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Original Scientific Article

THE EFFECT OF A PETIDE INHIBITOR OF CYSTEINE PEPTIDASES PRODUCED BY THE DERMATOPHYTE *Trichophyton mentagrophytes* ON A MOUSE IMMUNE SYSTEM

Blanka Premrov Bajuk[1]*, Marinka Drobnič Košorok[1], Tadej Malovrh[2]

[1]Institue of Physiology, Pharmacology and Toxicology, [2]Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia

*Corresponding author, E-mail: blanka.premrovbajuk@vf.uni-lj.si

Summary: A mouse model was used to determine a possible influence of the peptide cysteine peptidase inhibitor isolated from the dermatophyte fungus *Trichophyton mentagrophytes* (*T. mentagrophytes*) on a host immune system. The inhibitor was isolated from cytosol of mechanically and ultrasonically disintegrated fungal mycelia. BALB/c mice were divided into 4 groups. On days 0, 5 and 15, mice were treated with physiological fluid (Control group), isolated fungal inhibitor (I Group), fungal inhibitor and ovalbumin (I + OVA Group) and ovalbumin only (OVA Group). On day 29, blood samples were taken, animals were euthanized and their spleens were aseptically removed. Harvested spleen cells were cultured in microtiter plates and used for the measurements of cell proliferation and the level of apoptosis with commercially available kits. Mice sera were used in Western blot procedure to study the effect of the fungal inhibitor on the processing and presentation of ovalbumin as antigen. The method was used to determine whether antibodies from differently treated mice differ in their specificity. Experiments showed that the *T. mentagrophytes* inhibitor decreases apoptosis of mouse spleen cells, but has no effect on the proliferation of lymphocytes among them. It was concluded from the immunoblotting results that the fungal inhibitor probably affects the processing of ovalbumin.

Key words: cysteine peptidase; inhibitor; immune system; cell proliferation; apoptosis; antigen presentation

Introduction

Peptidases are enzymes that catalyse the cleavage of peptide bonds of proteins, digesting them into peptides or free amino acids and thus regulating their function and fate. On the basis of the mode of action and their active sites peptidases are classified into several groups, namely aspartic, cysteine, glutamine, metallo, asparagine, serine, threonine peptidases and those with an unknown catalytic mechanism (1, 2, 3, 4, 5). Despite their essential role, proteolytic enzymes can be potentially extremely damaging in living systems. Proteolysis is therefore stringently regulated by several distinct mechanisms. One of them is reversible or irreversible suppression of peptidase activity by an inhibitor which binds in the catalytic cleft of the protease and thereby prevents access to substrates (6, 7, 8, 9).

The endosome/lysosome compartments, together with the cytosolic proteasomes, are the two major

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protein degradation systems in cells. Endosome/lysosome–located proteases play key roles in antigen processing and presentation, cytokine regulation, natural killer T cell development, activation of serine protease zymogens in regulated secretory granules, integrin activation, induction of apoptosis and the Toll-like receptor signalling. Cysteine, partly serine as well as aspartic peptidases and their inhibitors play an important role in two processes in the immune system that require proteolysis, i.e. apoptosis and endocytotic pathway of antigen processing. They are involved in the regulation of apoptosis in neutrophils, monocytes and dendritic cells, in lymphocyte maturation in primary lymphatic organs and in killing of target cells by cytotoxic T cells and NK cells. In antigen processing, aspartic and cysteine proteases introduce cleavages in endocytosed antigens, which trigger unfolding of the polypeptide chains and the capture of processed antigens by newly synthesised MHC class II molecules. In addition, MHC class II molecules are processed by the same type of enzymes to degrade the invariant chain (Ii) that occupies the peptide-binding site (1, 10, 11, 12, 13, 14).

The described experiment assesses the effect of a peptide inhibitor isolated from the dermatophyte species *Trichophyton mentagrophytes (T. mentagrophytes)* on a mouse immune system. The research was focused on measuring its effect on immune cell proliferation and apoptosis as well as on antigen presentation and consequently on the specificity of antibody binding by the defined antigens.

Materials and methods

Peptide dermatophyte inhibitor

Fungal homogenate, prepared from mechanically and ultrasonically disintegrated dermatophyte mycelia, was centrifuged for 20 minutes at 10.000 × g and for 1 hour at 25.000 × g to remove cell debris (ultracentrifuge Beckman, Avanti J-301). The cytosol extract was ultrafiltered using Centriprep YM-3 concentrator (Amicon) with the nominal molecular weight limit of 3.000 Da. The obtained filtrate with inhibitory activity against cysteine peptidase papain was pooled and diluted with physiological solution.

