

Characterization of equine CD4⁺CD25⁺ lymphocytes expressing Treg transcription factor FoxP3 and their role in insect bite hypersensitivity

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Equine insect bite hypersensitivity (IBH) is a recurrent seasonal IgE-mediated dermatitis caused by bites of *Culicoides spp.* We have previously shown that PBMC from healthy horses exposed to *Culicoides* bites produce higher levels of IL-10 and TGF- β 1 than those from IBH-affected horses. In humans, regulatory T cells (Treg) are an important source of IL-10 and TGF- β 1 which control allergy. The aim of this study is to examine the existence of horse Treg cells and to investigate if there are differences in the proportion and function of Treg cells between healthy and IBH-affected horses.

Freshly isolated PBMC from healthy horses were first examined for FoxP3 expression using flow cytometry. FoxP3 expressing cells were predominantly detected in CD4⁺CD25^{high} and in small proportion of CD4⁺CD25^{dim} lymphocytes. In contrast, CD4⁺CD25⁻ lymphocytes showed marginal expression of FoxP3. Proliferation of CD4⁺CD25⁻ sorted cells stimulated with irradiated allogenic PBMC was significantly suppressed in a dose dependent manner by CD4⁺CD25⁺ sorted cells.

Mechanism of suppression by CD4⁺CD25⁺ was also investigated.

The proportion of FoxP3⁺CD4⁺CD25⁺ cells was then determined in PBMC from IBH-affected and healthy Icelandic horses. No difference was detected between healthy and IBH-affected horses in freshly isolated PBMC. In contrast, upon stimulation of PBMC with *Culicoides* extract, expression of FoxP3 by CD4⁺CD25^{high} as well as CD4⁺CD25^{dim} cells was significantly higher in healthy compared to IBH horses. In a pilot experiment we have examined whether regulatory function of CD4⁺CD25⁺ in IBH horses are impaired. Preliminary data show that CD4⁺CD25^{high} of some IBH horses were not able to suppress proliferation of CD4⁺CD25⁻ cells following stimulation with *Culicoides* extract.

In conclusion, equine FoxP3 is constitutively expressed by a relatively small proportion of CD4⁺CD25⁺ lymphocytes and these are suppressor cells, likely resembling nTreg. These results also show that there is no difference in the proportion of nTreg cells between healthy and allergic horses. In contrast, healthy horses induce more Treg cells than IBH-affected horses upon stimulation by the allergen and the suppressive function of these Treg cells may be impaired in IBH-affected animals.