Localization of Cytokines in Tendinocytes of the Superficial Digital Flexor Tendon in the Horse

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ABSTRACT. Although inflammatory activation of cytokines have been analyzed in various tissues, there have only been a few and as-yet-inconclusive studies on cytokines in equine tendons. In this study, the localizations of 4 cytokines (IL-1α, IL-1β, TNFα and IFNγ) in tendinocytes of the equine superficial digital flexor tendon (SDFT) were analyzed by the use of an immunohistochemical method. In inflamed tendons positive staining for all 4 cytokines antibodies were detected in endotendinum cells and vascular epithelial cells. In contrast, negative or trace immunoreactions were obtained in many tendinocytes in the normal tendon. The variation in cellular immune responses depending on the kind of cytokine may reflect the physiological/pathological condition of the SDFT.

KEY WORDS: cytokine, superficial digital flexor tendonitis, tendinocyte.

NOTE

Anatomy

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Superficial digital flexor (SDF) tendonitis of the forelimb in horses is one of the most widespread and career-threatening injuries in racehorses such as Thoroughbred and Standard bred [5]. This injury usually takes long time for treatment and recovery, and frequently reoccurs after affected horses return to training or racing [5, 6, 8]. In most cases, this injury accompanies the formation of scars in tendons and very frail, and often reoccur at the scar. The mechanism of the occurrence of this injury has not yet been elucidated although a number of etiological factors have been implicated for the mechanism [13]. On the other hand, cytokines act in various ways in an inflammatory condition: either to activate or repress immune cells, cause the degeneration of tissues, promote and induce the cellular differentiation, and induce the secretion of cytokines by other cells [3, 14, 17, 18]. Moreover, some kinds of cytokines directly or indirectly induce the cell death, apoptosis or necrosis [7, 11]. Although inflammatory activations of cytokines have been investigated in various tissues, there have only been a few and as-yet-inconclusive studies on cytokines in equine tendons. The aim of the present study was therefore to determine the distribution of cytokines in tendinocytes of the equine SDF tendon (SDFT).

This study was carried out by the use of 4 normal SDFTs obtained from 3 male and 1 female Thoroughbred horses (aged 2–7 years) and 5 inflamed SDFTs obtained from 2 male and 3 female Thoroughbred horses (aged 1–6 years) housed at the Laboratory of Veterinary Pathology, Rakuno Gakuen University, Japan. All inflamed SDFTs formed scar lesions. After sedation with medetomidin hydrochloride injection (16 mg/kg i.m.) and induction of anesthesia with thiopental sodium (6 mg/kg i.v.) injection, tissue samples were removed, fixed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, embedded in Tissue-Tec O.C.T. Compound (Sakura Finetechnical, Tokyo, Japan), and rapidly frozen in liquid nitrogen. Frozen sections were cut at 10 µm and examined with the avidin-biotin complex method. After pretreatment with a normal rabbit serum, sections were incubated in the presence of 4 monoclonal antibodies (house-made: detailed description of the preparation of these antibodies to be presented in a subsequent paper): mouse anti-equine interleukin (IL)-1α (diluted 1:100 with PBS) IL-1β (1:40), tumor necrosis factor-α (TNFα) (1:100) and interferon-γ (IFNγ) (1:100). The antigen-antibody reactions were visualized by biotin-labeled rabbit anti-mouse IgG, IgA and IgM and peroxidase-streptavidin complex (Histofine; Nichirei, Tokyo, Japan). An enzyme reaction was developed with a mixture of diaminobenzidine (0.04%) and H2O2 (0.002%) in 0.05 M Tris-HCl buffer (pH 7.6). Sections were counterstained with methyl green, washed, dehydrated, mounted with Balsam Canada (Kanto Chemical, Tokyo, Japan), and then examined under a light microscope. Specificity of all antibodies was confirmed by the antigen-antibody adsorption test. To determine the frequencies of immunopositive cells, at least 4 sections were used for morphometrical analysis in each experiment. Immunopositive cells and tendinocytes were counted under a light microscope (× 200 magnification) in each microscopic area to calculate the cell densities. Cell density is expressed as percent of immunopositive cells (number of immunopositive cells/number of tendinocytes).

In the inflamed tendons, tendinocytes showed the immunopositive reactions to IL-1α IL-1β TNFα and IFNγ (Figs. 1A, B, C and D, respectively). Endotendinum cells and vascular epithelial cells also showed immunopositive reactions to all antibodies (IL-1β, Fig. 2). The percentages of immunopositive cells varied on the ground of cytokines; IFNγ (54%), TNFα (64%), IL-1α (74%) and IL-1β (84%) (Fig. 3). Immunopositive cells were distributed near the endotendinum with higher percentage, and tended to
decrease toward the inside of the tendon fascicle. Moreover, some of the positive tendinocytes showed a strong immunoreaction in the cytoplasm (Fig. 4A). In the normal tendon, in contrast, a large number of tendinocytes in the normal tendon showed negative or trace immunoreactions.

Fig. 1. Sections of inflamed tendons immunostained for IL-1α, IL-1β, TNFα and IFNγ (A, B, C and D, respectively). Immunoreactivity for these cytokines was found in tendinocytes (arrows). × 420.

Fig. 2. Immunoreactivity for IL-1β in endotendinieum (arrows) and vascular epithelial (asterisk and arrowheads) cells. × 160. The inset shows a magnification of the vascular epithelial cells indicated by the asterisk. × 360.

Fig. 3. Ratio of cytokine-immunopositive cells in inflammatory tendons. Each points represents the mean of 4 experiments. Vertical lines indicate the standard error.
In general, the tendon is a tissue composed of tendinocytes, extracellular matrices (collagen fibers, elastin, glycosaminoglycan, etc.), expiation blood vessels, and nerves [4, 12, 15, 16]. Tendinocytes are thought to differentiate from fibroblasts in the middle lamella [15]. It is well known that fibroblasts synthesize matrix components, secrete various kinds of cytokines and stimulate neighboring cells (paracrine) or by itself (autocrine) to enhance their function in the case of inflammation in the connective tissue [10, 19]. In the inflamed connective tissue, fibroblasts actively absorb and resolve the injured collagen fibers and synthesize and secrete fresh fibers and matrix components [2, 17]. In this study, many tendinocytes in the inflamed tendon showed cytokine-immunopositive reactions. These findings suggest that tendinocytes as well as fibroblasts actively secrete cytokines in the case of tendinitis. Thus, it is possible that tendinocytes function in immunoreactions of the tendon in the same way as fibroblasts do in the connective tissue. Accumulation of immunopositive tendinocytes near the region of the endotenon may be a sign of release of a cytokine-inducing substance from the endotenon toward the inside of the endotenon.

Recent studies have shown that the equine SDF tendonitis in the acute phase starts with the degeneration of microvascular hemorrhage in the endotendineum and that the region of degeneration is extended toward the endotenon in the next phase [1, 9]. The variation in the cellular immune response on the ground of cytokines may reflect the physiological/pathological conditions of the SDFT. To the best of our knowledge, this is the first report on the histological immunodetection of cytokines in tendinocytes in an equine tendon.

REFERENCES