https://doi.org/10.1038/nri.2015.1

Rossi, M. A., Messinger, L., Olivry, T., & Hoontrakoon, R. (2013). A pilot study of the validation of percutaneous testing in cats. *Veterinary Dermatology*, *24*(5). https://doi.org/10.1111/vde.12054

Said, A., & Weindl, G. (2015). Regulation of Dendritic Cell Function in Inflammation. *Journal of Immunology Research*. https://doi.org/10.1155/2015/743169

Sakamoto, M., Asahina, R., Kamishina, H., & Maeda, S. (2016). Transcription of thymic stromal lymphopoietin via Toll-like receptor 2 in canine keratinocytes: A possible association of Staphylococcus spp. in the deterioration of allergic inflammation in canine atopic dermatitis. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12301

Salzmann, C. A., Olivry, T. J., Nielsen, D. M., Paps, J. S., Harris, T. L., & Olby, N. J. (2011). Genome-wide linkage study of atopic dermatitis in West Highland White Terriers. *BMC Genetics*, *12*(37). https://doi.org/10.1186/1471-2156-12-37

Sánchez-caraballo, J., Diez-zuluaga, S., & Ricardo, C. (2012). Sensibilización a aeroalergenos en pacientes alérgicos de Medellín, Colombia. *Revista Alergia Mexico*, *59*(3), 139–147.

Santoro, D., Bunick, D., Graves, T. K., & Segre, M. (2013). Evaluation of canine antimicrobial peptides in infected and noninfected chronic atopic skin. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01091.x

Santoro, D., Marsella, R., Bunick, D., Graves, T. K., & Campbell, K. L. (2011). Expression and distribution of canine antimicrobial peptides in the skin of healthy and atopic beagles. *Veterinary Immunology and Immunopathology*, *144*(3–4), 382–388. https://doi.org/10.1016/j.vetimm.2011.08.004

Santoro, D., Marsella, R., Pucheu-Haston, C. M., Eisenschenk, M. N. C., Nuttall, T., & Bizikova, P. (2015). Review: Pathogenesis of canine atopic dermatitis: Skin barrier and host-micro-organism interaction. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12197

Santoro, D., & Rodrigues Hoffmann, A. (2016). Canine and Human Atopic Dermatitis: Two Faces of the Same Host-Microbe Interaction. *Journal of Investigative Dermatology*. https://doi.org/10.1016/j.jid.2016.03.015

Schindler, C., Levy, D. E., & Decker, T. (2007). JAK-STAT signaling: From interferons to cytokines. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.R700016200

Schlotter, Y. M., Rutten, V. P. M. G., Riemers, F. M., Knol, E. F., & Willemse, T. (2011). Lesional skin in atopic dogs shows a mixed Type-1 and Type-2 immune responsiveness. *Veterinary Immunology and Immunopathology*, *143*(1–2), 20–26.

https://doi.org/10.1016/j.vetimm.2011.05.025

Schmidt-Weber, C. B., Akdis, M., & Akdis, C. A. (2007). TH17 cells in the big picture of immunology. *Journal of Allergy and Clinical Immunology*, *120*(2), 247–254. https://doi.org/10.1016/j.jaci.2007.06.039

Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., … Kastelein, R. A. (2005). IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. https://doi.org/10.1016/j.immuni.2005.09.015

Schoenebeck, J. J., & Ostrander, E. A. (2014). Insights into Morphology and Disease from the Dog Genome Project. *Annual Review of Cell and Developmental Biology*, *30*(1), 535–560. https://doi.org/10.1146/annurev-cellbio-100913-012927

Shaw, S. C., Wood, J. L. N., Freeman, J., Littlewood, J. D., & Hannant, D. (2004). Estimation of heritability of atopic dermatitis in Labrador and Golden Retrievers. *American Journal of Veterinary Research*. https://doi.org/10.2460/ajvr.2004.65.1014

Shi, Y., Liu, C. H., Roberts, A. I., Das, J., Xu, G., Ren, G., … Devadas, S. (2006). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. *Cell Research*. https://doi.org/10.1038/sj.cr.7310017

Shibata, S., Maeda, S., Kondo, N., Chimura, N., Inoue, A., & Fukata, T. (2011). Identification of the signaling pathway of TNF-??-induced CCL17/TARC transcription in a canine keratinocyte cell line. *Veterinary Immunology and Immunopathology*, *139*(2–4), 90–98. https://doi.org/10.1016/j.vetimm.2010.08.008

Shida, M., Kadoya, M., Park, S. J., Nishifuji, K., Momoi, Y., & Iwasaki, T. (2004). Allergen-specific immunotherapy induces Th1 shift in dogs with atopic dermatitis. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2004.06.003

Shimada, K., Yoon, J. S., Yoshihara, T., Iwasaki, T., & Nishifuji, K. (2009). Increased transepidermal water loss and decreased ceramide content in lesional and non-lesional skin of dogs with atopic dermatitis. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2009.00847.x

Sinke, J. D., Rutten, V. P. M. G., & Willemse, T. (2002). Immune dysregulation in atopic dermatitis. In *Veterinary Immunology and Immunopathology*.

https://doi.org/10.1016/S0165-2427(02)00066-1

Sinke, J. D., Thepen, T., Bihari, I. C., Rutten, V. P. M. G., & Willemse, T. (1997). Immunophenotyping of skin-infiltrating T-cell subsets in dogs with atopic dermatitis. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(96)05763-7

Ständer, S., Raap, U., Weisshaar, E., Schmelz, M., Mettang, T., Handwerker, H., & Luger, T. A. (2011). Pathogenesis of pruritus. *JDDG: Journal Der Deutschen Dermatologischen Gesellschaft*. https://doi.org/10.1111/j.1610-0387.2011.07585.x

Strid, J., & Strobel, S. (2005). Skin barrier dysfunction and systemic sensitization to allergens through the skin. *Current Drug Targets. Inflammation and Allergy*, *4*(5), 531–41. https://doi.org/10.2174/156801005774322199

Suter, M. M., Schulze, K., Bergman, W., Welle, M., Roosje, P., & Müller, E. J. (2009). The keratinocyte in epidermal renewal and defence. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2009.00819.x

Thomsen, K. (1991). The role of neutrophil-activating mediators in canine health and disease (with special reference to the role of leukotrienes in inflammatory dermatoses). *Journal of Veterinary Pharmacology Therapy*, *1963*(1973), 113–133.

Tokura, Y. (2010). Extrinsic and intrinsic types of atopic dermatitis. *Journal of Dermatological Science*. https://doi.org/10.1016/j.jdermsci.2010.02.008

Tsai, K. L., Clark, L. A., & Murphy, K. E. (2007). Understanding hereditary diseases using the dog and human as companion model systems. *Mammalian Genome*, *18*(6–7), 444–451. https://doi.org/10.1007/s00335-007-9037-1

Turner, M. J., & Zhou, B. (2014). A new itch to scratch for TSLP. *Trends in Immunology*, *35*(2), 49–50. https://doi.org/10.1016/j.it.2013.12.001

Two, A. M., Nakatsuji, T., Kotol, P. F., Arvanitidou, E., Du-Thumm, L., Hata, T. R., & Gallo, R. L. (2016). The Cutaneous Microbiome and Aspects of Skin Antimicrobial Defense System Resist Acute Treatment with Topical Skin Cleansers. *Journal of Investigative Dermatology*. https://doi.org/10.1016/j.jid.2016.06.612

Watson W, & Kapur S. (2011). Atopic dermatitis. *Allergy, Asthma and Clinical Immunology*, *7*(Suppl. 1), S4. https://doi.org/10.1186/1710-1492-7-S1-S4

Weidinger, S., & Novak, N. (2016). Atopic dermatitis. In *The Lancet* (Vol. 387, pp. 1109–

1122). https://doi.org/10.1016/S0140-6736(15)00149-X

Wilson, S. R., Thé, L., Batia, L. M., Beattie, K., Katibah, G. E., McClain, S. P., … Bautista, D. M. (2013). XThe epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. *Cell*. https://doi.org/10.1016/j.cell.2013.08.057

Wong, R., Geyer, S., Weninger, W., Guimberteau, J., & Wong, J. K. (2016). The dynamic anatomy and patterning of skin. *Experimental Dermatology*, *25*(2), 92–98. https://doi.org/10.1111/exd.12832

Wood, S. H., Clements, D. N., Ollier, W. E., Nuttall, T., McEwan, N. A., & Carter, S. D. (2009). Gene expression in canine atopic dermatitis and correlation with clinical severity scores. *Journal of Dermatological Science*. https://doi.org/10.1016/j.jdermsci.2009.03.005

Wood, S. H., Ollier, W. E., Nuttall, T., McEwan, N. A., & Carter, S. D. (2010). Despite identifying some shared gene associations with human atopic dermatitis the use of multiple dog breeds from various locations limits detection of gene associations in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2010.07.020

Wüthrich B, S.-G. P. (2003). The atopic eczema/dermatitis syndrome. Epidemiology, natural course, and immunology of the IgE-associated (“extrinsic”) and the nonallergic (“intrinsic”) AEDS. *J Investig Allergol Clin Immunol.*, *13*(1), 1–5.

