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In order to improve the diagnostic value of histopathologic examination of skin biopsy samples from dogs with atopic dermatitis and, perhaps, to identify any differences from the normal state that may predispose to this skin condition, we compared the anatomic and cellular morphology of skin from three standard sites in 21 normal and 15 atopic dogs. The standard sites were lateral neck, dorsal rump, and craniolateral abdomen. No differences between the two groups were found in the means of area or thickness of the stratum corneum or the remainder of the epidermis at any site. The area of sebaceous glands, but not apocrine sweat glands, was larger in the atopic group (P less than or equal to 0.05 for the lateral neck skin and P less than or equal to 0.1 for the dorsal rump skin). The mean number of non-metachromatic mononuclear cells in combined skin samples (126 microns²) in atopic dogs (91.0 +/- 28.7) was significantly greater (P less than or equal to 0.01) than for the control normal dogs (65.3 +/- 19.3); the mean number of mast cells in atopic dogs (12.39 +/- 6.44) was similarly greater than in the controls (8.48 +/- 5.14; P less than or equal to 0.1). Eosinophils were significantly increased in atopic dog skin (P less than or equal to 0.01) with the mean for all three sites combined of 0.81 +/- 0.90 compared with a mean of 0.06 +/- 0.15 for normal dogs. Numbers of circulating blood eosinophils were not significantly different in the atopic and normal group.

Neurotrophin-4 production by human epidermal keratinocytes: increased expression in atopic dermatitis.

Grewe M, Vogelsang K, Ruzicka T, Stege H, Krutmann J.

Chronic inflammatory conditions of human skin, such as prurigo lesions of atopic dermatitis, are characterized clinically by intense pruritus and histologically by increased innervation. Regulation of skin innervation is thought to depend on neurotrophic factors. In this study, human skin cells were identified as a source of neurotrophins. Cultured keratinocytes expressed neurotrophin-4, whereas dermal fibroblasts expressed neurotrophin-3. In vitro stimulation with interferon-gamma, a marker cytokine for atopic eczema, induced keratinocyte neurotrophin-4 production, which was able to support growth of a neuroglioblastoma-derived cell line. In vivo, immunohistochemistry of human skin for neurotrophins showed neurotrophin-4 staining in the epidermal layer and neurotrophin-3 staining in the dermal compartment. Neurotrophin-4 but not neurotrophin-3 expression was markedly increased in interferon-gamma-injected skin. Prurigo lesions of atopic dermatitis skin were characterized by intense epidermal staining for neurotrophin-4, suggesting a pathophysiologic role for this neurotrophin in the increased innervation characteristic for these skin lesions. This study demonstrates differential expression and regulation of neurotrophins in human skin. It also identifies keratinocyte-derived neurotrophin-4 as a possible link between the immune and the nerve system of human skin.

Cutaneous nerves in atopic dermatitis. A histological, immunohistochemical and electron microscopic study.

Urashima R, Mihara M.

Although pruritus is the cardinal symptom of atopic dermatitis, its mechanism is not well understood. Free nerve endings in the skin are involved in pruritus as itching receptors. We studied the cutaneous nerve fibres in lichenified lesions of 16 patients with adult atopic dermatitis. On immunohistochemistry, fibres immunoreactive for neurofilament, neuron-specific enolase, and protein gene product 9.5 were observed in the papillary dermis and dermoepidermal junctions as well as in the epidermis. In these areas, no fibres stained positively for substance P, neuropeptide Y, vasoactive intestinal peptide, beta endorphin, somatostatin or serotonin. On electron microscopy, the ultrastructure of subepidermal and intraepidermal free nerve endings appeared to be essentially normal. However, the distribution density of the cutaneous nerve fibres was much higher than in normal controls, and the diameter of these fibres was much larger, because of the large number of axons in each nerve fibre. Degranulation of mast cells was not seen. These findings suggest that pruritus in lichenified atopic skin is probably not caused by damage to the cutaneous free nerve endings. In such lesions, the number of the cutaneous free nerve endings is greatly increased, but they may have a normal function.

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Allergy development and the intestinal microflora during the first year of life.

Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M.

