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EFFECTS OF DEXAMETHASONE ON A PEPTIDE BASED IMMUNOTHERAPY REGIME FOR TREATING AUTOIMMUNE DISEASE

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Based on successful treatment of experimental models of autoimmunity with soluble synthetic peptides representing key target epitopes in the affected tissue, human clinical trials in Type 1 diabetes and multiple sclerosis (MS) are imminent. Disease remission relies on antigen-specific in-vivo induction of regulatory T-lymphocytes (T-reg), capable of antigen non-specific suppression in the target tissue via production of IL-10. T-reg generated in vitro by exposure of naïve T-cells to a combination of dexamethasone (dex) and vitamin D have also been used in vivo. We hypothesized that in vivo dex treatment during peptide therapy might facilitate tolerance induction.

In a model for MS, the Tg4 transgenic mouse, >90% of T-cells recognise the N-terminal epitope of myelin basic protein (MBP Ac1-9, [4K]). Previous investigators established that ten intranasal (IN) treatments with 4Y, a modified synthetic analogue of 4K, creates a T-reg phenotype in the T-cells: they are tolerant, no longer proliferating in-vivo or in vitro in response to stimulation with 4K (assayed by CFSE dye intensity on flow cytometry or by H^3-thymidine incorporation respectively). They also suppress proliferation of naïve cells in both settings. Comparing IN tolerised mice with naïve mice, serum IL-10 is higher and serum IL-2 is lower following IN 4Y. Transfer of T-reg from tolerant mice to naïve mice followed by IN 4Y results in suppression of serum IL-2. We used these same readout systems, but evaluated Tg4 mice after partial tolerance induction (4 IN 4Y treatments) with additional groups receiving high or low dose intraperitoneal dex (1mg/kg or 0.01mg/kg) 2 hours before each 4Y treatment. Increases in serum IL-10 and IL-2 following 4 IN 4Y treatments were completely or partially ablated by high and low dose dex respectively. When CFSE-labelled naïve T-cells were transferred into mice that had been treated with 4 x IN 4Y +/- dex, antigen induced proliferation of naïve cells was greater in the mice exposed to high dose dex and lower in the low dose dex group, compared with group treated with 4Y only. Transfer of T-cells from mice treated with 4 x 4Y +/- dex into naïve mice followed by antigenic challenge, showed that cells treated with 4Y + low dose dex markedly suppress serum IL-2 and IL-10 in recipient mice compared with cells treated with 4Y alone. High dose dex had a less profound effect.

These results suggest that high dose dex may be deleterious to peptide based immunotherapy, but that low dose dex may enhance tolerance with respect to the ability of cells exposed to dex and peptide to suppress T-cell proliferation and IL-2 production in response to antigen. This effect is not mediated by enhanced IL-10 production.

AN IMMUNOHISTOCHEMICAL STUDY OF EOSINOPHILIC BRONCHOPNEUMOPATHY IN DOGS

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Canine eosinophilic bronchopneumopathy (EBP) is a disease characterised by eosinophilic inflammation of the bronchi and lungs. To date, the only effective therapy is the long-term use of glucocorticoids. In order to develop new therapeutic strategies, the immunopathogenesis of the disease must be elucidated. In this study, we have used immunohistochemistry to characterise the leucocyte populations in bronchial tissue from dogs suffering from EBP.

Perendoscopic bronchial biopsies were taken from 11 dogs with EBP. Tissues were fixed in 10% neutral-buffered formalin. Primary antibodies used were directed against MHC class II (HLA-DR), myelomonocytic antigen L1, IgA, IgG and IgM.

Intraepithelial MHC class II$^+$ cells with a dendritic morphology were found in low numbers. In the lamina propria (LP), MHC class II$^+$ cells were found in moderate numbers, mostly immediately beneath the epithelium, occasionally forming large clusters. Those cells were morphologically either dendritic-like cells or macrophages, but many macrophages were MHC class II$^-$. MHC class II antigens were expressed by few fibroblasts and eosinophils but not by epithelial cells. L1$^+$ cells were a relatively small component of the LP inflammatory response. L1 positive cells were morphologically either macrophages or polymorphonuclear cells likely to be eosinophils based on comparison with serial H&E stained sections. L1$^+$ polymorphonuclear cells were found mainly within blood vessels but were also present in the LP or epithelium. IgA$^+$ plasma cells were found in varying numbers in the LP, mostly in association with glandular tissue. IgG$^+$ cells were relatively fewer, and IgM$^+$ plasma cells were present in very low numbers.