Yoon, J. S., Nishifuji, K., Sasaki, A., Ide, K., Ishikawa, J., Yoshihara, T., & Iwasaki, T. (2011). Alteration of stratum corneum ceramide profiles in spontaneous canine model of atopic dermatitis. *Experimental Dermatology*. https://doi.org/10.1111/j.1600-0625.2011.01306.x

Yoshida, K., Kubo, A., Fujita, H., Yokouchi, M., Ishii, K., Kawasaki, H., … Amagai, M. (2014). Distinct behavior of human Langerhans cells and inflammatory dendritic epidermal cells at tight junctions in patients with atopic dermatitis. *Journal of Allergy and Clinical Immunology*. https://doi.org/10.1016/j.jaci.2014.08.001

Yuki, T., Tobiishi, M., Kusaka-Kikushima, A., Ota, Y., & Tokura, Y. (2016). Impaired tight junctions in atopic dermatitis skin and in a skin-equivalent model treated with interleukin-17. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0161759

Zajac, M., Szczepanik, M. P., Wilkolek, P. M., Adamek, L. R., Pomorski, Z. J. H.,

Sitkowski, W., & Goly??ski, M. G. (2014). Assessment of the relationship between transepidermal water loss (TEWL) and severity of clinical signs (CADESI-03) in atopic dogs. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12150

Zhang, Q., Putheti, P., Zhou, Q., Liu, Q., & Gao, W. (2008). Structures and biological functions of IL-31 and IL-31 receptors. *Cytokine and Growth Factor Reviews*. https://doi.org/10.1016/j.cytogfr.2008.08.003

Zheng, X., Ma, P., de Paiva, C. S., Cunningham, M. A., Hwang, C. S., Pflugfelder, S. C., & Li, D. Q. (2010). TSLP and downstream molecules in experimental mouse allergic conjunctivitis. *Investigative Ophthalmology and Visual Science*. https://doi.org/10.1167/iovs.09-4122

CAPITULO 4

CONCLUSIONES GENERALES

La dermatitis atópica (DA), es una patología compleja y multifactorial que afecta a humanos y caninos, y que reviste importancia desde múltiples áreas de las ciencias básicas. Tanto en medicina humana como en medicina veterinaria, se ha evidenciado un incremento en la incidencia de la enfermedad. La DA afecta el 15% de la población total canina, lo cual implica que es una de las patologías con mayor representación en medicina veterinaria general. Las similitudes encontradas entre la DA canina y la humana han permitido hacer uso de la medicina traslacional para generar grandes avances investigativos en la fisiopatogenia y el tratamiento de la misma.

Factores ambientales, nutricionales, genéticos e inmunológicos se conjugan para contribuir a la fisiopatogenia de la DA, lo cual dificulta establecer el punto de partida de la enfermedad. La evidencia actual indica que los factores desencadenantes de la DA difieren entre individuos, por variaciones raciales, ambientales y de exposición a diversos antígenos bacterianos, parasitarios y proteicos. De estas observaciones se deriva la hipótesis de la higiene, donde se teoriza que el incremento exponencial en la incidencia de la DA en los últimos años, obedece a los esquemas eficientes de desparasitación gastrointestinal y desinfección cutánea, aunada a la falta de contacto del individuo con antígenos medioambientales que estimulen una respuesta adaptativa protectora.

La perspectiva de la inmunopatogenia de la DA ha variado a medida que se han ido descubriendo nuevos mediadores bioquímicos de la enfermedad. Actualmente se considera que la respuesta inmune dentro de la DA varía según el estadío en el que se encuentre el paciente. En la fase aguda, la respuesta predominante será de tipo Th2, y posteriormente, en la fase crónica, se convertirá en una respuesta mixta tipo Th1/Th2. Dentro de la gran cantidad de citoquinas involucradas en el proceso de respuesta

inmune de la DA, se le ha dado mayor importancia a aquellas que inducen respuestas inflamatorias, pruriginosas, o ambas. Dentro de este pool de citoquinas se resaltan las interleucinas 4, 5, 13 y 31. Las tres primeras, hacen parte de la respuesta adaptativa mediada por IgE y la cascada de inflamación en la fase aguda, mientras que la IL31 es una de las citoquinas más recientemente descubiertas, siendo una de las principales estimuladoras del prurito en DA.

Aún hay muchas preguntas sin resolver dentro de la respuesta inmune adaptativa y de regulación en la DA. Se desconoce si el papel de los linfocitos Treg se encuentra bloqueado por las demás citoquinas en la fase activa de la enfermedad, o si por el contrario, su funcionamiento es normal pero no logra contrarrestar los efectos proinflamatorios y proliferativos de la respuesta adaptativa. Otras células que hacen parte de la integración de la respuesta inmune, y de las cuales se conoce poco dentro de la inmunopatogenia de la DA canina son los LTγδ, Th22 y Th17. Muchas de las interacciones y respuestas inmunológicas que se dan en el desarrollo de la DA, tanto humana como canina, continúan siendo una incógnita. Es necesario por tanto, el desarrollo de estudios que logren analizar la influencia de cada uno de los factores expuestos anteriormente, con el fin de obtener resultados extrapolables a un porcentaje significativo de la población afectada por DA.

La investigación relacionada con estrategias diagnósticas y terapéuticas de la DA, se da en paralelo con los descubrimientos recientes dentro de la inmunopatogenia de la enfermedad. Por más de 10 años, la prueba intradérmica ha sido la principal herramienta utilizada para identificar los alérgenos específicos causales de la respuesta de hipersensibilidad en caninos, a pesar de los evidentes beneficios que el prick test ha reportado en alergología humana. Otra prueba que ha cobrado importancia a través del tiempo, es la determinación de IgE sérica, que a pesar de tener muchos reportes de sensibilidad y especificidad, aún no logra correlacionarse con los resultados de las pruebas intradérmicas. Con base en esta información, y mediante el uso de la medicina traslacional, se debería considerar al prick test como una herramienta complementaria y confirmatoria dentro del diagnóstico de la DA canina, comparando los resultados

obtenidos con las pruebas intradérmicas y la medición de IgE sérica. Todo esto, con el fin de lograr una estandarización de conceptos aplicables en dermatología veterinaria.

El presente trabajo permitió integrar los avances más recientes dentro de la inmunopatogenia de la DA humana y canina, para evidenciar diferencias, similitudes y vacíos investigativos en ambas especies. Adicionalmente, analizó mediante una prueba piloto, la viabilidad del prick test como herramienta para la detección de sensibilización alergénica en caninos diagnosticados con DA en la ciudad de Medellín. Teniendo en cuenta la limitada bibliografía relacionada con el prick test en medicina veterinaria hasta el momento, fue necesario hacer uso de las experiencias reportadas en medicina humana, para extrapolarlas y ajustarlas a los requerimientos de los caninos con DA. Los resultados obtenidos en este trabajo, abren el camino para avanzar en la investigación de la DA, tanto en medicina humana como en medicina veterinaria, fortaleciendo así el área de la dermatoinmunología. Esto permitirá ofrecer en un futuro, mayores elementos diagnósticos y terapéuticos, que mejoren la calidad de vida de los individuos que padecen de esta enfermedad.

Referencias generales del trabajo de investigación.

Andrian, U., & Mackay, C. (2000). T-cell function and migration Two Sides of the Same Coin. *New England Journal of Medicine*, *343*, 1020–1034.

Angelbeck-Schulze, M., Mischke, R., Rohn, K., Hewicker-Trautwein, M., Naim, H. Y., & Bäumer, W. (2014). Canine epidermal lipid sampling by skin scrub revealed variations between different body sites and normal and atopic dogs. *BMC Veterinary Research*, *10*. https://doi.org/10.1186/1746-6148-10-152

Asahina, R., Kamishina, H., Kamishina, H., & Maeda, S. (2015). Gene transcription of pro-inflammatory cytokines and chemokines induced by IL-17A in canine keratinocytes. *Veterinary Dermatology*, *26*(6), 426–432. https://doi.org/10.1111/vde.12244

Asahina, R., & Maeda, S. (2017). A review of the roles of keratinocyte-derived cytokines and chemokines in the pathogenesis of atopic dermatitis in humans and dogs. *Veterinary Dermatology*, *28*(1), 16-e5. https://doi.org/10.1111/vde.12351

Aslund, N., Thomsen, S. F., Mølgaard, E., Nolte, H., & Backer, V. (2008). Changes in skin test reactivity among adults with atopic disease: a 3-year prospective study. *Annals of Allergy, Asthma & Immunology : Official Publication of the American College of Allergy, Asthma, & Immunology*, *101*(5), 524–8. https://doi.org/10.1016/S1081-1206(10)60292-X

Auriemma, M., Vianale, G., Amerio, P., & Reale, M. (2013). Cytokines and T cells in atopic dermatitis. *European Cytokine Network*. https://doi.org/10.1684/ecn.2013.0333

Auxilia, S. T., & Hill, P. B. (2000). Mast cell distribution, epidermal thickness and hair follicle density in normal canine skin: possible explanations for the predilection sites of atopic dermatitis? *Veterinary Dermatology*, *11*(4), 247–254. https://doi.org/10.1046/j.1365-3164.2000.00193.x

Ballauf, B. (1991). Comparison of the intradermal and prick tests for diagnosis of allergy in the dog. *Tierarztliche Praxis*, *19*(4), 428–430.

