

Immunoglobulin E-bearing cells and mast cells in skin biopsies of horses with urticaria

SILVIA RÜFENACHT*, ELIANE MARTI†, CLAUDIA VON TSCHARNER‡, MARCUS G. DOHERR§, URSULA FORSTER‡, MONIKA WELLE‡ and PETRA J. ROOSJE*

*Dermatology Unit, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland

†Division of Clinical Veterinary Immunology, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland

‡Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland

§Division of Clinical Research, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland

(Received 24 May 2004; accepted 10 September 2004)

Abstract The pathogenesis of equine urticaria is not well understood. In man, urticaria has been associated with immunological and nonimmunological mechanisms leading to the release of various mediators by mast cells. Skin biopsies of 32 horses with a history of urticaria were stained with toluidine blue, a double-labelling method for chymase and tryptase, and immunohistochemistry for immunoglobulin (Ig)E. These horses were compared with horses with pemphigus foliaceus, insect bite hypersensitivity and control horses with healthy skin. Neither formalin fixation time nor biopsy site influenced the staining methods. No chymase-positive cells were found. In all groups of horses, cells staining with toluidine blue and for tryptase and IgE were found in the epidermis and hair follicle papilla and significantly more positively staining cells were observed in the subepidermal dermis compared with the deep dermis. Horses with urticaria had significantly more IgE-bearing cells in the subepidermal dermis than control horses. However, horses with urticaria had significantly fewer toluidine-blue-stained mast cells in both subepidermal and deep dermis compared with the insect bite hypersensitivity and pemphigus foliaceus groups. This study suggests that IgE-mediated reactions play a role in the pathogenesis of urticaria.

INTRODUCTION

Urticaria (hives) is a common dermatological condition in horses. It occurs in horses of all ages, is often recurring and can be pruritic. Lesions appear suddenly, typically regressing within 24 h, but they can also be persistent.^{1,2} In horses, the differentiation between acute and chronic urticaria is not well defined. Chronic urticaria in humans is defined as recurrent urticaria occurring at least twice a week for 6 weeks.^{3,4}

Characteristic signs of urticaria in horses are acutely appearing wheals of variable diameter, distributed bilaterally symmetrically on the trunk, neck and proximal extremities. Less common signs are papules, and giant or exudative wheals or polycyclic (gyrate) forms.^{2,5} Most horses with urticaria respond favourably to glucocorticoids or antihistamines but if the underlying cause is not identified and eliminated, lesions often recur when therapy is stopped. As a result of the disease, pruritus and lesions that occur beneath the tack can make the horse unsuitable for working purposes.

Urticaria is considered to be a cutaneous reaction pattern for which there are multiple potential causes in

both humans^{3,6} and horses.^{7,8} In analogy to causes of urticaria induction in humans, hypersensitivity reactions towards aero-allergens, food or insect bites, contact allergens, irritants or drugs have been reported as possible underlying causes in horses.^{1,9–12} In addition, possible physical factors such as heat or cold, exercise or idiopathic causes have been described.⁵ The evidence, however, for a causal relationship between these factors and the occurrence of urticaria in horses is limited.

The pathophysiology of urticaria is poorly understood in both humans and horses.^{10,13} In man, urticaria has been associated with immunological and nonimmunological mechanisms leading to the release of various mediators by the major effector cell, the mast cell.^{10,13,14} However, basophils, mononuclear cells, platelets and endothelial cells have also been implicated.^{10,13–15}

The histopathology of equine urticaria is characterized by variable dermal oedema with or without a perivascular to diffuse infiltrate with eosinophils and lymphocytes.^{2,5} Equine mast cells are not easily seen without special stains. Although equine urticaria is often described as an example of a type I hypersensitivity reaction, the presence of mast cells has not been reported in histological sections from horses with the disease. Many horses with recurring urticaria undergo intra-dermal testing for the detection of immediate-type reactions but the sensitivity and specificity of this test are low.^{1,9,16} The distribution pattern and type of cellular

Correspondence: Silvia Rüfenacht, Dermatology Unit, Department of Clinical Veterinary Medicine, PO Box 8466, Länggassstrasse 128, 3001 Bern, Switzerland. E-mail: silvia.ruefenacht@itz.unibe.ch

infiltrate are similar to that observed in horses with insect bite hypersensitivity (IBH). In the latter, however, an immunoglobulin (Ig)E-mediated response has clearly been demonstrated *in situ* at the mRNA level and also at the protein level with immunohistochemistry.¹⁷

The aims of our study were, first, to investigate the occurrence of IgE-bearing cells and to characterize the mast cell infiltrate in lesional skin of horses with urticaria. Second, we aimed to compare the findings with those of horses with IBH and pemphigus foliaceus (PF) and horses without skin lesions. Finally, the immunohistochemistry was related to the clinical data.

MATERIALS AND METHODS

Case material

From biopsies sent for diagnostic purposes to the dermatopathology service of the Institute of Animal Pathology between 1991 and 2003, we selected 32 biopsies from 32 horses with a diagnosis of urticaria for examination. The horses ranged in age from 2 to 28 years. There were 16 mares, 10 geldings, 3 stallions and 3 of unknown gender. The horses were 17 European Warmblood, 2 Andalusians (Pura Raza Espanol) and 1 of each of the following breeds: Arabian, Akhal Teke, American Quarter Horse, Anglo-Arabian, Freiburger (small Swiss draft breed), Lusitano, Thoroughbred, 1 pony and 4 horses of unknown breed. The histopathological changes comprised a superficial to deep, perivascular to diffuse cellular infiltrate with eosinophils and mononuclear cells with variable dermal oedema (Fig. 1a).