Experimental animals, immunization and cell preparation

Adult female BALB/c mice were acquired at the Centre for animal genomics of the Veterinary Faculty, University of Ljubljana, Slovenia. They were kept in standard animals housing facilities and given free access to water and food. 12 BALB/c mice between 92 and 98 days old, weighing between 21.1 and 25.1 g were formed into 4 groups. On days 0, 5 and 15, mice in the Control group were i.p. treated with 300 µL of physiological fluid and mice in Group I with 300 µL of fungal inhibitor. At the same time, mice in the Group OVA were s.c. given 300 µL of ovalbumin (Chicken egg white, Grade V, Sigma; final concentration of 15 µg/L) and mice in the Group I + OVA were treated i.p. with 300 µL of fungal inhibitor and also s.c. with 300 µL of ovalbumin. On day 29, mice were anesthetized and their blood was removed from the retroorbital plexus using a glass Pasteur pipette (Brand, Wertheim). Blood was collected in Microtainer tubes with gel (Becton, Dickinson and Company). After centrifugation sera were frozen at –20 °C until analysis. Mice were then euthanized in accordance with the Slovene legislation on animal euthanasia and their spleens were aseptically removed. Animal experiment was approved by the Veterinary Administration of Republic of Slovenia (permit no. 34401-38/2007/3).

Aseptically removed spleens were teased apart between the frosted ends of two sterile microscope slides and resuspended in Lymphocyte Culture Medium (LCM). The cell suspension was centrifuged and the pellet resuspended in the ACK buffer for 5 minutes. Cells were washed twice with MEM (Gibco) and resuspended in LCM. Cells were quantified in Neubauer haemocytometer and appropriate cell dilutions were prepared.

Dermatophyte inhibitor, cell proliferation and apoptosis

Lymphocyte proliferation was measured using a commercially available kit (BrdU Cell Proliferation Assay, Calbiochem). It is an immunoassay for the quantification of bromodeoxyuridine (BrdU) incorporation into the newly synthesized DNA of actively proliferating cells. 50 µL of mononuclear cells in concentration of 2×10^6 cells/mL were cultured in triplicates in 96 well culture dish

(TPP). 100 µL of either medium (RPMI 1640 and MEM, 1:1 (v/v), Gibco), ovalbumin (Chicken egg white, Grade V, Sigma; final concentration of 5 µg/L), fungal inhibitor, the mix of the inhibitor and ovalbumin 1:1 (v/v) or phytochemagglutinin (PHA, Sigma) in the final concentration of 10 µg/mL was added to the cells. Cells stimulated with PHA were incubated for 5 days, whereas others were incubated for 7 days in the incubator at 37 °C, 100 % humidity and in 5 % CO_2. 24 hours before ending the incubation, 20 µL of BrdU (diluted 1:2000 (v/v)) was added to the wells. After the incubation period the assay was performed following manufacturer's instructions. Briefly, cells were fixed, denatured and incubated in the presence of anti-BrdU antibodies. After the incubation with peroxidase goat anti-mouse IgG/HRP conjugate, substrate solution was added to each well. In the end, the reaction was stopped and absorbance measured at dual wavelengths of 450 – 540 nm using a spectrophotometric plate reader (Tecan, Sunrise).

To measure the effect of the dermatophyte inhibitor on apoptosis in a culture of spleen cells the commercial kit (Cell Death Detection ELISA, Roche) was used again. It is based on a quantitative sandwich enzyme-immunoassay principle using mouse monoclonal antibodies directed against DNA and histones. This allows the specific detection and quantitation of mono- and oligonucleosomes that are released into the cytoplasm of cells that die from apoptosis. For the detection of cell death, mononuclear cells were prepared and treated as described previously. The assay was performed according to the manufacturer's instructions. Briefly, cells were centrifuged and lysed. Samples (in duplicates) were pipetted into wells of a coated microtiter plate. After adding the conjugate solution, the microtiter plate was washed and the substrate solution added. A photometric analysis was performed with a spectrophotometric plate reader at 405 nm (Tecan, Sunrise).

The results of measurements of cell proliferation and apoptosis were statistically analysed using the GrafPad Prism computer programme. The applied method was the one-way ANOVA followed by the Tukey's multiple comparison test. Data are presented as the mean ± SD. The P-value of ≤ 0.05 was considered statistically significant.

Dermatophyte inhibitor and MHC class II antigen presentation

To study the effect of the *T. mentagrophytes* cysteine peptidase inhibitor on antigen presentation, the method of Western blotting was applied. The antigen (ovalbumin, Chicken egg white, Sigma) was first enzymatically processed with papain and cathepsins B and L. The processed peptide fragments were transferred on the membrane, and mouse sera from the Control and experimental groups were used as primary antibodies. Secondary antibodies (anti-mouse IgG and IgM) were conjugated with horseradish peroxidase.