Bao, L., Zhang, H., & Chan, L. S. (2013). The involvement of the JAK-STAT signaling

pathway in chronic inflammatory skin disease atopic dermatitis. *JAK-STAT*, *2*(3). https://doi.org/10.4161/jkst.24137

Bäsler, K., & Brandner, J. M. (2017). Tight junctions in skin inflammation. *Pflügers Archiv - European Journal of Physiology*, *469*(1), 3–14. https://doi.org/10.1007/s00424-016-1903-9

Bieber, T. (2010). Atopic dermatitis. *Annals of Dermatology*. https://doi.org/10.5021/ad.2010.22.2.125

Biedermann, T., Skabytska, Y., Kaesler, S., & Volz, T. (2015). Regulation of T cell immunity in atopic dermatitis by microbes: The Yin and Yang of cutaneous inflammation. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2015.00353

Bizikova, P., Santoro, D., Marsella, R., Nuttall, T., Eisenschenk, M. N. C., & Pucheu-Haston, C. M. (2015). Review: Clinical and histological manifestations of canine atopic dermatitis. *Veterinary Dermatology*, *26*(2), 79-e24. https://doi.org/10.1111/vde.12196

Black, A. P. B., Ardern-Jones, M. R., Kasprowicz, V., Bowness, P., Jones, L., Bailey, A. S., & Ogg, G. S. (2007). Human keratinocyte induction of rapid effector function in antigen-specific memory CD4. *European Journal of Immunology*, *37*, 1485–1493. https://doi.org/10.1002/eji.200636915

Boguniewicz, M., & Leung, D. Y. M. (2011). Atopic dermatitis: A disease of altered skin barrier and immune dysregulation. *Immunological Reviews*, *242*(1), 233–246. https://doi.org/10.1111/j.1600-065X.2011.01027.x

Bousquet, J., Heinzerling, L., Bachert, C., Papadopoulos, N. G., Bousquet, P. J., Burney, P. G., … Demoly, P. (2012). Practical guide to skin prick tests in allergy to aeroallergens. *Allergy: European Journal of Allergy and Clinical Immunology*, *67*(1), 18–24. https://doi.org/10.1111/j.1398-9995.2011.02728.x

Brandt, E. B., & Sivaprasad, U. (2011). Th2 Cytokines and Atopic Dermatitis. *J Clin Cell Inmunol.*, *2*(3). https://doi.org/10.4172/2155-9899.1000110

Brazís, P., De Mora, F., Ferrer, L., & Puigdemont, A. (2002). IgE enhances FcεRI expression and IgE-dependent TNF-α release from canine skin mast cells. *Veterinary Immunology and Immunopathology*, *85*(3–4), 205–212. https://doi.org/10.1016/S0165-2427(01)00428-7

Brazís, P., Queralt, M., De Mora, F., Ferrer, L., & Puigdemont, A. (2000). Stem cell factor enhances IgE-mediated histamine and TNF-α release from dispersed canine cutaneous mast cells. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(00)00188-4

Breuer, K., Werfel, T., & Kapp Nordseeklinik Norderney, A. (2006). Allergic Manifestations of Skin Diseases – Atopic Dermatitis. *Chem Immunol Allergy. Basel, Karger*, *91*, 76–86.

Brown, S. J., & Irwin McLean, W. H. (2012). One Remarkable Molecule: Filaggrin. *Journal of Investigative Dermatology*. https://doi.org/10.1038/jid.2011.393

Campbell, D. J. (2015). Control of regulatory T cell migration, function and homeostasis 1. *Journal of Immunology Research*, *196*(6), 2507–2513. https://doi.org/10.4049/jimmunol.1500801

Cesare, A. Di, Meglio, P. Di, & Nestle, F. O. (2008). A Role for Th17 Cells in the Immunopathogenesis of Atopic Dermatitis? *Journal of Investigative Dermatology*. https://doi.org/10.1038/jid.2008.283

Cork, M. J., Danby, S. G., Vasilopoulos, Y., Hadgraft, J., Lane, M. E., Moustafa, M., … Ward, S. J. (2009). Epidermal Barrier Dysfunction in Atopic Dermatitis. *Journal of Investigative Dermatology*, *129*, 1892–1908. https://doi.org/10.1038/jid.2009.133

Cornegliani, L., Vercelli, A., Sala, E., & Marsella, R. (2012). Transepidermal water loss in healthy and atopic dogs, treated and untreated: A comparative preliminary study. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2011.01003.x

Czogala, J. M., Marycz, K., Kuryszko, J. J., & Zawadzki, M. (2011). Cells of the skin immune system in dogs with atopy. *Acta Veterinaria Brno*. https://doi.org/10.2754/avb201180010011

DeBoer, D. J., & Hillier, A. (2001). The ACVD task force on canine atopic dermatitis (XVI): Laboratory evaluation of dogs with atopic dermatitis with serum-based “allergy” tests. *Veterinary Immunology and Immunopathology*, *81*(3–4), 277–287. https://doi.org/10.1016/S0165-2427(01)00304-X

Dubrac, S., Schmuth, M., & Ebner, S. (2010). Atopic dermatitis: the role of Langerhans cells in disease pathogenesis. *Immunology and Cell Biology*. https://doi.org/10.1038/icb.2010.33

Elmariah, S. B., & Lerner, E. A. (2013). The missing link between itch and inflammation in atopic dermatitis. *Cell*, *155*(2), 267–269. https://doi.org/10.1016/j.cell.2013.09.038

Eyerich, S., Eyerich, K., Pennino, D., Carbone, T., Nasorri, F., Pallotta, S., … Cavani, A. (2009). Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI40202

Flohr, C., & Yeo, L. (2011). Atopic dermatitis and the hygiene hypothesis revisited. *Curr.Probl.Dermatol.*, *41*(1662–2944 (Electronic)), 1–34.

Garrood, T., Lee, L., & Pitzalis, C. (2006). Molecular mechanisms of cell recruitment to inflammatory sites: General and tissue-specific pathways. *Rheumatology*, *45*(3), 250–260. https://doi.org/10.1093/rheumatology/kei207

Gonzales, A. J., Humphrey, W. R., Messamore, J. E., Fleck, T. J., Fici, G. J., Shelly, J. A., … Mccall, R. B. (2013). Interleukin-31: Its role in canine pruritus and naturally occurring canine atopic dermatitis. *Veterinary Dermatology*, *24*(1), 48-e12. https://doi.org/10.1111/j.1365-3164.2012.01098.x

Griffin, C. ., & DeBoer, D. . (2001). The ACVD task force on canine atopic dermatitis (XIV): clinical manifestations of canine atopic dermatitis. *Veterinary Immunology and Immunopathology*, *81*(3–4), 255–269. https://doi.org/10.1016/S0165-2427(01)00346-4

Gröne, A. (2002a). Keratinocytes and cytokines. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(02)00136-8

Gröne, A. (2002b). Keratinocytes and cytokines. *Veterinary Immunology and Immunopathology*, *88*(1–2), 1–12. https://doi.org/10.1016/S0165-2427(02)00136-8

Halliwell, R. E. W., & DeBoer, D. J. (2001). The ACVD task force on canine atopic dermatitis (III): The role of antibodies in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*, *81*(3–4), 159–167. https://doi.org/10.1016/S0165-2427(01)00309-9

Hammerberg, B. (2009). Canine immunoglobulin E. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2009.09.009

Hänel, K. H., Pfaff, C. M., Cornelissen, C., Amann, P. M., Marquardt, Y., Czaja, K., … Baron, J. M. (2016). Control of the Physical and Antimicrobial Skin Barrier by an IL-

31–IL-1 Signaling Network. *The Journal of Immunology*, *196*(8), 3233–3244. https://doi.org/10.4049/jimmunol.1402943

Hari, A., Flach, T. L., Shi, Y., & Mydlarski, P. R. (2010). Toll-like receptors: Role in dermatological disease. *Mediators of Inflammation*. https://doi.org/10.1155/2010/437246

Hayashiya, S., Tani, K., Morimoto, M., Hayashi, T., Hayasaki, M., Nomura, T., … Taura, Y. (2002). Expression of T Helper 1 and T Helper 2 Cytokine mRNAs in Freshly Isolated Peripheral Blood Mononuclear Cells from Dogs with Atopic Dermatitis. *Journal of Veterinary Medicine Series A*, *49*(1), 27–31. https://doi.org/10.1046/j.1439-0442.2002.00413.x

Hensel, P., Santoro, D., Favrot, C., Hill, P., & Griffin, C. (2015a). Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Veterinary Research*, *11*(1), 196. https://doi.org/10.1186/s12917-015-0515-5

Hensel, P., Santoro, D., Favrot, C., Hill, P., & Griffin, C. (2015b). Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Veterinary Research*, *11*, 196. https://doi.org/10.1186/s12917-015-0515-5

Hiedi Wood, S., Ke, X., Nuttall, T., McEwan, N., Ollier, W. E., & Carter, S. D. (2009). Genome-wide association analysis of canine atopic dermatitis and identification of disease related SNPs. *Immunogenetics*, *61*, 765–772.

Hill, P. B., & Olivry, T. (2001). The ACVD task force on canine atopic dermatitis (V): biology and role of in¯ammatory cells in cutaneous allergic reactions. *Veterinary Immunology and Immunopathology*, *81*(3–4), 187–98.