Based on history, clinical signs and histopathological diagnosis, seven horses with PF (age 3–12 years, four mares, three geldings, various breeds) and eleven with IBH (age 1.5–11 years, three mares, seven geldings, one of unknown gender, various breeds) were selected for comparison.

Clinical data were obtained from the covering letter accompanying the biopsies and by mailing a questionnaire to the owners and veterinarians. Desired information included signalment, duration of disease, sites and characterization of cutaneous lesions, presence or absence of pruritus, and occurrence of lesions in relationship to season, exercise, sweating, drugs, feed and bedding.

Nine control horses had neither history nor clinical signs of skin disease. They ranged from in age 1 to 27 years with five mares and nine geldings of various breeds. Seven of the horses had acute abdominal crises, one had bacterial joint disease, and one had a congenital nasal anatomical abnormality. Six-millimetre punch biopsies were taken within 1 h of euthanasia from six different body sites: the lateral neck, lateral shoulder, lateral thorax, fetlock, and base of the mane and tail. To match the fixation times of the specimens of the skin disease groups, biopsies from four horses were fixed in 10% formalin for < 24 h and from five horses for > 48 h before paraffin embedding.

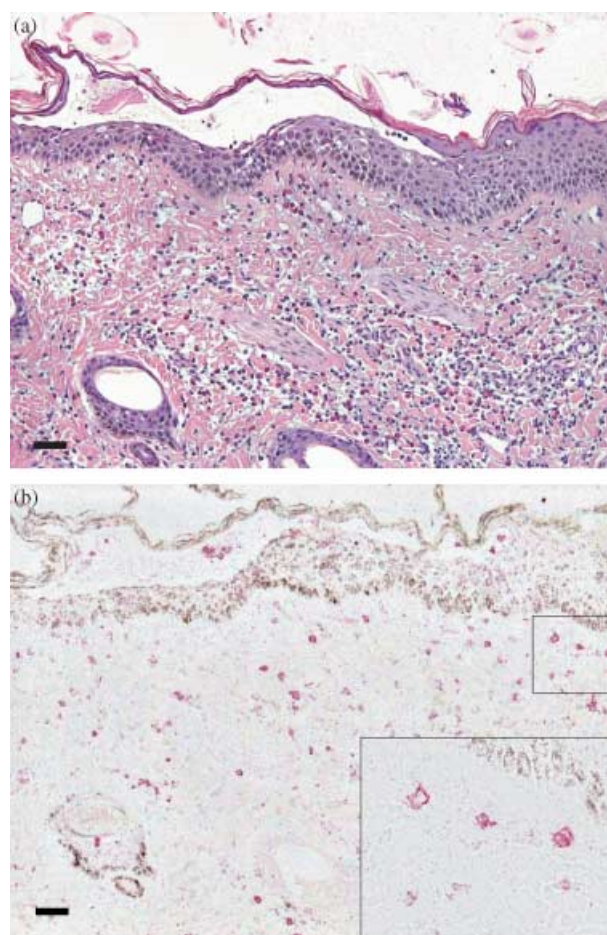


Figure 1. Photomicrograph of the skin from an urticaria-affected horse stained with H&E (a) and a polyclonal anti-horse IgE (b). Bar = 100 μ m.

Histopathology and immunohistochemistry

Serial sections were cut from all tissue samples. One section was stained with toluidine blue to detect mast cells. For IgE immunohistochemistry, slides were deparaffinized in xylene, fixed in ethanol and rehydrated in Tris-buffered saline (pH 7.6). Enzymatic digestion was performed with 0.2% trypsin. The nonspecific binding sites were blocked with 2% porcine skin gelatine. A polyclonal chicken antibody, specific for a recombinant equine IgE domain 3–4 fragment and reacting with native horse IgE¹⁸ was used as primary antibody. Staining was performed according to the method described by van der Haegen *et al.*¹⁷ As a secondary antibody an alkaline phosphatase-labelled goat anti-chicken IgG (Kirkegaard and Perry Laboratory Inc., MD, USA) was applied. Colour processing occurred with naphthol-AS-Mx-phosphate (Sigma Chemical Co., St. Louis, MO, USA) and Fast Red salt (Chroma-Gesellschaft, Schmid GmbH & Co, Köngen, Germany). Preimmune chicken IgY was used as negative control. Slides were mounted with Glycergel (Dako, Carpinteria, CA, USA).

A double-labelling procedure for the detection of chymase and tryptase was performed according to Welle *et al.*¹⁹ First, chymase detection was carried out with an enzyme–histochemical reaction for

naphthol-AS-D-chloroacetate, using a commercially available kit (Sigma). The use of Fast Blue BB salt (Sigma) instead of Fast Violet Luria–Bertani salt was our only modification to the company's protocol.¹⁹ Tryptase-positive mast cells were detected by immunohistochemistry with a rabbit anti-(human skin tryptase) (kindly provided by Prof Dr I. Harvima, Kupio, Finland).²⁰ A rabbit preimmune serum and an equine mast cell tumour served as the negative and positive control, respectively. Colour processing occurred with naphthol-AS-Mx-phosphate and Fast Red salt.