The SDS-PAGE was performed with the method of Laemmli using a 10 % resolving gel on a mini-Protean II Slab cell apparatus (Bio-Rad). Ovalbumin (Sigma) was digested *in vitro* with papain, cathepsin B or cathepsin L. 6 mg of ovalbumin was digested with papain (final concentration 0.2 mg/mL) in 0.1 M phosphate buffer and 1.5 mM EDTA, pH 6, for two hours at 37 °C. For papain activation, 1.4 mg of cysteine was added to the solution (all chemicals were from Sigma). 1.86 mg of ovalbumin was digested with cathepsin B (0.1 mg/250 µL) in 0.1 M of acetate buffer and 1.0 mM EDTA, pH 5.0 for 23 hours at 30 °C. 30 µL of cathepsin L (1.0 IU) was added to 0.65 mg of ovalbumin in 0.1 M acetate buffer with 2.0 mM EDTA and 20 mM DTT (dithiothreitol), pH 5.5 (all chemicals were purchased from Sigma). The digestion was performed for 20 hours at 30 °C. Prior to loading the samples onto the gel, fractions were mixed (1:1) with SDS Laemmli buffer (100 mM Tris/HCl buffer, pH 6.8; 4 % (w/v) SDS; 20 % (v/v) glycerol; 20 % (v/v) 2-mercaptoethanol; 0.025 % (w/v) bromophenol blue) and boiled for 5 minutes. The samples were visualized with Coomassie brilliant blue R-350. Molecular masses were determined using the LMW standards of 11 – 170 kDa (Fermentas).

The separated digested protein samples were transferred from the SDS-PAGE gels to polyvinylidene difluoride membranes (Immobilon-P, Millipore) using the same apparatus as mentioned above. Blots were incubated overnight at 4 °C in a blocking solution containing 2 % (w/v) Tween 20 in phosphate buffer (Sigma). After being washed three times with phosphate buffer, the membranes were incubated for 1.5 hours with primary antibodies

from the sera of mouse groups denoted Control, Group I, Group OVA and Group I + OVA (diluted 1:200). After the washing, blots were incubated with detection secondary goat HRP-conjugated anti-mouse IgG, diluted 1:1000 (Sigma) or goat HRP-conjugated anti-mouse IgM, diluted 1:1000 (Sigma). Antigen – antibody complexes were visualised by 3-amino-9-ethyl-carbazole (Sigma).

Results

The effect of the peptide T. mentagrophytes inhibitor on cell proliferation

Results depicted in Figure 1 show that the detected proliferation was the highest following the non-specific stimulation of cells with the plant lecithin PHA. The average O.D. values measured in cells of all groups of mice ranged from 2.596 (Group OVA) to 3.421 (Group I). As far as other treatments of cell cultures of all four groups of mice are concerned no statistically significant differences were observed. The measured O.D. values were very low ranging from 0.028 to 0.048.

The effect of the peptide T. mentagrophytes inhibitor on apoptosis

Results depicted in Figure 2 show that apoptosis was the strongest in all four groups of mice following the stimulation of cell cultures with ovalbumin. The average measured O.D. values in all groups of mice ranged from 0.622 (Group OVA) to 1.013 (Group I + OVA). Group I + OVA also had the highest standard deviation (0.588). Apoptosis was the lowest in all groups of mice after the cells were treated with the inhibitor or the mix of the inhibitor and ovalbumin. The average O.D. values were lower than 0.482 (Group OVA after the cells were treated with the inhibitor). The detected apoptosis was statistically significantly lower ($P \leq 0.05$) in Group I between the cells stimulated with ovalbumin and those treated with the inhibitor or the mix of ovalbumin and the inhibitor.