Hillier, A., & DeBoer, D. J. (2001). The ACVD task force on canine atopic dermatitis (XVII): Intradermal testing. *Veterinary Immunology and Immunopathology*, *81*(3–4), 289–304. https://doi.org/10.1016/S0165-2427(01)00313-0

Hillier, A., & Griffin, C. E. (2001). The ACVD task force on canine atopic dermatitis (I): Incidence and prevalence. *Veterinary Immunology and Immunopathology*, *81*(3–4), 147–151. https://doi.org/10.1016/S0165-2427(01)00296-3

Hoffmann, A. R., Patterson, A. P., Diesel, A., Lawhon, S. D., Ly, H. J., Stephenson, C. E., … Suchodolski, J. S. (2014). The skin microbiome in healthy and allergic dogs. *PLoS ONE*. https://doi.org/10.1371/journal.pone.003197

Irvine, A. D., McLean, W. H. I., & Leung, D. Y. M. (2011). Filaggrin Mutations Associated with Skin and Allergic Diseases. *New England Journal of Medicine*, *365*(14), 1315–1327. https://doi.org/10.1056/NEJMra1011040

Ito, T., Wang, Y.-H., Duramad, O., Hori, T., Delespesse, G. J., Watanabe, N., … Liu, Y.-J. (2005). TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *The Journal of Experimental Medicine*, *202*(9), 1213–1223. https://doi.org/10.1084/jem.20051135

Jassies-Van Der Lee, A., Rutten, V. P. M. G., Bruijn, J., Willemse, T., & Broere, F. (2014). CD4+ and CD8+ skin-associated T lymphocytes in canine atopic dermatitis produce interleukin-13, interleukin-22 and interferon-?? and contain a CD25+FoxP3+ subset. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12140

Jun, S. H., Lee, J. H., Kim, S. I., Choi, C. W., Park, T. I., Jung, H. R., … Lee, J. C. (2017). *Staphylococcus aureus* -derived membrane vesicles exacerbate skin inflammation in atopic dermatitis. *Clinical & Experimental Allergy*. https://doi.org/10.1111/cea.12851

Justo, X., Díaz, I., Gil, J. J., & Gastaminza, G. (2016). Prick test: evolution towards automated reading. *Allergy: European Journal of Allergy and Clinical Immunology*, *71*(8), 1095–1102. https://doi.org/10.1111/all.12921

Keppel, K. E., Campbell, K. L., Zuckermann, F. A., Greeley, E. A., Schaeffer, D. J., & Husmann, R. J. (2008). Quantitation of canine regulatory T cell populations, serum interleukin-10 and allergen-specific IgE concentrations in healthy control dogs and canine atopic dermatitis patients receiving allergen-specific immunotherapy. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2008.02.008

Kim, H.-J., Cronin, M., Ahrens, K., Papastavros, V., Santoro, D., & Marsella, R. (2016). A comparative study of epidermal tight junction proteins in a dog model of atopic dermatitis. *Veterinary Dermatology*, *27*(1), 40-e11. https://doi.org/10.1111/vde.12276

Kim, J. E., Kim, J. S., Cho, D. H., & Park, H. J. (2016). Molecular Mechanisms of Cutaneous Inflammatory Disorder: Atopic Dermatitis. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms17081234

Klukowska-Rötzler, J., Chervet, L., Müller, E. J., Roosje, P., Marti, E., & Janda, J. (2013). Expression of thymic stromal lymphopoietin in canine atopic dermatitis. *Veterinary Dermatology*, *24*(1). https://doi.org/10.1111/j.1365-3164.2012.01096.x

Kobayashi, T., Glatz, M., Horiuchi, K., Kawasaki, H., Akiyama, H., Kaplan, D. H., … Nagao, K. (2015). Dysbiosis and Staphylococcus aureus colonization drives inflammation in atopic dermatitis HHS Public Access microbiota-host immunity axis as a possible target for future therapeutics in eczematous dermatitis. *Immunity. April*, *21*(424), 756–766. https://doi.org/10.1016/j.immuni.2015.03.014

Kong, H. H., Oh, J., Deming, C., Conlan, S., Grice, E. A., Beatson, M. A., … Segre, J. A. (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Research*. https://doi.org/10.1101/gr.131029.111

Lauber, B., Molitor, V., Meury, S., Doherr, M. G., Favrot, C., Tengvall, K., … Marti, E. (2012). Total IgE and allergen-specific IgE and IgG antibody levels in sera of atopic dermatitis affected and non-affected Labrador- and Golden retrievers. *Veterinary Immunology and Immunopathology*, *149*(1–2), 112–118. https://doi.org/10.1016/j.vetimm.2012.05.018

Lee, J., Noh, G., Lee, S., Youn, Y., & Rhim, J. (2012). Atopic Dermatitis and Cytokines: Recent Patents in Immunoregulatory and Therapeutic Implications of Cytokines in Atopic Dermatitis -Part I: Cytokines in Atopic Dermatitis. *Recent Patents on Inflammation & Allergy Drug Discovery*, *6*, 222–247.

Leonard, B. C., Affolter, V. K., & Bevins, C. L. (2012). Antimicrobial peptides: Agents of border protection for companion animals. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01037.x

Liu, F. T., Goodarzi, H., & Chen, H. Y. (2011). IgE, mast cells, and eosinophils in atopic dermatitis. *Clinical Reviews in Allergy and Immunology*, *41*(3), 298–310. https://doi.org/10.1007/s12016-011-8252-4

Liu, Y.-J. (2006). Thymic stromal lymphopoietin: master switch for allergic inflammation. *The Journal of Experimental Medicine*. https://doi.org/10.1084/jem.20051745

Looringh van Beeck., F. (2014). Host and environmental contribution to atopic dermatitis in dogs. *Doctoral Dissertation , Utrecht University*.

Maeda, S., Fujiwara, S., Omori, K., Kawano, K., Kurata, K., Masuda, K., … Tsujimoto, H. (2002a). Lesional expression of thymus and activation-regulated chemokine in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(02)00140-X

Maeda, S., Fujiwara, S., Omori, K., Kawano, K., Kurata, K., Masuda, K., … Tsujimoto, H. (2002b). Lesional expression of thymus and activation-regulated chemokine in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*, *88*(1–2), 79–87. https://doi.org/10.1016/S0165-2427(02)00140-X

Maeda, S., Okayama, T., Omori, K., Masuda, K., Sakaguchi, M., Ohno, K., & Tsujimoto, H. (2002). Expression of CC chemokine receptor 4 (CCR4) mRNA in canine atopic skin lesion. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(02)00232-5

Majewska, A., Gajewska, M., Dembele, K., Maciejewski, H., Prostek, A., & Jank, M. (2016). Lymphocytic, cytokine and transcriptomic profiles in peripheral blood of dogs with atopic dermatitis. *BMC Veterinary Research*, *12*(174). https://doi.org/10.1186/s12917-016-0805-6

M Leung, D. Y., Ledford, D. K., Kuo, I.-H., Yoshida, T., De Benedetto, A., & Beck, L. A. (2013). Clinical reviews in allergy and immunology The cutaneous innate immune response in patients with atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *131*, 266–278. https://doi.org/10.1016/j.jaci.2012.12.1563

Manriquez, J., Andino-Navarrete, R., Cataldo-Cerda, K., & Harz-Fresno, I. (2015). Bibliometric characteristics of systematic reviews in dermatology: A cross-sectional study through Web of Science and Scopus. *Dermatologica Sinica*, *33*(3), 154–156. https://doi.org/10.1016/j.dsi.2014.12.007

Marsella, R. (2012). Are transepidermal water loss and clinical signs correlated in canine atopic dermatitis? A compilation of studies. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01055.x

Marsella, R. (2013a). Does filaggrin expression correlate with severity of clinical signs in dogs with atopic dermatitis? *Veterinary Dermatology*. https://doi.org/10.1111/vde.12007

Marsella, R. (2013b). Fixing the skin barrier: Past, present and future - man and dog

compared. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01073.x

Marsella, R., & Girolomoni, G. (2009). Canine Models of Atopic Dermatitis: A Useful Tool with Untapped Potential. *Journal of Investigative Dermatology*, *129*(10), 2351–2357. https://doi.org/10.1038/jid.2009.98

Marsella, R., & Olivry, T. (2003). Animal models of atopic dermatitis. *Clinics in Dermatology*. https://doi.org/10.1016/S0738-081X(02)00369-3

Marsella, R., Olivry, T., & Carlotti, D.-N. (2011). Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2011.00967.x

McCandless, E. E., Rugg, C. A., Fici, G. J., Messamore, J. E., Aleo, M. M., & Gonzales, A. J. (2014). Allergen-induced production of IL-31 by canine Th2 cells and identification of immune, skin, and neuronal target cells. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2013.10.017

Meury, S., Molitor, V., Doherr, M. G., Roosje, P., Leeb, T., Hobi, S., … Favrot, C. (2011). Role of the environment in the development of canine atopic dermatitis in Labrador and golden retrievers. *Veterinary Dermatology*, *22*(4), 327–334. https://doi.org/10.1111/j.1365-3164.2010.00950.x

Michels, G. M., Ramsey, D. S., Walsh, K. F., Martinon, O. M., Mahabir, S. P., Hoevers, J. D., … Dunham, S. A. (2016). A blinded, randomized, placebo-controlled, dose determination trial of lokivetmab (ZTS-00103289), a caninized, anti-canine IL-31 monoclonal antibody in client owned dogs with atopic dermatitis. *Veterinary Dermatology*, *27*(6), 478-e129. https://doi.org/10.1111/vde.12376