Morphometric analysis was performed under a standard light microscope at $\times 500$ magnification using a square eyepiece reticule (0.296×0.296 mm). For larger biopsies, two regions with typical histopathological changes were selected on haematoxylin and eosin (H&E)-stained sections and the same regions were assessed for the different stainings. The skin was subdivided into four different areas: epidermis, subepidermal layer, dermis and hair follicles. The entire surface of the epidermis and hair follicles were evaluated separately and classified as positive when one or more positive cells were detected. The subepidermal layer was defined as the dermal part just under the epidermis (0.296 mm depth, corresponding to one side of the square reticule) and was evaluated separately. Cells were counted in six adjacent square reticules. The deeper part of the dermis (named further dermis) was defined as a rectangular area of 6×5 adjacent square reticules located just under the subepidermal layer.

From each paraffin block (selected biopsy tissue), a series of adjacent sections was cut. On these serial sections, cells stained for IgE-protein, chymase and tryptase, and toluidine blue were counted in the same (corresponding) areas in order to allow direct comparison. Results were expressed as numbers of cells/mm².

Whenever multiple biopsies of one horse and anatomic region were available, all were initially evaluated (prepared, stained and counted). In total 45, 17, 11 and 54 biopsies of urticaria-, PF-, IBH-affected and control horses were processed. In order to avoid repeated (nonindependent) measures, those biopsy sections per horse and anatomic region that had the highest cell count per staining were selected and used for the comparative statistics between the different horse groups.

Statistical analysis

Data were stored in an MS EXCEL spreadsheet. Descriptive statistics (frequencies, measures of central tendency and spread) and graphs were generated for data exploration. For comparison of cell counts between biopsy sites within individual horses, nonparametric paired *t*-tests were used. Spearman's rank correlation coefficient was used to assess the correlation of different staining methods in control horses, and to describe the correlation between horse age and cell counts. Kruskal–Wallis analysis of variance (ANOVA) on Ranks with Bonferroni correction for multiple comparison was chosen to compare cell counts between the different groups of horses (disease groups, gender, etc.). Whenever

multiple nonindependent samples from individual horses were included into these comparisons, a repeated-measures ANOVA approach was chosen. The overall *P*-value for statistical significance was 0.05. All statistics were performed using the software package NCSS 2001 (www.ncss.com).

RESULTS

The skin lesions of urticarial horses were variable (Fig. 2; Table 1). Information about clinical signs could not be obtained from every horse but they often exhibited pruritus (12/26) and oozing (17/30). The neck (14/29) and trunk (14/29) were most commonly involved. Most horses (15/20) had recurring symptoms, often for more than 6 weeks (11/27). A seasonal influence was reported in 7/11 horses.

Horses with urticaria did not show any significant association between positively staining cells and pruritus or duration of clinical signs at the time of biopsy. There was no significant correlation between age and positive staining for IgE in control horses but with toluidine blue and tryptase staining, there was a tendency for the cell number to decrease with age. The only significant negative correlation ($r = -0.7$) was found with toluidine blue staining in the dermis. Gender did not influence cell counts within control and urticaria-affected horses.



Figure 2. Horse with chronic urticarial lesions on the cranial shoulder region and the lateral thorax. (a) Lateral aspect of the horse. (b) Detail of the lesion on the dorsolateral thorax with focal gyrate alopecia, scales and crusts.

Table 1. Clinical information for horses with urticaria

Sign	N	%	Duration of clinical signs	N	%	Localization of lesions	N	%	Cause/season of clinical signs	N	%
Wheals	25/30	83	< 1 week	7/27	26	Head	5/29	17		11/20	55
Oozing	17/30	57	1–6 weeks	9/27	33	Neck	14/29	48	Season	7/11	64
Papules	12/30	40	> 6 weeks	11/27	41	Shoulder	4/29	14	Winter	3/7	43
Crusts	10/30	33	recurring	15/20	75	Thorax	4/29	14	Spring + fall	2/7	29
Alopecia	5/30	17				Abdomen	3/29	10	Summer + fall	2/7	29
Hyperkeratosis, scales	4/30	13				Whole body	7/29	24	Food and bedding	6/11	56
Pruritus	12/26	46				Trunk	14/29	48	Stress	1/11	9
						Prox. extremities	3/29	10	Riding/sweating	1/11	9

N, number of horses with the symptom compared with the number of horses with obtained information.

Fixation times of < 24 h and > 48 h did not significantly affect the numbers of positively stained cells. There were no significant differences between sites within individual control horses. However, the fetlock IgE-staining tended to be higher in the dermis and fewer cells were seen with toluidine blue staining in the subepidermal layer (data not shown).

With all staining methods, the number of cells per mm² had a skewed (non-normal) distribution. In the horses with IBH, PF or urticaria, marked variations in cell counts were found in all skin biopsy sites and staining techniques between individual horses. In the control horse group, a high variation of cell counts was found, especially with the tryptase staining in the subepidermal layer.

Positively staining cells were mainly located in the subepidermal layer of the dermis. Tryptase-positive mast cells, but not chymase-positive mast cells, were detected in skin biopsies, although a control mast cell tumour stained for both tryptase and chymase. IgE-positive cells were found in a similar distribution pattern (Fig. 1b) with a few within the subcorneal vesicles in some urticaria-affected horses. Horses of all groups had cells in the epidermis and hair follicle papillae which were positive for IgE, toluidine blue and tryptase (20.3, 6.8 and 1.5%, respectively, for epidermis and (16.5, 11.5 and 9.8% for papillae) (Fig. 3). Serial sections indicated that the same cells stained for IgE-protein, tryptase and toluidine blue. IgE-positive cells were mainly located in the basal layer of the epidermis. Most staining was on the outer surface of the cells and most cells were round, occasionally with staining of possible dendrites. Mast cells identified by toluidine blue staining always stained positively for IgE but this should be confirmed using a double-labelling method.