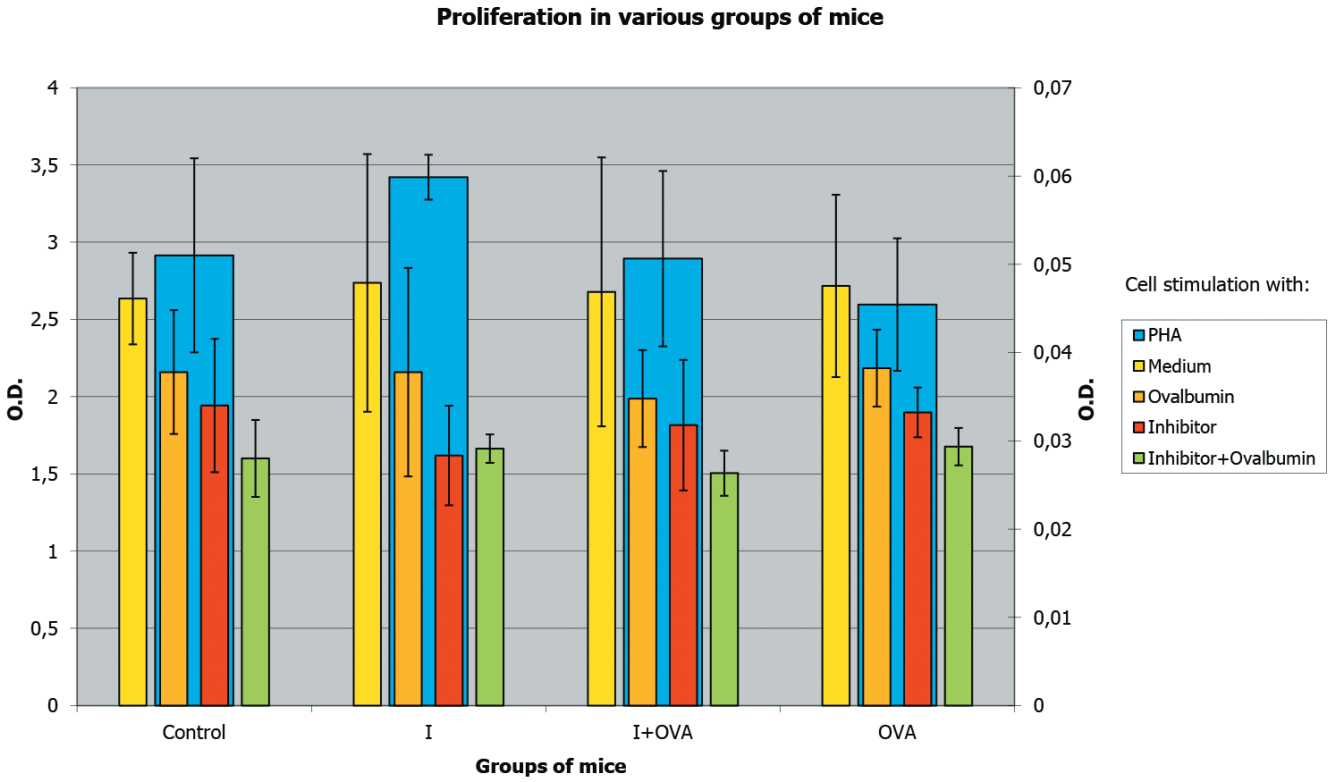

Figure 1: Comparison of results of proliferation of lymphocytes in the Control and experimental groups of mice. The results are represented as the mean OD value ± SD. Different columns represent treatments of cells in cultures with a medium (yellow), ovalbumin (orange), the inhibitor (red) or the mix of ovalbumin and the inhibitor (green) (OD scale on the right side). The wider blue-colored columns at the back (the OD scale on the left side) show unspecific proliferation of cells after on-plate stimulation with PHA