BACKGROUND: The intestinal microflora is a likely source for the induction of immune deviation in infancy.

OBJECTIVE: The purpose of this study was to prospectively relate the intestinal microflora to allergy development in 2 countries differing with respect to the prevalence of atopic diseases. METHODS: Newborn infants were

followed prospectively through the first 2 years of life in Estonia (n = 24) and Sweden (n = 20). By that age, 9 Estonian and 9 Swedish infants had developed atopic dermatitis and/or positive skin prick test results. Stool samples were obtained at 5 to 6 days and at 1, 3, 6, and 12 months, and 13 groups of aerobic and anaerobic microorganisms were cultivated through use of standard methods. RESULTS: In comparison with healthy infants, babies who developed allergy were less often colonized with enterococci during the first month of life (72% vs 96%; P <.05) and with bifidobacteria during the first year of life (17% to 39% vs 42% to 69%; P <.05). Furthermore, allergic infants had higher counts of clostridia at 3 months (median value, 10.3 vs 7.2 log(10); P <.05). The prevalence of colonization with Staphylococcus aureus was also higher at 6 months (61% vs 23%; P <.05), whereas the counts of Bacteroides were lower at 12 months (9.9 vs 10.6 log(10); P <.05). CONCLUSION: Differences in the composition of the gut flora between infants who will and infants who will not develop allergy are demonstrable before the development of any clinical manifestations of atopy. Because the observations were made in 2 countries with different standards of living, we believe that our findings could indicate a role for the intestinal microflora in the development of and protection from allergy.

Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age.
Owby DR, Johnson CC, Peterson EL.

CONTEXT: Childhood asthma is strongly associated with allergic sensitization. Studies have suggested that animal exposure during infancy reduces subsequent allergic sensitization. OBJECTIVE: To evaluate the relationship between dog and cat exposure in the first year of life and allergic sensitization at 6 to 7 years of age. DESIGN, SETTING, AND SUBJECTS: Prospective birth cohort study of healthy, full-term infants enrolled in a health maintenance organization in suburban Detroit, Mich, who were born between April 15, 1987, and August 31, 1989, and followed up yearly to a mean age of 6.7 years. Of 835 children initially in the study at birth, 474 (57%) completed follow-up evaluations at age 6 to 7 years. MAIN OUTCOME MEASURES: Atopy, defined as any skin prick test positivity to 6 common aeroallergens (dust mites [Dermatophagoides farinae, D pteronyssinus], dog, cat, short ragweed [Ambrosia artemisiifolia], and blue grass [Poa pratensis]); seroatopy, defined as any positive allergen-specific IgE test result for the same 6 allergens or for Alternaria species. RESULTS: The prevalence of any skin prick test positivity (atopy) at age 6 to 7 years was 33.6% with no dog or cat exposure in the first year of life, 34.3% with exposure to 1 dog or cat, and 15.4% with exposure to 2 or more dogs or cats (P =.005). The prevalence of any positive allergen-specific IgE test result (seroatopy) was 38.5% with no dog or cat exposure, 41.2% with exposure to 1 dog or cat, and 17.9% with exposure to 2 or more dogs or cats (P =.003). After adjustment for cord serum IgE concentration, sex, older siblings, parental smoking, parental asthma, bedroom dust mite allergen levels at 2 years, and current dog and cat ownership, exposure to 2 or more dogs or cats in the first year of life was associated with a significantly lower risk of atopy (adjusted odds ratio, 0.23; 95% confidence interval, 0.09-0.60) and seroatopy (adjusted odds ratio, 0.33; 95% confidence interval, 0.13-0.83). CONCLUSION: Exposure to 2 or more dogs or cats in the first year of life may reduce subsequent risk of allergic sensitization to multiple allergens during childhood.

Immune dysregulation in atopic dermatitis.
Sinke JD, Rutten VP, Willemse T.