Mitsdoerffer, M., Lee, Y., Jager, A., Kim, H.-J., Korn, T., Kolls, J. K., … Kuchroo, V. K. (2010). Proinflammatory T helper type 17 cells are effective B-cell helpers. *Proceedings of the National Academy of Sciences*, *107*(32), 14292–14297. https://doi.org/10.1073/pnas.1009234107

Mizuno, T., Kanbayashi, S., Okawa, T., Maeda, S., & Okuda, M. (2009a). Molecular cloning of canine interleukin-31 and its expression in various tissues. *Veterinary Immunology and Immunopathology*, *131*(1–2), 140–143. https://doi.org/10.1016/j.vetimm.2009.03.014

Mizuno, T., Kanbayashi, S., Okawa, T., Maeda, S., & Okuda, M. (2009b). Molecular cloning of canine interleukin-31 and its expression in various tissues. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2009.03.014

Mu, Z., Zhao, Y., Liu, X., Chang, C., & Zhang, J. (2014). Molecular biology of atopic dermatitis. *Clinical Reviews in Allergy & Immunology*. https://doi.org/10.1007/s12016-014-8415-1

Mullin, J., Carter, S., Williams, N., McEwan, N., & Nuttall, T. (2013). Transcription of canine toll-like receptor 2, ??-defensin 1 and ??-defensin 103 in infected atopic skin, non-infected atopic skin, healthy skin and the CPEK cell line. *Veterinary Microbiology*. https://doi.org/10.1016/j.vetmic.2012.09.017

Murota, H., & Katayama, I. (2017). Exacerbating factors of itch in atopic dermatitis. *Allergology International*. https://doi.org/10.1016/j.alit.2016.10.005

Nestle, F. O., Di Meglio, P., Qin, J.-Z., & Nickoloff, B. J. (2009). Skin immune sentinels in health and disease. *Nature Reviews Immunology*. https://doi.org/10.1038/nri2622

Nishifuji, K., & Yoon, J. S. (2013). The stratum corneum: The rampart of the mammalian body. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01090.x

Novak, N., Bieber, T., & Leung, D. Y. M. (2003). Immune mechanisms leading to atopic dermatitis. *Journal of Allergy and Clinical Immunology*. https://doi.org/10.1016/j.jaci.2003.09.032

Nuttall, T. (2013). The genomics revolution: Will canine atopic dermatitis be predictable and preventable? *Veterinary Dermatology*, *24*(1). https://doi.org/10.1111/j.1365-3164.2012.01094.x

Nuttall, T. J., Knight, P. A., McAleese, S. M., Lamb, J. R., & Hill, P. B. (2002a). Expression of Th1, Th2 and immunosuppressive cytokine gene transcripts in canine atopic dermatitis. *Clinical and Experimental Allergy*, *32*(5), 789–795. https://doi.org/10.1046/j.1365-2222.2002.01356.x

Nuttall, T. J., Knight, P. A., McAleese, S. M., Lamb, J. R., & Hill, P. B. (2002b). T-helper 1, T-helper 2 and immunosuppressive cytokines in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*, *87*(3–4), 379–384. https://doi.org/10.1016/S0165-2427(02)00076-4

Ogg, G., & Ogg, G. (2009). Role of T cells in the pathogenesis of atopic dermatitis

Clinical and Experimental Allergy. *Clinical and Experimental Allergy*, (39), 310–316. https://doi.org/10.1111/j.1365-2222.2008.03146.x

Okada, H., Kuhn, C., Feillet, H., & Bach, J. F. (2010). The “hygiene hypothesis” for autoimmune and allergic diseases: An update. *Clinical and Experimental Immunology*, *160*(1), 1–9. https://doi.org/10.1111/j.1365-2249.2010.04139.x

Olivry, T. (2011). Is the skin barrier abnormal in dogs with atopic dermatitis? *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2011.07.014

Olivry, T. (2012). What can dogs bring to atopic dermatitis research? *Chemical Immunology and Allergy*. https://doi.org/10.1159/000331884

Olivry, T., Dunston, S. M., Murphy, K. M., & Moore, P. F. (2001). Characterization of the inflammatory infiltrate during IgE-mediated late phase reactions in the skin of normal and atopic dogs. *Veterinary Dermatology*, *12*(1), 49–58. https://doi.org/10.1046/j.1365-3164.2001.00230.x

Olivry, T., & Hill. (2001a). The ACVD task force on canine atopic dermatitis (VIII): is the epidermal lipid barrier defective?, *81*, 159–167.

Olivry, T., & Hill, P. B. (2001b). The ACVD task force on canine atopic dermatitis (XVIII): Histopathology of skin lesions. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(01)00305-1

Olivry, T., Mayhew, D., Paps, J. S., Linder, K. E., Peredo, C., Rajpal, D., … Cote-Sierra, J. (2016). Early Activation of Th2/Th22 Inflammatory and Pruritogenic Pathways in Acute Canine Atopic Dermatitis Skin Lesions. *Journal of Investigative Dermatology*, *136*(10), 1961–1969. https://doi.org/10.1016/j.jid.2016.05.117

Olivry, T., Mueller, R., Nuttall, T., Favrot, C., & Prélaud, P. (2008). Determination of CADESI-03 thresholds for increasing severity levels of canine atopic dermatitis. *Veterinary Dermatology*, *19*(3), 115–119. https://doi.org/10.1111/j.1365-3164.2008.00668.x

Olivry, T., Naydan, D., & Moore, P. (1997). Characterization of the cutaneous inflammatory infiltrate in canine atopic dermatitis. *The American Journal of Dermatopathology*, *9*(5), 477–486.

Ong, P. Y., & Leung, D. Y. M. (2016). Bacterial and Viral Infections in Atopic Dermatitis: a Comprehensive Review. *Clinical Reviews in Allergy & Immunology*.

https://doi.org/10.1007/s12016-016-8548-5

Oyoshi, M. K., He, R., Kumar, L., Yoon, J., & Geha, R. S. (2009). Chapter 3 Cellular and Molecular Mechanisms in Atopic Dermatitis. *Advances in Immunology*. https://doi.org/10.1016/S0065-2776(09)01203-6

Peng, W., & Novak, N. (2015). Pathogenesis of atopic dermatitis. *Clinical and Experimental Allergy*, *45*, 566–574. https://doi.org/10.1111/cea.12495

Pierezan, F., Olivry, T., Paps, J. S., Lawhon, S. D., Wu, J., Org, J., … Hoffmann, A. R. (2016). The skin microbiome in allergen-induced canine atopic dermatitis. *Veterinary Dermatology*, *27*, 332-e82. https://doi.org/10.1111/vde.12366

Pucheu-Haston, C. M. (2016). Atopic dermatitis in the domestic dog. *Clinics in Dermatology*. https://doi.org/10.1016/j.clindermatol.2015.10.010

Pucheu-Haston, C. M., Bizikova, P., Eisenschenk, M. N. C., Santoro, D., Nuttall, T., & Marsella, R. (2015). Review: The role of antibodies, autoantigens and food allergens in canine atopic dermatitis. *Veterinary Dermatology*, *26*(2), 115-e30. https://doi.org/10.1111/vde.12201

Pucheu-Haston, C. M., Bizikova, P., Marsella, R., Santoro, D., Nuttall, T., & Eisenschenk, M. N. C. (2015). Review: Lymphocytes, cytokines, chemokines and the T-helper 1-T-helper 2 balance in canine atopic dermatitis. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12205

Pucheu-Haston, C. M., Santoro, D., Bizikova, P., Eisenschenk, M. N. C., Marsella, R., & Nuttall, T. (2015). Review: Innate immunity, lipid metabolism and nutrition in canine atopic dermatitis. *Veterinary Dermatology*, *26*(2), 104-e28. https://doi.org/10.1111/vde.12199

Pucheu-Haston, C. M., Shuster, D., Olivry, T., Brianceau, P., Lockwood, P., McClanahan, T., … Hammerberg, B. (2006). A canine model of cutaneous late-phase reactions: Prednisolone inhibition of cellular and cytokine responses. *Immunology*. https://doi.org/10.1111/j.1365-2567.2005.02276.x

Reiter, L. V., Torres, S. M. F., & Wertz, P. W. (2009). Characterization and quantification of ceramides in the nonlesional skin of canine patients with atopic dermatitis compared with controls. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2009.00759.x

Ricklin, M. E., Roosje, P., & Summerfield, A. (2010). Characterization of canine dendritic cells in healthy, atopic, and non-allergic inflamed skin. *Journal of Clinical Immunology*. https://doi.org/10.1007/s10875-010-9447-9

Rodrigues Hoffmann, A. (2017a). The cutaneous ecosystem: the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12408

Rodrigues Hoffmann, A. (2017b). The cutaneous ecosystem: the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. *Veterinary Dermatology*, *28*(1), 60-e15. https://doi.org/10.1111/vde.12408

Romagnani, S. (2000). T-cell subsets (Th1 versus Th2). *Annals of Allergy, Asthma & Immunology*. https://doi.org/10.1016/S1081-1206(10)62426-X

Romagnani, S. (2004). Immunologic influences on allergy and the TH1/TH2 balance. *Journal of Allergy and Clinical Immunology*. https://doi.org/10.1016/j.jaci.2003.11.025