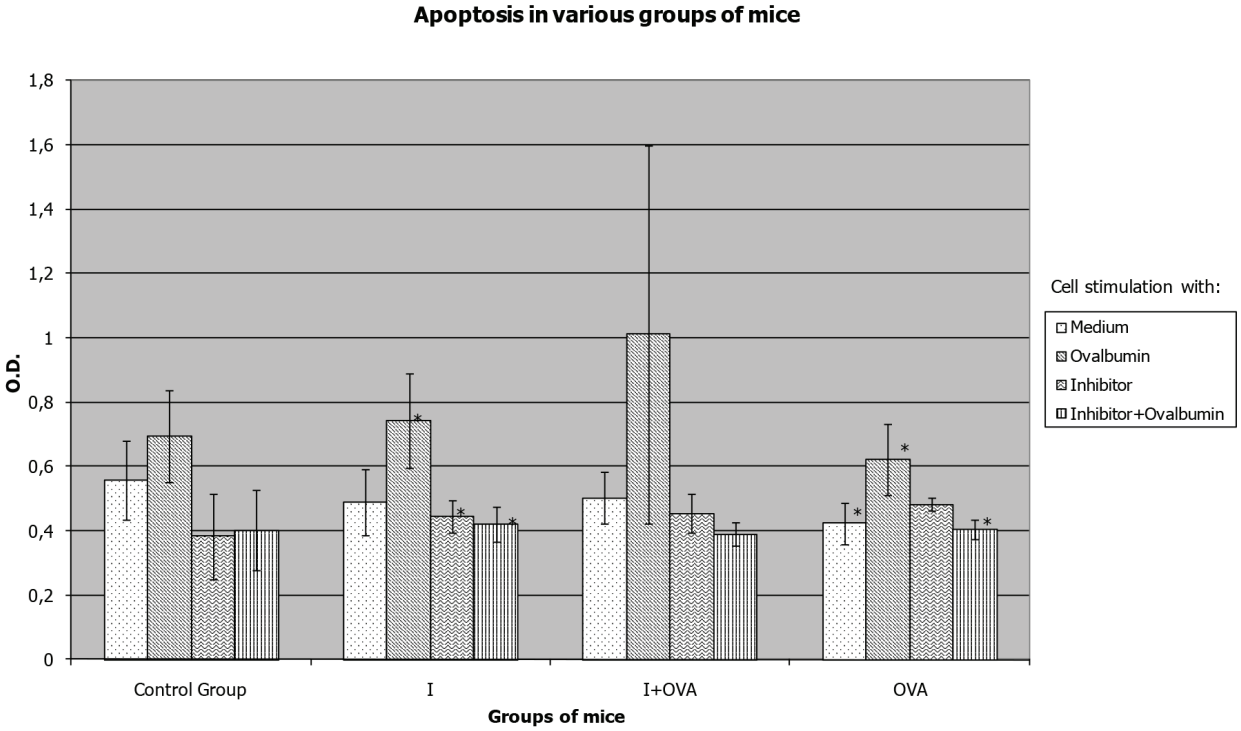

Figure 2: Comparison of results of apoptosis of spleen cells in the Control and experimental groups of mice. The results are represented as the mean OD value ± SD. Different columns represent treatments of cell cultures with a medium, ovalbumin, the inhibitor or the mix of ovalbumin and the inhibitor. * denotes statistically significant difference ($P \leq 0.05$)

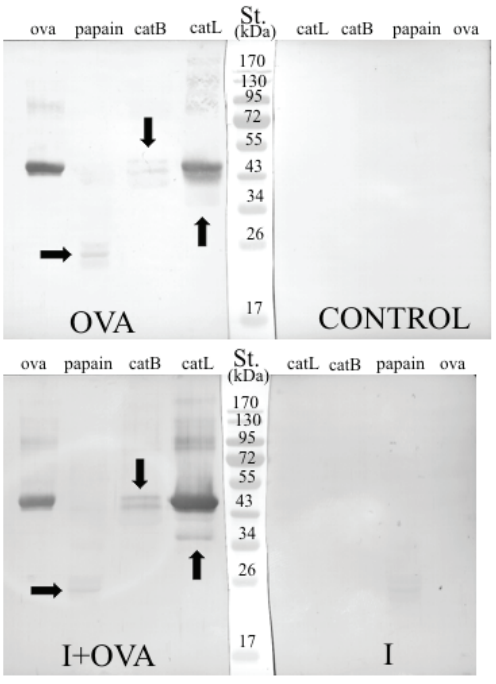

Figure 3: Immunoblot of unprocessed and processed ovalbumin visualised with goat HRP-conjugated anti-mouse IgG. Arrows represent a different pattern of bands in Groups OVA and I + OVA. Representative results are shown.

The detected apoptosis was also statistically significantly lower in the Group OVA between the cells stimulated with ovalbumin and those treated with a medium or the mix of the inhibitor and ovalbumin. The lowest detected average O.D. value was 0.384 in the cells of the Control group after the addition of the inhibitor.

Western blot analysis

The effect of the peptide inhibitor of *T. mentagrophytes* on antigen presentation was indirectly analysed by immunoblotting. Ovalbumin was processed *in vitro* with papain and cathepsins B and L. The processed peptides were loaded on the SDS-PAGE gel (the data not shown). Unprocessed ovalbumin with the molecular mass of 45 kDa was used as control. Digestion of ovalbumin was the greatest with papain since the highest number of fragments was observed there. The fragments had molecular masses from around 11 to 43 kDa. Digestion with cathepsin L gave fewer fragments with molecular masses of around 30 to 43 kDa and it appeared that some of the ovalbumin was not processed at all. Digestion

with cathepsin B (catB) resulted in only 3 bands with molecular masses of around 30 to 40 kDa.

Figure 3 shows the results of Western blotting. Fragments which were obtained after ovalbumin digestions *in vitro* were transferred from the SDS-PAGE gel to the PVDF membrane and detected with antibodies from control and experimental animals. As it can be observed, the number and intensity of the protein bands is different on the membrane where we used sera of Groups OVA (mice that were immunised with ovalbumin) and I + OVA (mice that were immunised with ovalbumin and treated with the fungal inhibitor).