Atopic dermatitis (AD) is a chronic inflammatory skin disease in humans and dogs with comparable clinical features. Comparative studies of immunological events in the pathogenesis of AD may contribute to understanding of the disease in dogs and to development and evaluation of immunomodulatory strategies of relevance to both species. Both allergen-specific as well as non-specific mechanisms contribute to the disease development. AD skin lesions are proposed to be initiated by activation of allergen-specific Th2-type cells, potentially influenced by local cutaneous factors. In the chronic stage of skin lesions reactivity may change into a Th1-type, e.g. driven by eosinophil derived IL-12. Analyses of these processes in course of time were performed in both spontaneous as well as in experimentally induced lesions (i.e. atopy patch test (APT) lesions). In the present paper, the immunological events as reported for human and canine AD are summarized and compared.

Th1/Th2 cytokine imbalance in a family with hyper-IgE syndrome.

Netea MG, Schneeberger PM, de Vries E, Kullberg BJ, van der Meer JW, Koolen MI.

BACKGROUND: Hyperimmunoglobulin E (hyper-IgE) syndrome is a rare immunodeficiency characterised by recurrent skin and respiratory tract infections, skeletal and dental abnormalities, chronic eczema, and elevated serum IgE. We describe a family with four hyper-IgE syndrome patients (38, 37, 30 and 7 years old), in which we investigated the cytokine response to both specific and non-specific stimulation. **METHODS:** Whole blood from patients and volunteers was stimulated for either 24 or 48h at 37 degrees C with heat-killed *Staphylococcus*, *C. albicans* or a combination of IL-12 and IL-18. Cytokine concentrations in the plasma were measured by specific radioimmuno-assays or ELISA. **RESULTS:** Serum IgE ranged from 5,000 to 16,670 IU/ml, and neutrophil chemotaxis was normal in all four patients. Tumour necrosis factor, interleukin (IL)-1beta, IL-6 and IL-8 production after stimulation of whole-blood cultures with lipopolysaccharide or heat-killed *S. aureus* did not differ between the adult patients and four healthy controls. In contrast, when blood from patients and controls was stimulated with heat-killed *S. aureus* or *C. albicans*, a severe imbalance towards a Th2 phenotype was found, with 10- to 30-fold reduction in the IFNgamma/IL-10 ratios in the hyper-IgE syndrome patients. The IFNgamma production in the patients was less severely impaired when blood was non-specifically stimulated with a combination of IL-18 and IL-12. **CONCLUSION:** In this family with hyper-IgE syndrome, the imbalance in the Th1/Th2 cytokine production may have been involved in the pathogenesis of the recurrent infections and/or chronic eczema characteristic of this disease.

Coincidence of immune-mediated diseases driven by Th1 and Th2 subsets suggests a common aetiology. A population-based study using computerized general practice data.

Simpson CR, Anderson WJ, Helms PJ, Taylor MW, Watson L, Prescott GJ, Godden DJ, Barker RN.

BACKGROUND: The recent rise in the prevalence of immune-mediated diseases has been attributed to environmental factors such as a lack of microbial challenge, or dietary change, that deviate the overall balance between mutually antagonistic subsets of T helper (Th) cells. **OBJECTIVE:** An alternative proposal is that recent environmental changes have resulted in an immune system that is more likely to produce both Th1 and Th2 responses against benign antigens. The prediction of this hypothesis, that Th1 and Th2-mediated diseases are not mutually exclusive, and may be positively associated, is tested here in a whole population. **METHODS:** Data from General Practices participating in the Scottish Continuous Morbidity Recording (CMR) project were used to determine the coincidence of the major Th2-mediated atopic diseases; asthma, eczema and allergic rhinitis, with the Th1-mediated autoimmune conditions; type I diabetes, rheumatoid arthritis and psoriasis. We also identified the prescription rates of inhaled therapy for asthma in patients with Th1-mediated disease. **RESULTS:** There was a significant increase in the risk of presenting with a Th1-mediated autoimmune condition in patients with a history of allergic disease (standardized prevalence ratio (95% confidence interval) 1.28 (1.18-1.37)). Likewise, the standardized prevalence ratios of presenting with either eczema (1.67 (1.48-1.87)) or allergic rhinitis (1.22 (1.02-1.44)) were significantly increased in subjects with a history of Th1-mediated disease. There was a particularly strong association between current psoriasis and current eczema (standardized prevalence ratio of psoriasis in subjects with eczema 2.88, 95% confidence interval (CI) 2.38-3.45). There was also a significant increase in prescriptions for inhaled asthma therapy in patients with Th1 disease. **CONCLUSION:** It is concluded that Th1- and Th2-mediated diseases are significantly associated in a large General Practice population. This finding supports the proposal that autoimmune and atopic diseases share risk factors that increase the propensity of the immune system to generate both Th1- and Th2-mediated inappropriate responses to non-pathological antigens.

Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations.

Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH.

Sib-pair analysis of 170 individuals from 11 Amish families revealed evidence for linkage of five markers in chromosome 5q31.1 with a gene controlling total serum immunoglobulin E (IgE) concentration. No linkage was found between these markers and specific IgE antibody concentrations. Analysis of total IgE within a subset of 128 IgE antibody-negative sib pairs confirmed evidence for linkage to 5q31.1, especially to the interleukin-4 gene (IL4). A combination of segregation and maximum likelihood analyses provided further evidence for this linkage. These analyses suggest that IL4 or a nearby gene in 5q31.1 regulates IgE production in a nonantigen-specific (noncognate)

fashion.

Long term maintenance of IgE-mediated memory in mast cells in the absence of detectable serum IgE.

Kubo S, Nakayama T, Matsuoka K, Yonekawa H, Karasuyama H.

Mast cells and basophils involved in allergic responses do not have clonotypic Ag receptors. However, they can acquire Ag specificity through binding of Ag-specific IgE to FcepsilonRI expressed on their surface. Previous studies demonstrated that IgE binding induced the stabilization and accumulation of FcepsilonRI on the cell surface and resulted in up-regulation of FcepsilonRI. In this study we have further analyzed the maintenance of IgE-mediated memory in mast cells and basophils in vivo by comparing kinetics of serum IgE levels, FcepsilonRI expression, and ability to induce systemic anaphylaxis. A single i.v. injection of trinitrophenyl-specific IgE induced 8-fold up-regulation of FcepsilonRI expression on peritoneal mast cells in B cell-deficient (micro m(-/-)) mice. Serum IgE levels became undetectable by day 6, but the treatment of mice with anti-IgE mAb induced a significant drop in body temperature on days 14, 28, and 42. The administration of trinitrophenyl -BSA, but not BSA, in place of anti-IgE mAb gave similar results, indicating the Ag specificity of the allergic response. This long term maintenance of Ag-specific reactivity in the allergic response was also observed in normal mice passively sensitized with IgE even though the duration was shorter than that in B cell-deficient mice. The appearance of IgE with a different specificity did not interfere with the maintenance of IgE-mediated memory of mast cells and basophils. These results suggest that IgE-mediated stabilization and up-regulation of FcepsilonRI enables mast cells and basophils not only to acquire Ag specificity, but also to maintain memory in vivo for lengthy periods of time.

Tacrolimus ointment: a new therapy for atopic dermatitis--review of the literature.

Pustisek N, Lipozencic J, Ljubojevic S.

Atopic dermatitis is a chronic inflammatory skin disease characterized by severe pruritus, typical morphology and distribution of skin lesions, and personal and family history of atopy. The management of atopic dermatitis is directed at preventing the inflammation, itch, and secondary lesions. Therapy relies on general management measures, anti-inflammatory agents, antipruritics, antibiotics, and immunosuppressants. Treatment options for patients with severe or longstanding disease, extensive body surface area involvement of facial lesions are limited. Tacrolimus ointment is the first in the class of topical immunomodulators that has been formulated for the treatment of atopic dermatitis in children (2 to 15 years of age) and adult patients. The mechanism of action of tacrolimus in atopic dermatitis seems to involve T-cells, Langerhans cells, mast cells and basophiles. Experimental evidence suggests that tacrolimus inhibits T-lymphocytes activation by binding to an intracellular protein, FKBP-12. This binding phenomenon inhibits the ability of calcineurin to activate the promoter region of the gene for IL-2, IL-3, IL-4, IL-5, interferon gamma, tumor necrosis factor alpha, and granulocyte macrophage colony-stimulating factor, all of which participate in the early immune response and play a role in the pathogenesis of atopic dermatitis. Tacrolimus ointment is not atrophogenic, and is associated with minimal systemic absorption. There were no consistent changes in any laboratory variable during topical tacrolimus therapy. The most common adverse events associated with its use were transient skin burning and pruritus at the site of application. Tacrolimus ointment is safe and efficacious therapy for the treatment of pediatric and adult patients with atopic dermatitis on all skin regions including the face, neck and intertriginous areas. An overview is given of tacrolimus in atopic dermatitis.

