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STUDIES ON THE POSSIBLE ROLE OF HISTAMINE IN THE PATHOGENESIS OF RINGWORM INFECTIONS

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Abstract. Extracts of different Trichophyton strains did not induce histamine release from rat peritoneal mast cells. Neither did broth medium where *Trichophyton mentagrophytes* var. asteroides (TMA) and var. granulosum (TMG), *Epidermophyton floccusum* (EF) or *Trichophyton rubrum* (TR) had been grown. The same broth was also analysed for histamine, which could not be demonstrated. It is concluded that histamine does not seem to be involved in the inflammatory reaction of ringworm infections, histamine being neither released from mast cells, nor formed by some common dermatophytes.

Varying degrees of inflammation and itch occur in ringworm infections. As dermatophytes are incapable of growth in living tissues, it is stated that the inflammatory reactions result from the diffusion of metabolites, toxins or allergens from the fungus in the keratin into dermis (1, 7).

Histamine is considered to be one of the mediators of the inflammatory response in connective tissue (12). The histamine is probably released from the tissue mast cells, which are known to secrete their amine contents after various kinds of stimuli (14). Histamine injected into the skin produces redness, wheal and flare (the triple response) (8) and the same effect is obtained when histamine-liberating substances are injected causing histamine release from the dermal mast cells. Against this background it was considered of interest to study whether histamine or histamine-releasing factors are produced by dermatophytes and thus take part in the inflammatory reaction of dermatophytosis.

MATERIAL AND METHODS

Dermatophytes. As test species were chosen fresh isolates of *Trichophyton mentagrophytes* var asteroides (TMA), *Trichophyton mentagrophytes* var. granulosum (TMG), *Trichophyton rubrum (TR)* and *Epidermophyton floccosum (EF)*. Sabouraud's glucose agar was used as culture medium and poured into Petri dishes, 9 cm in diameter, each filled with 33 ml of the substrate. Each fungus species was inoculated on 10–20 plates and incubated for 10–30 days at $+25° \pm 1°C$. Thereafter the aerial mycelium from the fungi was removed—care was taken to avoid contamination from the agar plate—and transferred to retorts, filled with 50 ml broth (peptone 5, glycose 10, glycerol 5, NaCl 5, Mg $SO_4 \cdot 7\ H_2O$ 0.5, KH_2PO_4 1, asparagine 1 g/l) with or without antibiotics (benzylpenicillin 0.12 g/l, streptomycin 0.25 g/l and cyclohexemide 0.5 g/l). The fungi were incubated for 1 or 4 weeks at $+37°C$. The pH was measured before and after the incubation. Thereafter the fluid medium was filtered through a Seitz filter and stored at $-20°C$ until examined for histamine content and for histamine-releasing activity. The latter was obtained by incubating a fraction of the filtered broth with mast cell suspension and measuring the histamine release.

Mast cells. Rat peritoneal cell suspension containing 3–5% mast cells was obtained from male Sprague-Dawley rats (for details see refs. 2, 3). The rats were killed, and into the peritoneal cavity a salt solution was injected containing 154 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$ and 6.7 mM phosphate buffer (pH 7.0) 4.0 mM $Na_2HPO_4 + 2.7$ mM KH_2PO_4, human serum albumin 1 mg/ml. After massaging the abdomen for a few minutes the abdominal wall was cut open and the salt solution containing suspended cells was removed from the peritoneal cavity. The suspension was centrifuged at 350 *g*, the cells resuspended in fresh salt solution and incubated with the possible histamine releasers. As a rule 0.1 ml of releaser was mixed with 0.9 ml of cell suspension and incubated for 10 min at 37°C. The incubation was terminated by centrifugation at 350 *g* for 5 min. The supernatant containing released histamine was carefully decanted. The histamine remaining in the sedimented mast cells was extracted by suspending the cells in saline and heating in a boiling water bath for 3 min. Histamine released (supernatant) was expressed as a percentage of the total amount of histamine present (supernatant + sediment) in each incubation tube. The mast cells are the only histamine-containing structure in a suspension of rat peritoneal cells, i.e. all the histamine released originates from the mast cells (3). Thus the cell suspension containing 3–5% mast cells is referred to as mast cell suspension in the present paper.

Table I. *Fungi homogenizied at different pH in order to extract possible histamine-liberating factors*

Incubation with mast cells at pH 7.0. Histamine released from the mast cells is expressed as percentage of the total amount of histamine present in each incubation tube. (Histamine release is denoted as "0" when histamine in the supernatant is equal to or slightly less than the blank values)

	TMA			TMG			TMI			
pH	5.5	7.2	8.2	5.5	7.2	8.2	5.5	7.2	8.2	Comp. 48/80 0.5 μg/ml
Conc. of mycelsuspension (g/ml)	0.09	0.07	0.07	0.04	0.05	0.05	0.03	0.04	0.02	
Histamine release (%)	0.2	0	0	1.2	0	0	0	0	1.2	66

Histamine determination was made by fluorimetry after condensation with *o*-phthaldialdehyde (OPT) according to the method described by Shore et al. (11). In those samples where fungal culture medium was present, histamine was extracted and purified according to Shore et al. (11) prior to condensation with OPT. When only mast cells had been present (extracts from sedimented cells), the purification steps were omitted and OPT was added directly after alkalinization, since the mast cells contain no substance giving fluorescence except histamine (Fredholm & Hägermark, unpubl.).