The two dermal layers of control horses showed moderate to high ($r = 0.59$ – 0.70) correlation for numbers of positively stained cells by all methods. There was a moderate correlation of 0.69 between toluidine blue and tryptase staining in the dermis. All staining methods in all groups of horses demonstrated more positive cells in the subepidermal dermis than in the deep dermis (Fig. 4). Toluidine-blue-positive and tryptase-positive cells were not more common in the dermis of urticarial horses dermis than control horses. Horses with PF and IBH had more toluidine-blue-positive and tryptase-positive cells in the subepidermal

and deep dermis than urticaria-affected and control horses (Fig. 4a,b).

Horses with urticaria, IBH and PF all had significantly more IgE-bearing cells in the subepidermal dermis than the control group. Horses with IBH and PF also had significantly more IgE-bearing cells in the deep dermis (Fig. 4c).

DISCUSSION

Wheals, pruritus, oozing and recurrence of lesions were often described in these urticarial horses (83, 46, 57 and 75%, respectively). It is probable that biopsies are mainly taken from horses with lesions of longer duration or recurring lesions. This may skew the data towards chronic or recurring urticaria. It was often unclear whether lesions were new occurrences or older persistent lesions. Although many biopsies may have been from chronic urticaria, many were from oozing lesions. The histopathological findings were comparable with lesions described as spongiotic vesicular dermatitis,^{5,21} but with less clinical and histological oedema. In contrast to an earlier study,¹⁷ which used slaughter horses as controls, in this study, toluidine-blue-positive and IgE-bearing cells were observed in the epidermis of control horses. Although the control horses in this study often had other acute diseases, none is typically associated with IgE production.

There was variability in cell counts by all staining methods in all horses. This variability can be explained by differences in chronicity of the lesions in the diseased animals. We have no explanation, however, for the high variation in the number of tryptase-positive cells within the control group.

Many IgE-positive cells were in the subepidermal and deeper dermis of all horses. An unexpected finding was the significant increase in the tryptase-, toluidine-blue- and IgE-positive cells in the PF group. PF is generally regarded as an IgG-mediated disease characterized by the production of autoantibodies directed against the major antigen desmoglein I (specifically identified in humans and dogs) located in the intercellular spaces of the epidermis.²² In human endemic pemphigus foliaceus (EPF) increased serum IgE^{23,24} is said to be associated with mercury and certain black fly species,^{23,25} but no IgE hypersensitivity reactions have