Comparison of response to immunotherapy by intradermal skin test and antigen-specific IgE in canine atopy.

Park S, Ohya F, Yamashita K, Nishifuji K, Iwasaki T.

The intradermal skin test (IDST) and serologic allergy test (SAT) has been developed for confirming a diagnosis of canine atopy and determining allergens for immunotherapy. To determine the prevalence of causative allergens for canine atopic dermatitis in Japan, IDST and SAT were performed with the CMG Immunodot strips on 95 atopic dogs using 9 allergens. In addition, we compared agreement rate, sensitivity and specificity between them (using IDST as the standard). The allergen most commonly positive in both tests was house dust mites (IDST: 69.5%, SAT: 48.4%). Moreover, Japanese cedar, mugwort and grass mix were detected as attendant causative allergens. Agreement rates between the two tests ranged from 67.4% to 96.8%; the overall mean agreement rate were 81%. SAT was shown to have sensitivity to IDST ranging from 16.7 to 68.2%. The specificities were very high for all allergens, on the order of 94.9-100% (median=98.7%). Finally, the efficacy of immunotherapy was evaluated on 27 atopic dogs based on IDST (15 dogs) and SAT (12 dogs) results. Overall, 60% (9/15) of the IDST group and 66.8%

(8/12) of the SAT group experienced a 50% to 100% reduction in their symptomatology. No significant differences were found in response to immunotherapy during the follow-up period between allergen selection methods. These results indicate the value of serologic tests as an aid to identifying an allergen solution for immunotherapy.

Warming of feet elevates nasal mucosal surface temperature and reduces the early response to nasal challenge with allergen.

Assanasen P, Baroody FM, Naureckas E, Naclerio RM.

BACKGROUND: We have previously shown that hot, humid air partially reduces the early allergic response.

Mechanisms for this effect have been suggested, but none has gained universal acceptance. The most likely explanations are a modification of mucosal temperature or a reduction in nasal secretion osmolality. **OBJECTIVE:**

We sought to investigate whether increasing the nasal mucosal surface temperature by immersing feet in warm water (WW) could decrease the immediate nasal response to challenge with allergen. **METHODS:** We performed a randomized, 2-way crossover study on 14 subjects with seasonal allergic rhinitis outside of their allergy season. They immersed their feet in either WW (42 degrees C) or room-temperature water (RW; 30 degrees C) for 5 minutes before and during nasal challenge with diluent for the allergen extract, followed by 2 increasing doses of allergen.

RESULTS: There was a statistically significant increase in nasal mucosal temperature from baseline after warming of feet (WW, 1.9 +/- 0.1 degrees C, vs RW, 0.2 +/- 0.1 degrees C; P = .001), but there were no significant differences in body temperature (WW, 0.1 +/- 0.1 degrees C, vs RW, 0.4 +/- 0.1 degrees C; P = .1). Net changes from diluent challenge for all parameters were compared between immersion of feet in WW and RW. Immersion of feet in WW significantly inhibited allergen-induced sneezes (WW, 5.7 +/- 1.1, vs RW, 11.6 +/- 3.2; P < .01), human serum albumin levels (WW, 941.7 +/- 172.2 microg/mL vs RW, 1524.8 +/- 220.6 microg/mL; P < .01), and secretion weights (WW, 30.5 +/- 7.2 mg, vs RW, 41.8 +/- 6.8 mg; P < .01). **CONCLUSION:** Our data show that warming of feet decreases the early response to nasal challenge with antigen. This inhibitory effect is probably related to the increase in the nasal mucosal temperature.