RESULTS

Homogenisation of dermatophytes. Trichophyton mentagrophytes var. asteroides (TMA), *Trichophyton mentagrophytes* var. granulosum (TMG) and *Trichophyton mentagrophytes* var. interdigitale (TMI) were harvested from the agar plates and divided into approximately equal parts. In order to extract possible histamine releasing factors the fungal mycels were suspended in distilled water buffered with phosphate buffer at acid, neutral, and alkaline pH (5.5; 7.2; 8.2) and homogenized in a Potter-Elvehjem homogenizer. The homogenates were incubated with rat mast cells at 37°C for 10 min. To check the reactivity of the mast cells a control with the histamine liberating compound 48/80 was included (Table I). No histamine release was observed when homogenates had been added to the cell suspension. Thus, under these conditions no histamine releasing activity could be demonstrated in the contents of aerial mycelium.

Long term incubation in broth medium. This series of experiments was performed in order to study whether the dermatophytes produce either histamine or any histamine-releasing factor to the surroundings while growing in the medium. As described in Methods, the mycels were transferred to broth medium and incubated at 37°C for 7 or 30 days. The filtered broth was then analysed for histamine or histamine-releasing factors. No histamine was detected in the broth. It was found that mast cells incubated with filtered broth medium did not release histamine above the controls (Table II). Thus, no evidence was obtained of histamine-releasing factors being produced from the fungi examined.

The reactivity of the mast cells was controlled by incubation with compound 48/80. In addition, the mast cells were incubated with a mixture of filtered broth and compound 48/80 in order to exclude the possibility that the fungi might have excreted any factor inhibiting the histamine release. Approximately the same release values (about 70%) were obtained with compound 48/80 only, and with compound 48/80 in presence of broth medium.

DISCUSSION

The reason why certain fungi are pathogenic for animal and man is largely unknown. The production of keratinolytic enzymes may be essential in this respect (16). The factors causing inflammatory reaction in some dermatophytoses are also mainly unknown. Some of the fungal products may be allergens and some may be toxins initiating inflammation (7). As histamine is one mediator of inflammation, it might be expected to be involved in the pathogenesis of dermatophytosis, but to our knowledge it has not been studied whether histamine is released by fungal products or whether the fungi themselves produce histamine. This possibility seemed quite likely since histamine-liberating factors have been isolated from various biological sources, such as eelworm (*Ascaris lumbricoides*) and jelly fish (*Cyanea capillata*) (5, 13, 15), bee venom (3, 6) and lysosomes (10). As some bacteria contain histidine decar-

Table II. *Fungi incubated in broth medium in order to study production of possible histamine-liberating factors*

Filtered broth added to mast cells suspension. The reactivity of the mast cells checked by incubation with compound 48/80. Spontaneous histamine release is deducted

Fungal strain	Time on plates (days)	Incubation in broth: Mycel. conc. (g/ml broth)	Inc. time (weeks)	pH of broth: Before inc.	pH of broth: After inc.	Histamine release from mast cells (%): Filtered broth	48/80. 0, 5 µg/ml: In broth	48/80. 0, 5 µg/ml: In NaCl
EF	12	0.018[a]	1	5.4	6.2	0	71	68
	12	0.045	1	5.6	6.2	0		
	14	0.091[a]	1	5.3	5.8	2.2	37	32
	14	0.100	1	5.3	6.0	2.8		
	14	0.099[a]	4	5.3	6.8	5.1		
	14	0.099	4	5.3	6.5	3.7		
TMA	11	0.348[a]	1	5.4	5.3	0	71	68
	11	0.147	1	5.6	5.9	0		
	27	0.237[a]	1	5.5	6.2	0	68	73
	27	0.266	1	5.6	6.6	0		
	27	0.223[a]	4	5.5	7.3	2.7	60	57
	27	0.188	4	5.6	7.4	0.1		
TMG	23	0.463[a]	1	5.6	6.4	0	82	81
	23	0.531	1	5.6	6.3	0		
	14	0.549[a]	1	5.6	6.8	0	68	73
	14	0.604	1	5.5	6.8	0		
	14	0.544[a]	4	5.4	6.9	0		
	14	0.510	4	5.4	7.0	0		
TR	23	0.633[a]	1	5.6	6.4	0	82	81
	23	0.686	1	5.6	6.1	0		
	20	1.55[a]	1	5.4	6.0	0.1	60	57
	20	1.36	1	5.5	6.3	0		
	20	2.57[a]	4	5.4	6.3	2.8		
	20	2.79	4	5.4	7.0	2.9		

[a] Antibiotics (benzylpenicillin, streptomycin, cycloheximide) added to the incubation medium.

boxylase and synthesize histamine from histidine (4, 9), this might also be true for dermatophytes.

In the first part of the present investigation the histamine-releasing capacity of mycel extracts prepared from different strains of *Trichophyton mentagrophytes* was studied. No histamine-releasing activity could be demonstrated in the extracts, a finding which agrees with the general observation that trichophytin—an extract of dermatophytes—does not elicit a reaction of the immediate type upon i.c. injection. Thus it was considered more relevant to study the excretion products rather than the extracts of the dermatophytes. Therefore the fungi were grown in broth medium which subsequently was analysed for histamine-releasing properties and histamine content. The four most common dermatophytes in Sweden were chosen. However, in these experiments no histamine-releasing activity could be detected, nor was any histamine discovered.

Thus histamine does not seem to be involved in the inflammatory response caused by dermatophytes, though it should be kept in mind that these *in vitro* experiments may not reflect the events taking place *in vivo* in skin, and it cannot be excluded that under more favourable conditions the fungi may produce histamine-releasing agents and/or histamine.

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