Discussion

Dermatophyte colonization is characteristically limited to the dead keratinized tissue of the *stratum corneum.* Several steps are required for infection to take place, namely contact, adherence and invasion of keratin layers. The severity of the infection depends on the type of the agent, environmental factors and the host immunologic status and results in either a mild or intense inflammatory reaction. Although cornified layers of skin lack specific immune defense, both humoral and cell-mediated reactions as well as non-specific host defense mechanisms respond and eliminate the fungus (15, 16). There are reports on peptidases of various classes secreted mostly by parasitic organisms that functionally and structurally resemble the host endosomal proteases. In addition to their roles in the life cycle of pathogens, these peptidases act as virulence factors by proteolytically degrading components of the host immune system including immunoglobulins and components of the complement system. Helminth worms secrete proteases that can activate the Th2 type immune response, and, on the other hand, endogenous parasites and bacteria induce Th1 cell immune response (9, 17, 18). Among peptidase inhibitors expressed by pathogens, the best studied are those of filarial nematodes. Their immunoregulatory properties include inhibition of antigen presentation, induction of IL-10 expression and macrophage stimulation (9). For example, the intestinal nematode *Nippostrongylus brasiliensis* evades a host defense system by secreting the cysteine peptidase inhibitor nippocystatin, which modulates antigen processing in host antigen presenting cells (19). As for dermatophytes: certain dermatophyte fungi produce substances that supress the immune response. These mostly include mannans – glycoprotein constituents of the cell wall that bind to the cell surface of mononuclear phagocytes in suspension and can inhibit cell-mediated immunity and keratinocyte proliferation (20, 21).

Two important zoophilic dermatophyte fungi, namely *Microsporum canis* (*M. canis*) and *Trichophyton mentagrophytes* (*T. mentagrophytes*) were shown to produce *in vitro* peptide inhibitors that inhibit papain, cathepsins B and L, but not cathepsin H (22, 23). Different cysteine, serine and aspartic proteases and their inhibitors are involved in the immune response so we designed an experiment based on an animal model to study the effect of the fungal inhibitor on host immune response. Since the isolated peptide *T. mentagrophytes* inhibitor had no cytotoxic effect on baby hamster liver cells (BHL cells; the data not shown), its potential effect on proliferation of lymphocytes and apoptosis of immune cells in ovalbumin immunised mice was studied as well, as was its effect on the processing and presentation of ovalbumin as an antigen. To follow the 3 R rule in animal experiment, we used a minimal yet accepted number of mice which corresponds to small-sample statistics. We also tried to combine the *in vivo* and *in vitro* studies to test as much variables as possible.

Cell proliferation was studied using the commercial enzyme immune test BrdU Cell Proliferation Assay. The results (Figure 1) show that the measured proliferation was as expected the highest in cells that were stimulated with PHA. The PHA lecithin is a known mitogen that non-specifically stimulates the proliferation of lymphocytes T. Proliferation was not detected after all other specific stimulations. The results indicate that the treatment of mice with specific antigens did not activate the specific cellular immunity and that the peptide inhibitor had no effect on mice lymphocyte proliferation. The dermatophyte inhibitor was studied also regarding antibody immunity, where specificity of antibodies derived from mice of the Control and experimental groups was tested against the defined antigens applying the Western blot method.

Apoptosis was measured using a commercial photometric enzyme immunoassay for *in vitro* determination of cytoplasmic histone-associated DNA fragments. Figure 2 shows that the

measured apoptosis was the highest in all groups of mice (Control, Inhibitor, Ovalbumin, Inhibitor + Ovalbumin) after stimulation of cell cultures with ovalbumin. Cell cultures treated with the *T. mentagrophytes* inhibitor or the mix of ovalbumin and the fungal inhibitor exhibited lower detected apoptosis. The apoptosis between differently treated cell cultures was statistically significant. It can be concluded from the results of our experiment that the *T. mentagrophytes* peptide inhibitor supressed cell apoptosis. One could speculate that the role of the fungal inhibitor is to diminish cell apoptosis and thus influence the outcome of an infection and/or to modulate host inflammatory response during the infection. To elucidate the role of the *T. mentagrophytes* inhibitor further, the inhibition of caspases and not only cathepsins should be tested.