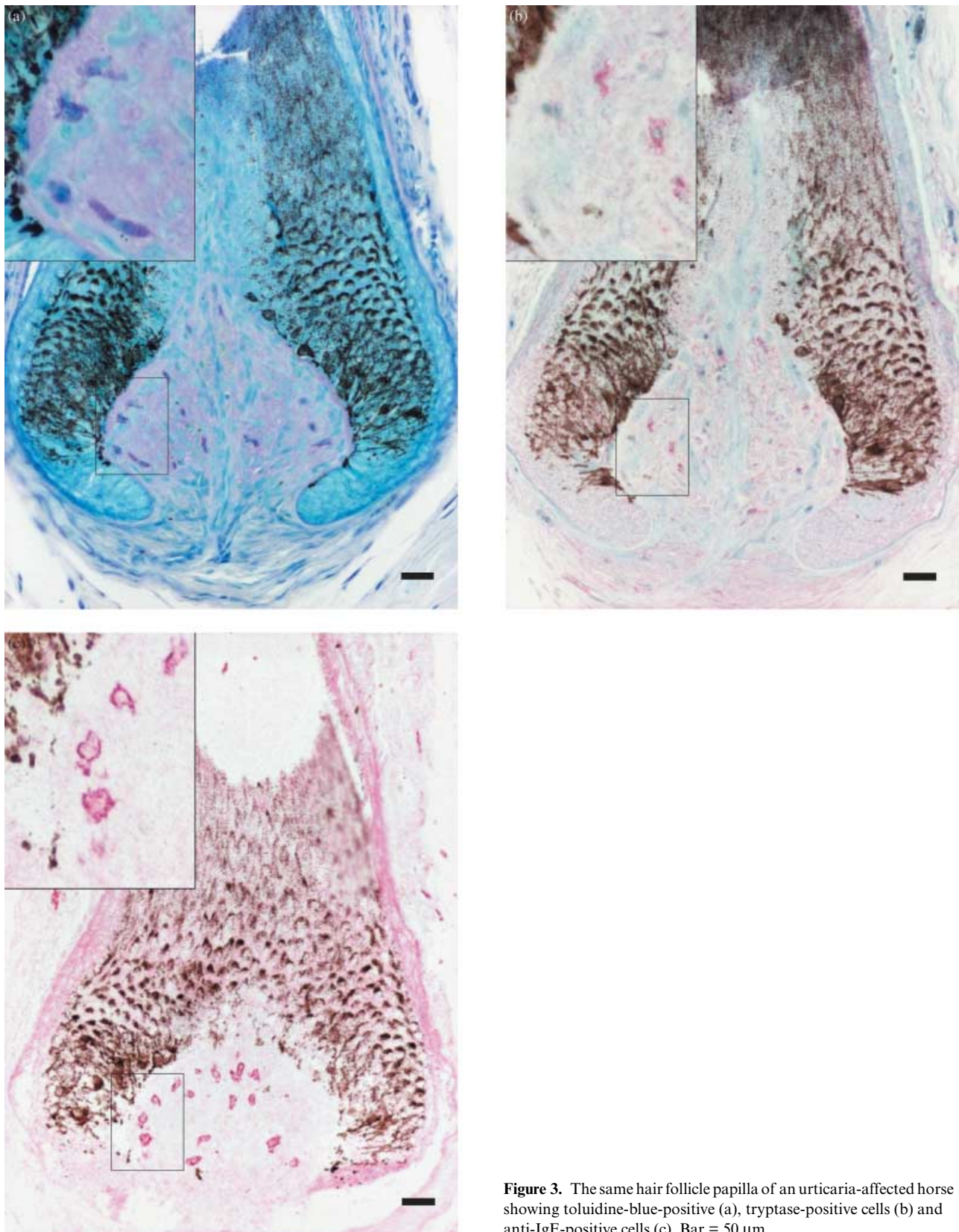


Figure 3. The same hair follicle papilla of an urticaria-affected horse showing toluidine-blue-positive (a), tryptase-positive cells (b) and anti-IgE-positive cells (c). Bar = 50 μ m.

been recorded, possibly because no black fly allergens were used in these tests.²³ Horses are commonly exposed to black flies but so far an association with PF has not been found.²⁶ To our knowledge, IgE-bearing cells have not been described in humans or dogs with PF. However, in cats and humans with PF, high numbers of skin mast cells are described.^{27,28}

Control horses had many IgE-bearing cells (median 180 cells/mm² in the subepidermal dermis and 20 cells/mm² in the deeper dermis) compared with dogs, which may also be one explanation for the false-positive reactions in intradermal testing in horses.^{1,9,16} Fewer IgE-, toluidine-blue-, and tryptase-positive cells were found in urticaria-affected horses compared with IBH

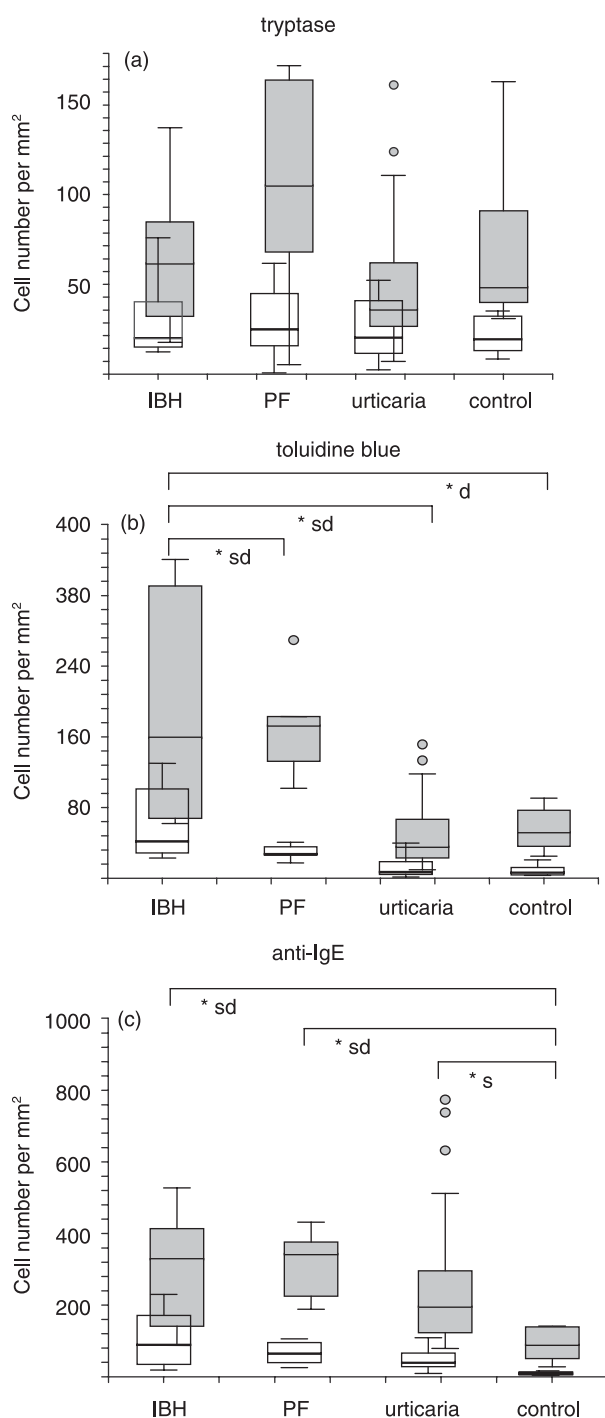


Figure 4. Cell counts with the different stainings. Number of tryptase- (a), toluidine-blue- (b) and IgE- (c) positive cells per mm² in the subepidermal (grey box plots) and dermal (white box plots) layers in horses with insect bite hypersensitivity (IBH), pemphigus foliaceus (PF), urticaria, and in horses without skin lesions (control). The central horizontal line of the box plots marks the median (50 percentile) of the sample. The edges of the box mark the first and third quartiles (interquartile range, IQR), and the whiskers represent the tail of the distribution. ? = outliers, * significant differences in cell counts in the subepidermal layer (s) and the dermis (d).

and PF horses. This may have been caused by oedema leading to spreading of all cells. Retrospectively, however, we could not find any significant difference in cell counts between biopsies with or without signs of oedema (data not shown).