In general, fewer inflammatory cells were labelled with these markers than the total inflammatory population within the LP of the biopsies. This was especially the case for antigen-presenting cells (APC) represented by dendritic-like cells and macrophages. The lack of L1 labelling suggests that these APC were not recent tissue immigrants, but the absence of uniform MHC class II labelling suggests low level of activation of these cells. This would be consistent with a Th2 dominated immunological milieu within the LP. By contrast, the expression of MHC class II by some eosinophils and fibroblasts suggests that these cells might act as non-professional APC in the canine respiratory mucosa when eosinophilic inflammation is present. Although plasma cells were generally increased in number within the LP of dogs with EBP compared to controls, the relative proportions of IgA, IgG and IgM cells were similar.

Further studies examining T lymphocyte subpopulations and cytokine expression are required to more fully elucidate the immunopathogenesis of EBP.

CANINE EHRLICHIOSIS: A RETROSPECTIVE STUDY OF CONCURRENCE WITH OTHER DISEASES

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Canine ehrlichiosis is a tick-borne disease caused by several species of the genus *Ehrlichia*. In the literature, there has been described with some frequency the concurrence of ehrlichiosis with many other illnesses, specially infectious and parasitic diseases. The objective of this retrospective study is to determine in a population of dogs infected by *Ehrlichia spp.*, which the more frequent agents that coinfect these dogs are. Besides that, this is also an attempt to evaluate if the existence of any concurrence in dogs suffering from canine ehrlichiosis affects the clinical response of these animals.

A total of 421 cases of canine ehrlichiosis attended from 1992 to 2003 in the Veterinary Clinic Hospital of Madrid (Spain) were included in this study. In these animals, the existence of concurrences with other infectious and parasitic agents was analysed. The possible association between this factor and the response after a specific treatment against ehrlichiosis was analysed using the Chi-square test.

No concurrence was found in 295 of these animals (70.07 %). The other 126 dogs (29.93%) showed simultaneous infection by *Ehrlichia spp* and other agents. In the studied population, the concurrence most frequently detected was canine ehrlichiosis and leishmaniasis, exactly in 87 animals (69.04 % of the animals with any concurrence). 4 of these animals, in addition to leishmaniasis and ehrlichiosis, presented multiple coinfections (one with filariasis, other with distemper, the other one with *Hepatozoon canis* infection and the last one parasitized by *Trichuris vulpis*).

Concurrences with other tick-borne diseases were also found. Exactly, 12 cases showed *Anaplasma platys* coinfection (one of them also suffering babesiosis), 10 animals presented hepatozoonosis, and 6, babesiosis.

Anecdotal concurrences with different diseases (distemper, different intestinal parasitosis, demodicosis, filariasis and aspergillosis) were also detected.

The dogs received treatment against all the diagnosed diseases. Regarding to the progress of dogs with ehrlichiosis, the existence of concurrences in the studied population was not statistically associated with a non-favourable evolution.

INDIRECT FLUORESCENT ANTIBODY TEST IN CANINE EHRLICHIOSIS: A COMPARATIVE STUDY USING DIFFERENT STRAINS OF *Ehrlichia canis* AS ANTIGEN

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Indirect fluorescent antibody test (IFAT) is considered the gold standard technique in the diagnosis of canine ehrlichiosis. Antigen used to develop the technique is derived from cell cultures infected by *Ehrlichia canis*. Antigenic differences between strains from different geographic origins have been described and the greater specificity of local isolates has also been suggested. However, the information available regarding to these differences is limited. *Ehrlichia canis* has been recently isolated by the first time in Spain. The goal of this study is to compare results obtained by IFAT in canine serum samples from Spain using three different antigens: 1) an autochthonous Spanish strain of *E. canis* cultured in the cell line DH82, 2) the cell line DH82 ECOK (infected by *E.canis*, Oklahoma strain) and 3) a commercial antigen (Aehrag ©, Synbiotics Europe).

149 serum samples submitted for diagnosis of canine ehrlichiosis were initially analysed using the Spanish strain: 60 of them were negative (antibody titer < 1:40) and 89 presented different antibody titers (≥1:40). Afterwards, the samples were also analysed using the other 2 antigens.

Correlations of the results obtained with the 3 strains were statistically significant ($p < 0.001$). Spearman's coefficients of rank correlation were: $r = 0.922$ (Spanish strain versus Oklahoma strain), $r = 0.978$ (Spanish strain versus commercial antigen), and $r = 0.932$ (Oklahoma strain versus commercial antigen). However, when comparing antibody titers results using the paired t-student test, differences between the local strain and Oklahoma strain, and between the local strain and the commercial antigen were statistically significant ($p<0.001$). On the other hand, no differences were found when comparing the Oklahoma strain to the commercial antigen.

In detail, the differences detected among antigens were in most cases subtle (one or two dilutions). All the samples negative to the local strain were also negative to the other two antigens. In 59 of the 89 positive sera, antibody titer was higher with the local strain than with the other 2 antigens. Differences in titer greater than three-fold were only found in 2 samples. The results obtained in this study suggest a higher sensitivity of the technique when using a local isolate as antigen.