Roosterman, D., Goerge, T., Schneider, S. W., Bunnett, N. W., & Steinhoff, M. (2006). Neuronal Control of Skin Function: The Skin as a Neuroimmunoendocrine Organ. *Physiological Reviews*. https://doi.org/10.1152/physrev.00026.2005

Rosenblum, M. D., Way, S. S., & Abbas, A. K. (2015). Regulatory T cell memory. *Nature Reviews Immunology*, *16*(2), 90–101. https://doi.org/10.1038/nri.2015.1

Rossi, M. A., Messinger, L., Olivry, T., & Hoontrakoon, R. (2013). A pilot study of the validation of percutaneous testing in cats. *Veterinary Dermatology*, *24*(5). https://doi.org/10.1111/vde.12054

Said, A., & Weindl, G. (2015). Regulation of Dendritic Cell Function in Inflammation. *Journal of Immunology Research*. https://doi.org/10.1155/2015/743169

Sakamoto, M., Asahina, R., Kamishina, H., & Maeda, S. (2016). Transcription of thymic stromal lymphopoietin via Toll-like receptor 2 in canine keratinocytes: A possible association of Staphylococcus spp. in the deterioration of allergic inflammation in canine atopic dermatitis. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12301

Salzmann, C. A., Olivry, T. J., Nielsen, D. M., Paps, J. S., Harris, T. L., & Olby, N. J. (2011). Genome-wide linkage study of atopic dermatitis in West Highland White

Terriers. *BMC Genetics*, *12*(37). https://doi.org/10.1186/1471-2156-12-37

Sánchez-caraballo, J., Diez-zuluaga, S., & Ricardo, C. (2012). Sensibilización a aeroalergenos en pacientes alérgicos de Medellín, Colombia. *Revista Alergia Mexico*, *59*(3), 139–147.

Santoro, D., Bunick, D., Graves, T. K., & Segre, M. (2013). Evaluation of canine antimicrobial peptides in infected and noninfected chronic atopic skin. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2012.01091.x

Santoro, D., Marsella, R., Bunick, D., Graves, T. K., & Campbell, K. L. (2011). Expression and distribution of canine antimicrobial peptides in the skin of healthy and atopic beagles. *Veterinary Immunology and Immunopathology*, *144*(3–4), 382–388. https://doi.org/10.1016/j.vetimm.2011.08.004

Santoro, D., Marsella, R., Pucheu-Haston, C. M., Eisenschenk, M. N. C., Nuttall, T., & Bizikova, P. (2015). Review: Pathogenesis of canine atopic dermatitis: Skin barrier and host-micro-organism interaction. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12197

Santoro, D., & Rodrigues Hoffmann, A. (2016). Canine and Human Atopic Dermatitis: Two Faces of the Same Host-Microbe Interaction. *Journal of Investigative Dermatology*. https://doi.org/10.1016/j.jid.2016.03.015

Schindler, C., Levy, D. E., & Decker, T. (2007). JAK-STAT signaling: From interferons to cytokines. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.R700016200

Schlotter, Y. M., Rutten, V. P. M. G., Riemers, F. M., Knol, E. F., & Willemse, T. (2011). Lesional skin in atopic dogs shows a mixed Type-1 and Type-2 immune responsiveness. *Veterinary Immunology and Immunopathology*, *143*(1–2), 20–26. https://doi.org/10.1016/j.vetimm.2011.05.025

Schmidt-Weber, C. B., Akdis, M., & Akdis, C. A. (2007). TH17 cells in the big picture of immunology. *Journal of Allergy and Clinical Immunology*, *120*(2), 247–254. https://doi.org/10.1016/j.jaci.2007.06.039

Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., … Kastelein, R. A. (2005). IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. https://doi.org/10.1016/j.immuni.2005.09.015

Schoenebeck, J. J., & Ostrander, E. A. (2014). Insights into Morphology and Disease from the Dog Genome Project. *Annual Review of Cell and Developmental Biology*, *30*(1), 535–560. https://doi.org/10.1146/annurev-cellbio-100913-012927

Shaw, S. C., Wood, J. L. N., Freeman, J., Littlewood, J. D., & Hannant, D. (2004). Estimation of heritability of atopic dermatitis in Labrador and Golden Retrievers. *American Journal of Veterinary Research*. https://doi.org/10.2460/ajvr.2004.65.1014

Shi, Y., Liu, C. H., Roberts, A. I., Das, J., Xu, G., Ren, G., … Devadas, S. (2006). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. *Cell Research*. https://doi.org/10.1038/sj.cr.7310017

Shibata, S., Maeda, S., Kondo, N., Chimura, N., Inoue, A., & Fukata, T. (2011). Identification of the signaling pathway of TNF-??-induced CCL17/TARC transcription in a canine keratinocyte cell line. *Veterinary Immunology and Immunopathology*, *139*(2–4), 90–98. https://doi.org/10.1016/j.vetimm.2010.08.008

Shida, M., Kadoya, M., Park, S. J., Nishifuji, K., Momoi, Y., & Iwasaki, T. (2004). Allergen-specific immunotherapy induces Th1 shift in dogs with atopic dermatitis. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2004.06.003

Shimada, K., Yoon, J. S., Yoshihara, T., Iwasaki, T., & Nishifuji, K. (2009). Increased transepidermal water loss and decreased ceramide content in lesional and non-lesional skin of dogs with atopic dermatitis. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2009.00847.x

Sinke, J. D., Rutten, V. P. M. G., & Willemse, T. (2002). Immune dysregulation in atopic dermatitis. In *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(02)00066-1

Sinke, J. D., Thepen, T., Bihari, I. C., Rutten, V. P. M. G., & Willemse, T. (1997). Immunophenotyping of skin-infiltrating T-cell subsets in dogs with atopic dermatitis. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/S0165-2427(96)05763-7

Ständer, S., Raap, U., Weisshaar, E., Schmelz, M., Mettang, T., Handwerker, H., & Luger, T. A. (2011). Pathogenesis of pruritus. *JDDG: Journal Der Deutschen Dermatologischen Gesellschaft*. https://doi.org/10.1111/j.1610-0387.2011.07585.x

Strid, J., & Strobel, S. (2005). Skin barrier dysfunction and systemic sensitization to allergens through the skin. *Current Drug Targets. Inflammation and Allergy*, *4*(5), 531–41. https://doi.org/10.2174/156801005774322199

Suter, M. M., Schulze, K., Bergman, W., Welle, M., Roosje, P., & Müller, E. J. (2009). The keratinocyte in epidermal renewal and defence. *Veterinary Dermatology*. https://doi.org/10.1111/j.1365-3164.2009.00819.x

Thomsen, K. (1991). The role of neutrophil-activating mediators in canine health and disease (with special reference to the role of leukotrienes in inflammatory dermatoses). *Journal of Veterinary Pharmacology Therapy*, *1963*(1973), 113–133.

Tokura, Y. (2010). Extrinsic and intrinsic types of atopic dermatitis. *Journal of Dermatological Science*. https://doi.org/10.1016/j.jdermsci.2010.02.008

Tsai, K. L., Clark, L. A., & Murphy, K. E. (2007). Understanding hereditary diseases using the dog and human as companion model systems. *Mammalian Genome*, *18*(6–7), 444–451. https://doi.org/10.1007/s00335-007-9037-1

Turner, M. J., & Zhou, B. (2014). A new itch to scratch for TSLP. *Trends in Immunology*, *35*(2), 49–50. https://doi.org/10.1016/j.it.2013.12.001

Two, A. M., Nakatsuji, T., Kotol, P. F., Arvanitidou, E., Du-Thumm, L., Hata, T. R., & Gallo, R. L. (2016). The Cutaneous Microbiome and Aspects of Skin Antimicrobial Defense System Resist Acute Treatment with Topical Skin Cleansers. *Journal of Investigative Dermatology*. https://doi.org/10.1016/j.jid.2016.06.612

Watson W, & Kapur S. (2011). Atopic dermatitis. *Allergy, Asthma and Clinical Immunology*, *7*(Suppl. 1), S4. https://doi.org/10.1186/1710-1492-7-S1-S4

Weidinger, S., & Novak, N. (2016). Atopic dermatitis. In *The Lancet* (Vol. 387, pp. 1109–1122). https://doi.org/10.1016/S0140-6736(15)00149-X

Wilson, S. R., Thé, L., Batia, L. M., Beattie, K., Katibah, G. E., McClain, S. P., … Bautista, D. M. (2013). XThe epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. *Cell*. https://doi.org/10.1016/j.cell.2013.08.057

Wong, R., Geyer, S., Weninger, W., Guimberteau, J., & Wong, J. K. (2016). The dynamic anatomy and patterning of skin. *Experimental Dermatology*, *25*(2), 92–98. https://doi.org/10.1111/exd.12832

Wood, S. H., Clements, D. N., Ollier, W. E., Nuttall, T., McEwan, N. A., & Carter, S. D.

(2009). Gene expression in canine atopic dermatitis and correlation with clinical severity scores. *Journal of Dermatological Science*. https://doi.org/10.1016/j.jdermsci.2009.03.005

Wood, S. H., Ollier, W. E., Nuttall, T., McEwan, N. A., & Carter, S. D. (2010). Despite identifying some shared gene associations with human atopic dermatitis the use of multiple dog breeds from various locations limits detection of gene associations in canine atopic dermatitis. *Veterinary Immunology and Immunopathology*. https://doi.org/10.1016/j.vetimm.2010.07.020

Wüthrich B, S.-G. P. (2003). The atopic eczema/dermatitis syndrome. Epidemiology, natural course, and immunology of the IgE-associated ("extrinsic") and the nonallergic ("intrinsic") AEDS. *J Investig Allergol Clin Immunol.*, *13*(1), 1–5.