Although horses with urticaria had significantly more IgE-bearing cells than control horses, decreased mast cell counts were noted. Mast cell degranulation may lead to the depletion of granules²⁹ and loss of staining. The results, therefore, suggest mast cell degranulation due to an IgE-mediated, type 1 hypersensitivity reaction. In contrast, nevertheless, only a small percentage of human patients with chronic urticaria have IgE-mediated mast cell degranulation. In 30–50% of humans with chronic idiopathic urticaria, autoantibodies (IgG) against the high-affinity receptor for IgE (FcεRI) or against IgE itself have been described.^{14,30–32} In horses, the existence of such autoantibodies is unknown. These autoantibodies may also exist in horses with urticaria. Although colocalization of IgE-, toluidine-blue- or tryptase-positive cells was often suggested in sequential sections, further studies are planned to define which cell types carry surface-bound IgE or produce IgE.

Mast cells were detected in the hair follicle papillae of most horses in all groups. In humans, mast cells are not present in the papilla but can be found aligned along the outside of the hair follicles.³³ The role of intrapapillary mast cells is unknown. Significantly fewer toluidine-blue-positive cells were seen in the urticaria group compared with the IBH and PF groups. This may have been caused by depletion of granules of mast cells,²⁹ and consequent loss of staining. Heterogeneity of mast cells has been demonstrated both in different species and in different tissues within one species.^{19,34} In man³⁵ and domestic animal species^{36,37} mast cells are distinguished by their proteases, tryptase and chymase. The three mast cell subtypes contain tryptase or chymase alone or both tryptase and chymase. With the double-labelling technique we could only detect tryptase-positive cells and no chymase-positive cells. The absence of chymase in mast cells has been described in equine skin¹⁶ and endometrial¹⁹ mast cells, but using the same technique, chymase-positive mast cells can be detected in formalin-fixed equine lung tissue.³⁸ We therefore conclude that the lack of chymase-positive cells in the skin is not due to formalin fixation. As in cattle,³⁷ the major mast cell subtype in equine skin does not contain chymase. This is in contrast to man,³⁹ dogs³⁶ and cats^{40,41} where mast cells containing both tryptase and chymase are the major subtype in the skin. An earlier report described how mast cells tended to be more numerous along the dorsal midline,⁴² but our findings were in agreement with the observations of Shearer⁴³ who found no significant differences in mast cell count in different body sites but significant differences between animals. We found the fetlock area had different mast cell counts but as this was not a biopsy site in any of the other disease groups it was not of significance in our study. Similarly, the fixation times tested in control horses did not influence toluidine blue staining or immunohistochemistry.

In conclusion, the results of this study suggest that IgE-mediated reactions may play a role in the pathogenesis of urticaria. Prospective studies on mast cell protease content and the cytokine profile of T-helper

cells may prove useful in elucidation of the pathogenesis of equine urticaria.

ACKNOWLEDGEMENTS

We appreciate the financial support of the ESVD grant 2003 and from the Swiss National Science Foundation grant no. 31-63449.00. We thank Professor Harvima, Kupio, Finland for providing the antibody against tryptase and Mrs Manuela Bozzo, Mrs Erika Garchi, Mrs Evelyn Rohrer and Mrs Lucie Palmieri for excellent technical assistance. We also like to thank Dr Lisa Harwood for critical reading of the manuscript.