Helper T cells recognize antigens in the form of peptides bound to the MHC class II molecules on the surface of antigen presenting cells. Those peptide determinants are generated in acidic compartments of APC by a series of events including denaturation, reduction and proteolysis which are collectively referred to as endocytic antigen processing (24). To establish if the peptide *T. mentagrophytes* inhibitor affects processes in antigen presentation of ovalbumin, the method of Western blotting was applied. The method was used to determine whether antibodies from differently treated mice differ in their specificity as a result of modified antigen processing. Ovalbumin processed by different proteolytic enzymes (papain, cathepsins B and L) was transferred onto a membrane using electroelution. Membranes with ovalbumin digestions were incubated with sera belonging to all four groups of mice (the Control Group, the Group Ovalbumin, the Group Inhibitor and the Group Inhibitor + Ovalbumin). When anti-mouse IgG were used as detector antibodies, no bands were visible on the membrane incubated with serums of the Control Group of mice (Figure 3). This was expected since mice in the Control Group were not in contact with the antigen and did not develop antibodies against ovalbumin or its fragments. Similarly, we observed only a few non-specific bands on the membranes incubated with sera belonging to mice that were treated with the fungal inhibitor. This can be explained in the manner already mentioned above – mice did not develop the antibody immune response. The bands appear on the spot where ovalbumin digested with papain was applied. The cause of such staining could be cross-reactivity against antigens that are similar to ovalbumin. In this case, the exact staining pattern should have appeared also in the presence of antibodies from the Control Group of mice. A more probable cause for the presence of bands on the membrane incubated with the sera of the group that was treated with the inhibitor is the effect of a dermatophyte peptidase inhibitor on "daily" antigen presentation. This effect could have changed the specificity of antibodies against antigens that mice were exposed to and the above mentioned cross-reactivity could have appeared. More obvious differences were observed in the staining pattern of protein fragments between the group of mice that was immunised with ovalbumin (the Group OVA) and the group of mice that was treated with the fungal inhibitor and immunised with ovalbumin (the Group I + OVA). Mice in both groups were immunised with the ovalbumin so antibodies against it could have developed. The only difference between the two groups was that the mice in the Group I + OVA were also treated with the fungal inhibitor. The inhibitor could have inhibited or altered the activity of peptidases which are involved in the antigen presentation of ovalbumin so the recognition of peptides and consequently specificity of antibodies can be different. The differences in the band pattern and intensity were observed in all samples digested with peptidases. In the case of papain digestions, we noticed 3 bands with molecular masses of around 20 kDa on the membrane incubated with the serum from mice in the Group OVA while only one band was visible on the membrane incubated with sera of the Group I + OVA. In cathepsin B digestions, the membrane incubated in the presence of sera from the Group OVA, 3 bands are visible at around 43 kDa. On the other hand only 2 bands are seen on the membrane incubated with the sera of mice from the Group I + OVA. Major differences were observed in cathepsin L digestions. Two bands are visible at around 43 kDa on the membrane incubated with sera of mice from the Group OVA, while only one band is seen on the membrane incubated in the presence of antibodies from the Group I + OVA. In addition, two protein fragments at around 34 and 95 kDa are visible on the same membrane. It appears as if ovalbumin was processed differently in the Groups OVA and I + OVA due to the selective inhibition of proteolytic enzymes by the fungal

inhibitor. The same effect was not observed when anti-mouse IgM antibodies were used (the data not shown). There are two reasons for such a result, namely IgM antibodies develop early in the primary immune response and are not so specific and, on the other hand, specific IgG antibodies result from the secondary, more specific and more abundant immune response.

The results of our research can not fully answer which stages of antigen presentation the dermatophyte cysteine peptidase inhibitor affects. It is possible that due to the selective peptidase inhibition the fungal inhibitor influences the degradation of ovalbumin so different antigen peptides appear in the MHC II molecules. On the other hand, the fungal inhibitor might affect the processes in maturation of the MHC II molecules – the removal of Ii chain or loading of antigen peptides into the binding groove.

The biological function of a peptide cysteine peptidase inhibitor from the dermatophyte fungus *T. mentagrophytes* is still unknown. Although our studies demonstrated the possible effect of the fungal inhibitor on antigen processing and apoptosis, further experiments are needed to elucidate the physiological mechanisms involved in the complex host – the dermatophyte interaction during the infection. Also, selective inhibition of cysteine peptidases with the purified *T. mentagrophytes* inhibitor may have important therapeutic potential in modulating immune responses in the future.

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References

1. Champan HA, Riese RJ, Shi GP. Emerging roles for cysteine proteases in human biology. Annu Rev Physiol 1997; 59: 63–88.

2. Monod M, Capoccia S, Léchenne B, Zaugg C, Holdom M, Jousson O. Secreted proteases from pathogenic fungi. Int J Med Microbiol 2002; 292(5/6): 405–19.

3. Dickinson DP. Cysteine peptidases of mammals: their biological roles and potential effects in the oral cavity and other tissues in health and disease. Crit Rev Oral Biol Med 2002; 13(3): 238–75.

4. Monod M. Secreted proteases from dermatophytes. Mycopathologia 2008; 166: 285–94.

5. Rawlings ND, Barett AJ, Bateman A. MEROPS: the peptidase database. Nucleic Acids Res 2010; 38: D227–33.

6. Turk B, Turk D, Salvesen GS. Regulating cysteine protease activity: essential role of protease inhibitors as guardians and regulators. Curr Pharm Des 2002; 8: 1623–37.

7. Leung-Toung R, Li W, Tam TF, Karimian K. Thiol-dependent enzymes and their inhibitors: a review. Curr Med Chem 2002; 9(9): 979–1002.

8. Rawlings ND, Tolle DP, Barrett AJ. Evolutionary families of peptidase inhibitors. Biochem J 2004; 378: 705–16.

9. Bird PI, Trapani JA, Villadangos JA. Endolysosomal proteases and their inhibitors in immunity. Nat Rev Immunol 2009; 9(12): 871–82.