Yoon, J. S., Nishifuji, K., Sasaki, A., Ide, K., Ishikawa, J., Yoshihara, T., & Iwasaki, T. (2011). Alteration of stratum corneum ceramide profiles in spontaneous canine model of atopic dermatitis. *Experimental Dermatology*. https://doi.org/10.1111/j.1600-0625.2011.01306.x

Yoshida, K., Kubo, A., Fujita, H., Yokouchi, M., Ishii, K., Kawasaki, H., … Amagai, M. (2014). Distinct behavior of human Langerhans cells and inflammatory dendritic epidermal cells at tight junctions in patients with atopic dermatitis. *Journal of Allergy and Clinical Immunology*. https://doi.org/10.1016/j.jaci.2014.08.001

Yuki, T., Tobiishi, M., Kusaka-Kikushima, A., Ota, Y., & Tokura, Y. (2016). Impaired tight junctions in atopic dermatitis skin and in a skin-equivalent model treated with interleukin-17. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0161759

Zajac, M., Szczepanik, M. P., Wilkolek, P. M., Adamek, L. R., Pomorski, Z. J. H., Sitkowski, W., & Goly??ski, M. G. (2014). Assessment of the relationship between transepidermal water loss (TEWL) and severity of clinical signs (CADESI-03) in atopic dogs. *Veterinary Dermatology*. https://doi.org/10.1111/vde.12150

Zhang, Q., Putheti, P., Zhou, Q., Liu, Q., & Gao, W. (2008). Structures and biological functions of IL-31 and IL-31 receptors. *Cytokine and Growth Factor Reviews*. https://doi.org/10.1016/j.cytogfr.2008.08.003

Zheng, X., Ma, P., de Paiva, C. S., Cunningham, M. A., Hwang, C. S., Pflugfelder, S. C., & Li, D. Q. (2010). TSLP and downstream molecules in experimental mouse allergic

conjunctivitis. *Investigative Ophthalmology and Visual Science*. https://doi.org/10.1167/iovs.09-4122

ANEXOS

Reglamentación artículo Capítulo II – Revista: Veterinary Immunology and Immunopathology

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

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• All references mentioned in the Reference List are cited in the text, and vice versa

• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements
Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Essential title page information

• Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration, e.g. "Y. Wang (王羽佳)". Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

· Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.

· Present/permanent address. If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. Your abstract should not be longer than 400 words.</>

Keywords

Immediately after the abstract, provide a maximum of 6 keywords avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with

abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

A number of standard accepted abbreviations can be used in the body of a manuscript without further explanation (see Appendix 1), but must be explained if used in the Title or Abstract. Non-standard abbreviations should be explained when first used in the Abstract, and again when first used in the body of a manuscript, and should also be explained in an abbreviations list to follow the list of keywords.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Nomenclature

1. Authors and Editors are, by general agreement, obliged to accept the rules governing biological nomenclature, as laid down in the International Code of Botanical Nomenclature, the International Code of Nomenclature of Bacteria, and the International

Code of Zoological Nomenclature. Virologists should consult the latest Report of the International Committee on Taxonomy of Viruses for proper nomenclature and spelling.

2. All biotica (crops, plants, insects, birds, mammals, etc.) should be identified by their scientific names when the English term is first used, with the exception of common domestic animals.

3. All biocides and other organic compounds must be identified by their Geneva names when first used in the text. Active ingredients of all formulations should be likewise identified.

4. For chemical nomenclature, the conventions of the International Union of Pure and Applied Chemistry and the official recommendations of the IUPAC-IUB Combined Commission on Biochemical Nomenclature should be followed.
References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles,

publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

A DOI can be used to cite and link to electronic articles where an article is in-press and full citation details are not yet known, but the article is available online. A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

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1. All publications cited in the text should be presented in a list of references following the text of the manuscript. The manuscript should be carefully checked to ensure that the spelling of author's names and dates are exactly the same in the text as in the reference list.

2. In the text refer to the author's name (without initial) and year of publication, followed – if necessary – by a short reference to appropriate pages. Examples: "Since Peterson (1988) has shown that..." "This is in agreement with results obtained later (Kramer, 1989, pp. 12–16)".

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4. References cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically on author's names, and chronologically per author. If an author's name in the list is also mentioned with co-authors the following order should be used: publications of the single author, arranged according to publication dates – publications of the same author with one co-author – publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1974a, 1974b, etc.

5. Use the following system for arranging your references:

a. For periodicals

Harp, J.A., Walters, T.E., Goff, J.P., 2004. Lymphocyte subsets and adhesion molecule expression in milk and blood of periparturient dairy cattle. Vet. Immunol. Immunopathol. 102, 9-17.

b. For edited symposia, special issues, etc., published in a periodical

Miller, L.C., Fox, J.M., 2004. Apoptosis and porcine reproductive and respiratory syndrome virus. In: Murtaugh, M.P., Rowland, R.R.R. (Eds), Immunology and Immunopathology of Porcine Reproductive and Respiratory Syndrome (PRRS). Vet. Immunol. Immunopathol. 102, 131-142.

c. For books

Gershwin, M., Naguwa, S., 2005. Allergy and Immunology Secrets, Elsevier, 352 pp.

d. For multi-author books

Butler, J.E., 1981. A concept of humoral immunity among ruminants and an approach to its investigation. In: Butler, J.E., Nielson, K., Duncan, J.R. (Eds.), The Ruminant Immune System, Plenum Press, New York, pp. 3-55.

Data references

[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. http://dx.doi.org/10.17632/xwj98nb39r.1.

6. Abbreviate the titles of periodicals mentioned in the list of references in accordance with BIOSIS Serial Sources, published annually by BIOSIS. The correct abbreviation for this journal is Vet. Immunol. Immunopathol.

7. In the case of publications in any language other than English, the original title is to be retained. However, the titles of publications in non-Latin alphabets should be transliterated, and a notation such as "(in Russian)" or "(in Greek, with English abstract)" should be added.

8. Work accepted for publication but not yet published should be referred to as "in press".

9. References concerning unpublished data and "personal communications" should not be cited in the reference list but may be mentioned in the text.

10. Web references may be given. As a minimum, the full URL is necessary. Any further information, such as Author names, dates, reference to a source publication and so on, should also be given.

11. Articles available online but without volume and page numbers may be referred to by means of their Digital Object identifier (DOI) code.

Reglamentación artículo : CAPITULO III

An analysis of skin prick test reactions to detect allergic sensitization in dogs clinically compatible with atopic dermatitis in Medellín, Colombia.

Revista Veterinary Dermatology

Format

Veterinary Dermatology operates a system of double-blinded review and the names of the authors will not be disclosed to the reviewers. Authors should therefore avoid including anything that could identify them within the text. This, for example, includes: the name of the institution at which the work was performed; initials of the authors; acknowledgements and names of institutions on illustrations, etc. To enable double-blinded review, contributors (including acknowledgements) should only be named on the title page or uploaded separately as a supplementary file, and not on the manuscript. Authors should also avoid statements that could identify them through references (e.g. instead of 'we have previously shown that black is white', authors should write 'previous studies have shown that black is white').

The manuscript (including references and figure legends) must be A4 or 8.5 x 11 inch format with 2.5 cm margins, single-spaced typed (please do not submit double line spaced), align text left, 12 point font using sans serif typeface such as Helvetica (Swiss), Arial or Verdana style (please do not use Times New Roman). Each line and page of the manuscript text should be numbered consecutively from the title page.

Authors are requested to write with the minimum of formatting and NOT to write over previous versions, which may contain hidden formatting. Do not enhance text and tables with unnecessary formatting (e.g. small capitals, headers). Software programmes that automatically create endnotes and footnotes should not be used.

Title Page

The title of the article should be concise but informative. Drug trade names will not be included in the title. The first name, middle initial(s), and last name of each author must be given. Professional affiliations of the authors at the time of the study should be indicated using the symbols *, †, ‡, §, ¶, then **, †† etc., in this order; these are not superscripts. Titles (e.g. professor) and qualifications (e.g. DipACVD) are not required.

Please provide full address details for all of the authors. If an author's affiliation has changed since the study was performed, the author's new affiliation should be identified. The name of the corresponding author, any conflicts of interest and sources of funding (see section 2.5) should be stated. If information in the text has been presented at a scientific meeting, this should be indicated. A short running title of no more than 40 characters (counting letters and spaces) should also be included. The short running title will be used in the journal at the top of the page, see current publications. Keywords are NOT required.

Abstract

The abstract should be no more than 250 words and must be constructed using the subheadings given below. While this format is most appropriate for scientific studies, the authors of reviews, brief communications and case reports are encouraged to also provide a structured abstract using the following:

Background – A brief explanation of why the study was performed.
Hypothesis/Objectives – A statement of the principal hypothesis tested in the study, a brief statement of the major objectives, or both.
Animals – A concise description of the number of animals used in the study including the population from which they were drawn (e.g. research colony, hospital population) and any special characteristics of the animals (e.g. disease status).