REFERENCES

1. Jose-Cunilleras E, Kohn CW, Hillier A *et al.* Intradermal testing in healthy horses and horses with chronic obstructive pulmonary disease, recurrent urticaria, or allergic dermatitis. *Journal of the American Veterinary Medical Association* 2001; 219: 1115–21.
2. Scott DW, Miller WH. *Equine Dermatology*. St. Louis, MO: W.B. Saunders, 2003: 422–7.
3. Blauvelt A, Hwang ST, Udey MC II. Allergic and immunologic diseases of the skin. *Journal of Allergy and Clinical Immunology* 2003; 111: 560–70.
4. Grattan CE, Sabroe RA, Greaves MW. Chronic urticaria. *Journal of the American Academy of Dermatology* 2002; 46: 645–57.
5. von Tscharner C, Kunkle G, Yager J. Immunologic diseases. *Veterinary Dermatology* 2000; 11: 163–78.
6. Zuberbier T. Urticaria. *Allergy* 2003; 58: 1224–34.
7. Logas DB, Barbet JL. Inflammatory, infectious, and immune disease. In: Colahan PT, Merritt AM, Moore JN eds. *Equine Medicine and Surgery*. St. Louis, MO: Mosby Year Book, 1999: 1868–73.
8. Moriello KA, DeBoer D, Semrad SD. Diseases of the skin. In: Reed SM, Bayly WM eds. *Equine Internal Medicine*. Philadelphia: W.B. Saunders, 1998: 513–57.
9. Evans AG, Paradis MR, O'Callaghan M. Intradermal testing of horses with chronic obstructive pulmonary disease and recurrent urticaria. *American Journal of Veterinary Research* 1992; 53: 203–8.
10. Fadok VA. Overview of equine papular and nodular dermatoses. *Veterinary Clinics of North America, Equine Practice* 1995; 11: 61–74.
11. Francqueville M, Sabbah A. Chronic urticaria in sports horses. *Revue Française d'allergologie et d'immunologie Clinique (Paris)* 1999; 31: 212–13.
12. Lorch G, Hillier A, Kwochka KW *et al.* Results of intradermal tests in horses without atopy and horses with atopic dermatitis or recurrent urticaria. *American Journal of Veterinary Research* 2001; 62: 1051–9.
13. Doutre M. Physiopathology of urticaria. *European Journal of Dermatology* 1999; 9: 601–5.
14. Miescher SM, Vogel M. Molecular aspects of allergy. *Molecular Aspects of Medicine* 2002; 23: 413–62.
15. Sabroe RA, Francis DM, Barr RM *et al.* Anti-Fc (epsilon) RI auto antibodies and basophil histamine releasability in chronic idiopathic urticaria. *Journal of Allergy and Clinical Immunology* 1998; 102: 651–8.
16. Lorch G, Hillier A, Kwochka KW *et al.* Results of intradermal tests in horses without atopy and horses with chronic obstructive pulmonary disease. *American Journal of Veterinary Research* 2001; 62: 389–97.
17. van der Haegen A, Griot-Wenk M, Welle MM *et al.* Immunoglobulin-E-bearing cells in skin biopsies of horses with insect bite hypersensitivity. *Equine Veterinary Journal* 2001; 33: 699–706.
18. Marti E, Peveri P, Griot-Wenk M *et al.* Chicken antibodies to a recombinant fragment of the equine immunoglobulin epsilon heavy-chain recognising native horse IgE. *Veterinary Immunology and Immunopathology* 1997; 59: 253–70.
19. Welle MM, Audige L, Belz JP. The equine endometrial mast cell during the puerperal period: evaluation of mast cell numbers and types in comparison to other inflammatory changes. *Veterinary Pathology* 1997; 34: 23–30.
20. Harvima IT, Naukkarinen A, Harvima RJ *et al.* Immunoperoxidase and enzyme-histochemical demonstration of human skin tryptase in cutaneous mast cells in normal and mastocytoma skin. *Archives of Dermatological Research* 1988; 280: 363–70.
21. Hargis AM, Clark EG, Duclos DD *et al.* Spongiotic vesicular dermatitis as a cutaneous reaction pattern in seven horses. *Veterinary Dermatology* 2001; 12: 291–6.
22. Olivry T, Chan LS. Autoimmune blistering dermatoses in domestic animals. *Clinics in Dermatology* 2001; 19: 750–60.
23. Abreu Velez AM, Warfvinge G, Herrera WL *et al.* Detection of mercury and other undetermined materials in skin biopsies of endemic pemphigus foliaceus. *American Journal of Dermatopathology* 2003; 25: 384–91.
24. Bruns GR, Ablin RJ, Guinan PD. Serum immunoglobulin E in pemphigus. *Journal of Investigative Dermatology* 1978; 71: 217–18.
25. Eaton DP, Diaz LA, Hans-Filho G *et al.* Comparison of black fly species (Diptera: Simuliidae) on an Amerindian reservation with a high prevalence of fogo selvagem to neighboring disease-free sites in the State of Mato Grosso do Sul, Brazil. The Cooperative Group on Fogo Selvagem Research. *Journal of Medical Entomology* 1998; 35: 120–31.
26. Pascal A, Shiebert J, Ihrke P. Seasonality and environmental risk factors for pemphigus foliaceus in animals. A retrospective study of 83 cases presented to the veterinary medical teaching hospital. University of California, Davis from 1976 to 1994. *Proceedings of the Eleventh Annual Memberships' Meeting of the American Academy of Veterinary Dermatology and the American College of Veterinary Dermatology, Sante Fe, 1995: 24–5.*
27. Preziosi DE, Goldschmidt MH, Greek JS *et al.* Feline pemphigus foliaceus: a retrospective analysis of 57 cases. *Veterinary Dermatology* 2003; 14: 313–21.
28. Caproni M, Giomi B, Cardinali C *et al.* Further support for a role for Th₂-like cytokines in blister formation of pemphigus. *Clinical Immunology* 2001; 98: 264–71.
29. Claman HN, Choi KL, Sujansky W *et al.* Mast cell 'disappearance' in chronic murine graft-vs-host disease (GVHD)-ultrastructural demonstration of 'phantom mast cells'. *Journal of Immunology* 1986; 137: 2009–13.
30. Friedmann PS. Assessment of urticaria and angio-oedema. *Clinical and Experimental Allergy* 1999; 29 (Suppl. 3): 109–12.
31. Greaves MW. Chronic urticaria. *Journal of Allergy and Clinical Immunology* 2000; 105: 664–72.

32. Greaves MW. Chronic idiopathic urticaria. *Current Opinion in Allergy and Clinical Immunology* 2003; 3: 363–8.
33. Christoph T, Muller-Rover S, Audring H et al. The human hair follicle immune system: cellular composition and immune privilege. *British Journal of Dermatology* 2000; 142: 862–73.
34. Hill PB, Martin RJ. A review of mast cell biology. *Veterinary Dermatology* 1998; 9: 145–66.
35. Irani AM, Schwartz LB. Human mast cell heterogeneity. *Allergy and Asthma Proceedings* 1994; 15: 303–8.
36. Kube P, Audige L, Kuther K et al. Distribution, density and heterogeneity of canine mast cells and influence of fixation techniques. *Histochemistry and Cell Biology* 1998; 110: 129–35.
37. Kuther K, Audige L, Kube P et al. Bovine mast cells: distribution, density, heterogeneity, and influence of fixation techniques. *Cell Tissue Research* 1998; 293: 111–19.
38. van der Haegen A, Welle M, Gerber V et al. Chymase/tryptase+ mast cells in lung tissue samples from RAO-affected and control horses after mouldy hay challenge. *Proceedings of the Sixth International Veterinary Immunology Symposium, Uppsala, 2001*: 74.
39. Schwartz LB. Mast cells: function and contents. *Current Opinion in Immunology* 1994; 6: 91–7.
40. Noli C, Welle M, Scarampella F et al. Quantitative analysis of tryptase- and chymase-containing mast cells in eosinophilic conditions of cats. *Veterinary Pathology* 2003; 40: 219–21.
41. Roosje PJ, Koeman JP, Thepen T et al. Mast cells and eosinophils in feline allergic dermatitis: a qualitative and quantitative analysis. *Journal of Comparative Pathology* 2004; 131: 61–9.
42. Morrow AN, Baker KP, Quinn PJ. Skin lesions of sweet itch and the distribution of dermal mast cells in the horse. *Journal of Veterinary Medicine Series B* 1987; 34: 347–55.
43. Shearer DH. A study of the number and distribution of cutane mast cells in the horse. *Proceedings of the Twelfth Annual Meeting of the European Society of Veterinary Dermatology, Barcelona: European Society of Veterinary Dermatology, 1995*: 250.

