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MHC class II expression by follicular keratinocytes in canine demodicosis—An immunohistochemical study

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Abstract

MHC class II proteins present fragments of extra cellular antigen to stimulate $CD4^+$ T lymphocytes. Aim of this study was the detection of MHC class II antigens on different cutaneous cells in canine demodicosis. Histopathological and immunohistochemical examination of skin biopsies from 44 dogs with demodicosis is reported. The control group consisted of skin biopsies taken from 10 necropsied dogs without obvious skin lesions.

The immunohistological assessment of the MHC class II expression revealed MHC class II proteins on different cell types of infiltrating inflammatory cells, i.e. APCs (antigen-presenting cells), macrophages, T lymphocytes and B lymphocytes. The plasma cells, however, only showed expression in 32 (73%) of 44 cases. Generally it was noticeable that most plasma cells but never all of them expressed MHC class II. Neutrophils, mast cells and eosinophils were MHC class II negative. Furthermore, in 39 biopsies (89%) from dogs with demodicosis MHC class II positive follicular keratinocytes were found. The control group did not show MHC class II expression on epithelial cells. Concerning the endothelial cells, a total of 25 biopsies (57%) showed MHC class II expression in which different vascular plexuses were affected by staining.

This examination shows that MHC class II expression in the skin of dogs suffering form demodicosis is elevated. Especially the MHC class II expression by follicular keratinocytes seems to be conspicuous. We hypothesize that this is in association with the development and the maintenance of follicular inflammation.

Keywords: Demodicosis; MHC II; Canine; Immunohistochemistry; Keratinocyte; Skin

1. Introduction

Demodicosis is a well-known parasitic skin disease characterized by the presence of larger than normal numbers of *Demodex* mites in the skin (Scott et al., 2001a). The mites detected in the skin are mostly of the *Demodex canis* type. Furthermore, the existence of a smaller mite form, whose habitat might be the superficial layers of the epidermis, has been documented (Chesney, 1999; Saridomichelakis et al., 1999). Desch and Hiller recently described a longer-bodied mite, called *Demodex injai* (Desch and Hillier, 2003; Hillier and Desch, 2002).

Canine demodicosis can be subdivided into the juvenile-onset and adult-onset forms, and both forms can occur in localized or generalized distribution. Histopathological changes in skin biopsies with demodicosis are characterized independently of the clinical picture by three main patterns that can occur in parallel or individually. These patterns are furunculosis, nodular

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dermatitis and mural folliculitis (Caswell et al., 1995). In addition, the occurrence of luminal folliculitis has been described (Scott et al., 2001a). Furunculosis is an inflammation of the hair follicle in which the follicle is destroyed. This can be recognized by the severe inflammatory reaction to expose keratin and hair as well as to extra follicular mites. In contrast, nodular dermatitis shows perifollicular granulomas in the dermis containing mites but no hair fragments (Caswell et al., 1995).

Mural folliculitis is characterized by lymphocytic infiltration of the follicular wall at the level of the infundibulum and isthmus. The inflammatory infiltrates can obscure the interface between follicle and dermis, which is called interface mural folliculitis. In such cases, the follicular keratinocytes of the outer root sheath especially show additional changes such as apoptosis and vacuolar degeneration (Caswell et al., 1995; Gross et al., 2005) that are also characteristic for interface dermatitis (Yager and Wilckock, 1994).

The findings of Caswell et al. (1997) and Day (1997) showed that the lymphocytes infiltrating the follicular wall were $CD8^+$ T lymphocytes, i.e. a cell-mediated immune response. In contrast, the perifollicular dermis contained approximately equal numbers of $CD8^+$ and $CD4^+$ T cells (Caswell et al., 1997; Day, 1997). The perifollicular infiltrates in cases with furunculosis were dominated by IgG-producing plasma cells (Day, 1997; Caswell et al., 1995). Hence, these authors suspected that in canine demodicosis a shift takes place from an early cell-mediated response toward a later humoral response.

MHC class II molecules play a crucial role in the mediation of an immune response. In healthy dogs, MHC class II proteins are present on constitutive APCs, i.e. on macrophages, dendritic cells and lymphocytes (Cobbold and Metcalfe, 1994; Doveren et al., 1985, 1986). They are inconsistently found on different epithelial and mesenchymal cells (Rabanal et al., 1995; Vilafranca et al., 1995). Their function is to present fragments of extra cellular antigen to $CD4^+$ T lymphocytes. After stimulation by foreign antigen, $CD4^+$ T cells secrete cytokines to direct and augment immune and inflammatory reactions. The expression of MHC II antigens can be increased via cytokines, which act through activation of transcription factors that in turn react with the class II transcription activator (CIITA) and bind to the class II promoter as a complex. CIITA can be increasingly generated under the influence of interferon γ (IFNγ) (Steimle et al., 1994) which is produced by $CD4^+$ and $CD8^+$ T lymphocytes as a result of the antigen contact.

In both human medicine (Auböck et al., 1986; Barker et al., 1988; Drijkoningen et al., 1988; Smolle, 1985) and veterinary medicine (Day, 1996; Carter et al., 2005; Fondevila et al., 1997; Papadogiannakis et al., 2005; Affolter et al., 1998; Huisinga and Reinacher, 2005 (preliminary data)) the presence of MHC class II proteins on keratinocytes has been described in conjunction with different dermatitides. These dermatitides are almost always inflammations accompanied by a lymphocytic infiltration of the epidermis. The MHC class II expression of keratinocytes is explained by infiltrating lymphocytes releasing IFNγ (Scott et al., 2001b).

Since epidermal keratinocytes seem to express MHC class II molecules in conjunction with lymphocytic infiltrates of the epidermis, we hypothesized that MHC class II antigens might be expressed by follicular keratinocytes due to the interface mural folliculitis and might be of special importance in canine demodicosis. The objective of this study was to examine the distribution of MHC class II proteins on epidermal and follicular keratinocytes as well as on infiltrating inflammatory cells and on endothelial cells.

2. Materials and methods

2.1. Tissue samples

The examination was performed on 44 skin biopsies of dogs that underwent routine diagnostics at the Institute of Veterinary Pathology of the Justus-Liebig-University of Giessen between 2003 and 2006. Animals were 6 months to 12 years of age (Table 1). The control group consisted of skin samples taken from 10 necropsied dogs from the Institute that did not show any macroscopic or microscopic pathological changes in the skin.

Demodicosis was diagnosed histologically in all 44 cases due to mite sections and the outlined inflammatory changes.

All tissue samples were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin (H E) as well as the periodic acid–Schiff (PAS) method for histopathological examination.

2.2. Immunohistochemical techniques

To perform immunohistochemical examination, sections were mounted on SuperFrost® Plus slides (Menzel Gläser, Braunschweig) and dried at room temperature.

After deparaffinization in xylene substitute (Roti-Histol®; Carl Roth GmbH & Co KG Karlsruhe) and rehydration using a descending alcohol series, the