Methods – A statement of overall study design (e.g. randomized, blinded, placebo-controlled clinical trial; retrospective study) and principal interventions or methods.
Results – Concise statement of important results including numerical description of critical variables and statement of statistical significance.
Conclusions and clinical importance – A summary of conclusions based on results of the study and statement of clinical importance of these conclusions. The results should not be restated.
Introduction

State the purpose of the article. Summarize the rationale for the study or observation. Give only strictly pertinent references and do not review the subject extensively.

Materials and Methods

These should be described in sufficient detail to allow other workers to reproduce the results. References for study design and statistical methods should be to standard works (with pages stated) when possible rather than to papers where designs or methods were originally reported. Specify any statistics computer programs used. Report losses to observation (such as dropouts from a clinical trial).

The methods of data collection and use of statistical analysis will be checked by the referees, editors and, if necessary, a statistician. It is highly recommended that authors consult a professional statistician for advice on complex statistical analyses. It is also recommended that authors provide details of which statistical methods and the P-value, if relevant, have been used for each component of the data set (e.g. P = 0.08; ANOVA).

Drugs and therapeutic agents should be given in the format: drug ingredient (trade name; manufacturer name, city, (state), country), e.g. fenbendazole (Panacur; Intervet-Schering Plough, Milton Keynes, UK).

Drug names should follow the recommended International Non-Proprietary Names (rINN). Common examples include cefalexin, ciclosporin, meticillin and rifampicin.

Products such as equipment or methods should be given as: Product name (Company name; town or city, (state) and country); e.g. Datex CD 200-02 (Datex; Hatfield, UK); or SuperScript® III First-Strand Synthesis kit (Invitrogen, Carlsbad, CA, USA). The detailed information about drugs, therapeutic agents and products need only be given once.

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Present your results in a logical sequence in the text, tables and illustrations. Do not repeat in the text data in the tables or illustrations. In manuscripts describing more than one animal, all animals should be assigned a case number.

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The discussion should emphasize the new and important aspects of the study and the conclusions that follow from them. Include the implications of the findings and their limitations, including implications for future research. Relate the observations to other relevant studies. Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly indicate them as such.

Recommendations, when appropriate, may be included.

Acknowledgements (should be made on the title page or as a separate supplementary file and not included on the manuscript). These are to indicate support, advice or technical help that does not justify authorship. Please use first and second (family) names, e.g. The authors would like to thank Fred Flintstone for assistance with statistical analysis.

Funding sources should be included in the declared sources of funding (see section 2.5).

Language and style

The language of publication is English. Authors for whom English is a second language must have their manuscript thoroughly, and preferably professionally, edited by an English speaking person before submission to make sure that the English is of high quality. A list of independent suppliers of editing services can be found at http://aplicacionesbiblioteca.udea.edu.co:2218/bauthor/english_language.asp. All services are paid for and arranged by the author; use of one of these services does not guarantee acceptance or preference for publication.

Units, Abbreviations and Nomenclature

All units of measurement must follow the SI system. Concentrations of solutions should be given as molar concentrations (e.g. mmol/L). All other concentrations should be expressed as percentages. Drug dosages should be given as: e.g. mg/kg; µg/kg; also use ‘once daily’, ‘twice daily’ etc. Spell out numbers one to nine, keep 10 onwards as numerals. However, use Arabic numerals for numbers used with units of measure (e.g. 9 kg, 5 h, 10 mmol/L). Use h, min, s, for hour, minute, second, respectively. Abbreviations of biological, medical, chemical and other terms should be used only when such abbreviations are both internationally recognized and unambiguous. The first use of an abbreviation must be explained by also giving the unabbreviated term.

All biological, medical, chemical and other names should be given in keeping with the latest international nomenclature. If an animal or micro-organism is being mentioned in the text for the first time, the binomial name should be given, e.g. carp (Cyprinus carpio). Thereafter, this can be abbreviated to C. carpio. Please check recent articles for information about the spelling of dog and cat breeds.

Figures (illustrations): Graphs, Tables, Clinical Photographs and Photomicrographs

Figure legends must be given at the end of the manuscript. Sufficient information should be included to allow the figure to be understood without reference to the text. Authors wishing to use any previously published figures must submit written permission from the copyright holder. Figure legends should be written in the following style:

1. Organ or tissue; animal identification, Case No. A sentence describing the change that is visible in the print. (For photomicrographs add: staining method with names of stains and counter stains and magnification, e.g. avidin–biotin– peroxidase complex method, Mayer's Haematoxylin counter stain, x40).

2. Graph or Table: statement of how data is expressed. Identification of symbols in table, graph, or photo: e.g. N, nucleus.

Graphs

To ensure high-quality reproduction, symbols should be clear and even throughout and of sufficient size, that when reduced for publication, each item will still be legible. Graph axes should be labelled in sans serif (Helvetica or Arial) font. Letters, Numbers and Titles belong in the legends for illustrations, not on the illustrations themselves.

Tables should be limited to those containing data important to understanding and interpreting results and reducing or clarifying the text. Tables may be include within the word file containing the main text, placed after the figure legends; alternatively, large tables should be submitted as separate table files (e.g. in Word or Excel). Number tables consecutively in the order of the first citation in the text and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory material in footnotes, not in the heading. Explain in the footnotes all non-standard abbreviations

that are used in each table. Identify statistical measures of variations such as standard deviation. Ensure that each table is cited in the text. If you use data from another published or unpublished source, obtain written permission and acknowledge fully.

Figures; Clinical Photographs & Photomicrographs (histopathology)

Such figures should be ideally be originally captured and submitted in a neutral data format such as TIFF or EPS. There is no requirement to convert an original uncompressed JPEG file to TIFF or EPS format. (JPEG format will be accommodated but must fulfil the format criteria given below and should be uncompressed). PowerPoint, PDFs and Word graphics are usually unsuitable for reproduction.

Figures should have a minimum resolution of 300 dpi; grey tone and line drawings require 600–1200 dpi. Photographic material should be of such quality that high-contrast that reproductions can be made. Poor-quality images may be removed from a manuscript and where critical to the content may lead to rejection of a manuscript.

Graphics created in the CMYK colour palette (print colours) are preferable to those created in RGB (screen colours) to maximize the consistency of print reproduction. Images supplied in RGB will be converted to CMYK for printing; this may lead to some variations in colour representation. Immunoflourescence images may be submitted in RGB.

When symbols, arrows, numbers or letters are used to identify parts of the illustrations, identify and explain each one clearly in the legend.

Clinical and histopathology (photomicrograph) figures must be no more than 19 cm in width and must be submitted at a resolution of 300 dpi.

Limit figures to those that reduce or clarify the text. These should be free of extraneous material and, where possible, if portions of the handler such as fingers or hands are to be included, particularly adjacent to lesions, they should be gloved.

Montage (composite plates) figures are allowed and should have no tooling (space bars) to separate the individual images. Each part should be labelled in the top left-hand corner in black, (or if appropriate for clarity in white) in Arial, in lower case, with no brackets, starting with a, b, c, etc.

References (Please note that EndNote™ and Refman™ software for the Journal of the American Veterinary Medical Association can be used for Veterinary Dermatology; please adapt to Vancouver style).

Software programs for creating reference lists may be used but they should be set up so that they generate in-text citations and reference lists according to the instructions and examples given below. Authors bear primary responsibility for the accuracy of all references. References must be limited to those that are necessary and must be cited in the text by superscript numbers in order of citation. Journal titles in the Reference section should be abbreviated in accordance with the National Library of Medicine (NLM website) and Index Medicus. For references with more than 3 authors, only the first 3 authors should be listed, followed by ‘et al.’ The following is the style used for common types of references:

Article in journal

1. Müntener T, Doherr MG, Guscetti F et al. The canine hair cycle – a guide for the assessment of morphological and immunohistochemical criteria. Vet Dermatol 2011; 22: 383-395.

Book

2. Scott DW. Large Animal Dermatology. Philadelphia, PA: Saunders, 1988; 457–458.

Book chapter

3. Muir P, Johnson KA, Manley PA. Fractures of the pelvis. In: Birchard SJ, Sherding RG, eds. Saunders Manual of Small Animal Practice. 2nd edition. Philadelphia: W.B. Saunders Co., 2000; 1126–1132.

Proceedings

4. Kunkle G, Hillier A, Beale K et al. Steroid effects on intradermal skin testing in sensitized dogs. In: Proceedings of the American Academy of Veterinary Dermatology & American College of Veterinary Dermatology. Charleston, SC, USA: 1994; 54–55.

Electronic Material

5. Animal and Plant Health Inspection Service website. Bovine spongiform encephalopathy (BSE). Available at: www.aphis.usda.gov/lpa/issues/bse/bse.html. Accessed Feb 18, 2003.

Animal Experiments

Animal experiments are to be undertaken only with the purpose of advancing knowledge and in a manner that avoids unnecessary discomfort to the animals by the use of proper management and laboratory techniques. They shall be conducted in compliance with federal, state and local laws and regulations, and in accordance with the internationally accepted principles and guidelines for the care and use of agricultural, laboratory or experimental animals. In the interests of the reproducibility of results, accurate information about any test animals used in the experiments (origin, inbreeding etc.), as well as information about the housing conditions (diet, environment etc.), should be given. For further information and guidance on how to report on animal experiments see: ARRIVE guidelines for reporting animal research see: ARRIVE guidelines for reporting animal research