10. Honey K, Rudensky AY. Lysosomal cysteine proteases regulate antigen presentation. Nat Rev Immunol 2003; 3(6):472–82.

11. Berdowska I. Cysteine proteases as disease markers. Clin Chim Acta 2004; 342: 41–69.

12. Colbert JD, Matthews SP, Miller G, Watts C. Diverse regulatory roles for lysososmal proteases in the immune response. Eur J Immunol 2009; 39: 2955–65.

13. Lennon-Duménil AM, Bakker AH, Wolf-Bryant P, Ploegh HL, Lagaudriére-Gesbert C. A closer look at proteolysis and MHC-class-II-restricted antigen presentation. Curr Opin Immunol 2002; 14: 15–21.

14. Van Eijk M, van Noorden CJF, de Groot C. Proteinases and their inhibitors in the immune system. Int Rev Cytol 2003; 222: 197–236.

15. Weitzman I, Summerbell RC. The dermatophytes. Clin Microbiol Rev 1995; 8(2): 240–59.

16. Mendez-Tovar LJ. Pathogenesis of dermatophytosis and tinea versicolor. Clin Dermatol 2010; 28: 185–9.

17. Lecaille F, Kaleta J, Bröme D. Human and parasitic papain-like cysteine proteases: their role in physiology and pathology and recent developments in inhibitor design. Chem Rev 2002; 102: 4459–88.

18. Atkinson HJ, Babbitt PC, Sajid M. The global cysteine peptidase landscape in parasites. Trends Parasitol 2009; 25(12): 573–81.

19. Dainichi T, Maekawa Y, Ishii K, et al.

Nippocystatin, a cysteine protease inhibitor from *Nippostrongylus brasiliensis*, inhibits antigen processing and modulates antigen-specific immune response. Infect Immun 2001; 69: 7380–6.

20. Dahl MV. Suppression of immunity and inflammation by products produced by dermatophytes. J Am Acad Dermatol 1993; 28(5 Pt 1): S19–23.

21. Dahl MV. Dermatophytosis and the immune response. J Am Acad Dermatol 1994; 31(3 Pt 2): S34–41.

22. Mihelčič D. Znotrajcelični inhibitorji cisteinskih proteaz dermatofita *Microsporum canis*: magistrsko delo. Ljubljana: Univerza v Ljubljani, Veterinarska fakulteta, 2003.

23. Premrov Bajuk Blanka. Izolacija in karakterizacija proteinskih in neproteinskih inhibitorjev cisteinskih peptidaz dermatofita *Trichophyton mentagrophytes* ter njihov vpliv na gostitelja: doktorska disertacija. Ljubljana: Univerza v Ljubljani, Veterinarska fakulteta, 2011.

24. Rodriguez GM, Diment S. Destructive proteolysis by cysteine proteases in antigen presentation of ovalbumin. Eur J Immunol 1995; 25: 1823–7.

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B. Premrov Bajuk, M. Drobnič Košorok, T. Malovrh

Povzetek: Za proučevanje vpliva zaviralca cisteinskih peptidaz iz dermatofita *Trichophyton mentagrophytes* (*T. mentagrophytes*) na gostitelja smo uporabili mišji model. Zaviralec smo izolirali iz citosola glive, ki smo ga pridobili z mehanskim in ultrazvočnim razbitjem micelija. Miši seva BALB/c smo razdellili v štiri skupine. Miši so bile 0., 5. in 15. dan tretirane s fiziološko raztopino (kontrolna skupina), izoliranim glivnim zaviralcem (skupina I), glivnim zaviralcem in ovalbuminom (skupina I + OVA), ali samo z ovalbuminom (skupina OVA). 29. dan smo mišim odvzeli vzorce krvi, jih nato evtanazirali in jim v aseptičnih pogojih odstranili vranico. Celice iz vranice smo gojili v mikrotitrskih ploščah in jih uporabili za meritve celične proliferacije in apoptoze. Meritve smo izvedli s komercialno dostopnimi testnimi kompleti. Serume mišk smo uporabili za imunski odtis, s katerim smo proučevali učinek glivnega zaviralca na procesiranje in predstavljanje ovalbumina kot antigena. Z omenjeno metodo smo poskušali preveriti, ali se protitelesa iz serumov različno tretiranih miši razlikujejo po svoji specifičnosti. S poskusi smo dokazali, da peptidni glivni zaviralec zavira apoptozo celic iz vranic miši, nima pa vpliva na proliferacijo limfocitov. Na podlagi rezultatov prenosa proteinov na membrano z imunodetekcijo smo ugotovili, da glivni zaviralec najverjetneje vpliva na procesiranje ovalbumina.

Ključne besede: cisteinska peptidaza; zaviralec; imunski sistem; celična proliferacija; predstavljanje antigenov; apoptoza