Résumé La pathogénie de l'urticaire n'est pas bien comprise chez le cheval. Chez l'homme, l'urticaire est associée à des mécanismes immunologiques et non immunologiques, responsables d'une libération de médiateurs variés par les mastocytes (MCs). Des biopsies cutanées de 32 chevaux présentant une urticaire ont été colorées avec le bleu de toluidine, une méthode de double marquage pour la chymase et la tryptase, et un marquage immunohistochimique des IgE. Ces chevaux ont été comparés avec d'autres chevaux présentant un pemphigus foliaceus (PF), une hypersensibilité aux piqûres d'insectes (IBH) et un groupe contrôle sans trouble cutané. Ni le temps de fixation dans le formol, ni la zone de biopsie n'ont influencé les résultats. Aucune cellule positive pour la chymase n'a été retrouvée. Dans tous les groupes de chevaux, des cellules colorées par le bleu de toluidine, réagissant à la tryptase et aux IgE ont été retrouvées dans l'épiderme, la papille folliculaire et significativement plus dans le derme superficiel que dans le derme profond. Les chevaux à urticaire avaient significativement plus de cellules IgE positives dans le derme superficiel que les chevaux du groupe contrôle. Cependant, les chevaux à urticaire avaient significativement moins de MCs colorés par le bleu de toluidine en comparaison avec ceux des groupes IBH et PF. Cette étude suggère que des réactions médiées par les IgE jouent un rôle dans la pathogénie de l'urticaire.

Resumen La patogenia de la urticaria equina no se conoce en su totalidad. En el hombre, la urticaria se ha asociado a mecanismos inmunológicos y no-inmunológicos que llevaban a la liberación de varios mediadores por parte de mastocitos (MCs). Se realizó una tinción de azul de toluidina en biopsias cutáneas de 32 caballos con una historia de urticaria, un método de marcaje doble para quimasa y triptasa, e inmunohistoquímica para IgE. Estos caballos fueron comparados con caballos con pénfigo foliáceo (PF), hipersensibilidad a picadura de insecto (HPI) y con caballos control con piel sana. Ni el tiempo de fijación con formalina ni el sitio de la biopsia influenció los métodos de tinción. No se encontraron células positivas a quimasa. Se encontraron en la epidermis y la papila del folículo del pelo, en todos los grupos de caballos, células teñidas con azul de toluidina y para triptasa e IgE, y mostraron una intensidad mayor las células de la dermis subepidérmica que las de la dermis profunda. Los caballos con urticaria tenían considerablemente más células con IgE en la dermis subepidérmica del cojinete que en caballos control. Sin embargo, los caballos con urticaria tenían perceptiblemente menos MCs teñidos con azul de toluidina en la dermis subepidérmica y profunda que los de los grupos de HPI y de PF. Este estudio sugiere que las reacciones mediadas por IgE participan en la patogenia de la urticaria.

Zusammenfassung Die Pathogenese der equinen Urikaria ist unklar. Beim Menschen ist Urtikaria mit immunologischen und nicht-immunologischen Mechanismen verbunden, die zur Freisetzung verschiedener Mediatoren durch Mastzellen führen. Hautproben von 32 Pferden mit Urtikaria wurden mit Toluidinblau, einem Doppellabelling für Chymase und Tryptase und Immunhistochemie für IgE untersucht. Diese Pferde wurden mit Pferden mit Pemphigus foliaceus (PF), Insektenbisshypersensitivität (IBH) und Kontrollpferden mit gesunder Haut verglichen. Weder die Formalinfixationszeit noch Lokalisation der Biopsie beeinflusste die Färbemethoden. Es wurden keine Chymase-positive Zellen gefunden. In allen Gruppen von Pferden wurden in der Epidermis und der Papille der Haarfollikel Zellen gefunden, die sich mit Toluidinblau anfärbten und positiv für Tryptase und IgE waren und es wurden signifikant mehr positive Zellen in der subepidermalen Dermis verglichen mit der tiefen Dermis beobachtet. Pferde mit Urtikaria hatten signifikant mehr IgE-beladene Zellen in der subepidermalen Epidermis als die Kontrollpferde. Jedoch hatten Pferde mit Urtikaria signifikant weniger Toluidinblau angefärbte Mastzellen sowohl in der subepidermalen als auch tiefen Dermis als die IBH und PF Gruppen. Diese Studie läßt vermuten, dass IgE-medierte Reaktionen eine Rolle bei der Pathogenese von Urtikaria spielen.