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Where do the immunostimulatory effects of oral proteolytic enzymes ('systemic enzyme therapy') come from? Microbial proteolysis as a possible starting point

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Summary Enteric-coated proteolytic enzyme preparations like Wobenzym® and Phlogenzym® are widely used for the so-called 'systemic enzyme therapy' both in humans and animals. Numerous publications reveal that oral proteolytic enzymes are able to stimulate directly the activity of immune competent cells as well as to increase efficiency of some of their products. But origins of the immunostimulatory effects of oral proteolytic enzymes are still unclear. The hypothesis described here suggests that it may be proteolysis of intestinal microorganisms that makes the immune competent cells to work in the immunostimulatory manner. The hypothesis was largely formed by several scientific observations: First, microbial lysis products (lipopolysaccharides, muropeptides and other peptidoglycan fragments, β-glucans, etc.) are well known for their immunostimulatory action. Second, a normal human being hosts a mass of intestinal microorganisms equivalent to about 1 kg. The biomass (mainly due to naturally occurring autolysis) continuously supplies the host's organism with immunostimulatory microbial cell components. Third, the immunostimulatory effects resulting from the oral application of exogenously acting antimicrobial (lytic) enzyme preparations, such as lysozyme and lysosubtilin, are likely to be a result of the action of microbial lysis products. Fourth, cell walls of most microorganisms contain a considerable amount of proteins/peptides, a possible target for exogenous proteolytic enzymes. In fact, several authors have already shown that a number of proteases possess an ability to lyse the microbial cells *in vitro*. Fifth, the pretreatment of microbial cells (at least of some species) *in vitro* with proteolytic enzymes makes them more sensitive to the lytic action of lysozyme and, otherwise, pretreatment with lysozyme makes them more susceptible to proteolytic degradation. Sixth, exogenous proteases, when in the intestines, may participate in final steps of food-protein digestion. The resulting food-borne peptides have recently been shown to be potential activators of microbial autolysis. The main question that needs to be answered in order to verify the hypothesis is whether oral proteases are able (and to what extent) to lyse/mediate lysis of intestinal microorganisms *in situ*. Methods based on up-to-date molecular biology techniques to allow investigation of the influence of exogenous proteases on microbial lysis processes *in vivo* (in the

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intestines) need to be developed. Research testing of this hypothesis may have an important impact in development of novel preparations for the systemic enzyme therapy.

Introduction

Enteric-coated proteolytic enzyme preparations like Wobenzym® and Phlogenzym® are widely used for the so-called 'systemic enzyme therapy' both in humans and animals. Numerous publications, including monographs [1,2] and specialized journal issues (e.g., Int J Immunother 1997;13(3/4) and 2001;17(2–4)), reveal that oral proteolytic enzymes are able to stimulate directly the activity of immune competent cells as well as to increase efficiency of some of their products. But origins of the immunostimulatory effects of oral proteolytic enzymes are still unclear.

The hypothesis

Recent research suggests that it may be proteolysis of intestinal microorganisms that makes the immune competent cells to work in the immunostimulatory manner. The facts in support of the hypothesis are as follows:

1. Microbial lysis products (lipopolysaccharides, muropeptides and other peptidoglycan fragments, β-glucans, etc.) are well known for their immunostimulatory action (see [3–6] for reviews). Their health benefits are confirmed by the fact that a list of immunostimulants applied in clinical practice [4] is composed for the main part of microbial cell-derived substances.
2. A normal human being hosts a mass of intestinal microorganisms equivalent to about 1 kg [7]. The biomass (mainly due to naturally occurring autolysis) continuously supplies the host's organism with immunostimulatory microbial cell components and thus according to Bocci [8] represents 'the neglected organ having a crucial immunostimulatory role'.
3. The immunostimulatory effects resulting from the oral application of exogenously acting antimicrobial (lytic) enzyme preparations, such as lysozyme and lysosubtilin, are thought to be based on the action of microbial lysis products [9–12]. The same holds true for the oral application of some food-grade substances stimulating microbial autolysis, such as food-protein hydrolysates [13–16].
4. Cell walls of most microorganisms contain a considerable amount of proteins/peptides, a possible target for exogenous proteolytic enzymes [17,18]. In fact, several authors have already shown that a number of proteases possess an ability to lyse the microbial cells *in vitro* [19–25]. Some proteolytic enzymes of bacterial origin have even been given a name of 'lytic proteases' [26]. It remains to speculate whether the lysis might result from direct action of proteases on proteinous microbial cell wall components responsible for integrity of the cell (e.g., covalently linked proteins in fungal cell walls) [27] or from proteolytic activation of the microbial autolytic enzymes [27,28].
5. It is known that pretreatment of microbial cells (at least of some species) *in vitro* with proteolytic enzymes makes them more sensitive to the lytic action of lysozyme [21] and, otherwise, pretreatment with lysozyme makes them more susceptible to proteolytic degradation [29]. Thus, it is quite realistic to suppose that (in the presence of intestinal lysozyme) the above sequences of events also occur in *in vivo* systems. It may be added, that, in general, a combination of lysozyme and trypsin (an authorized protease) is one of the most powerful systems for enzymatic lysis of microorganisms [27].
6. Exogenous proteases, when in the intestines, may participate in final steps of food-protein digestion. The resulting food-borne peptides (these may differ from the ones released by the action of endogenous gastrointestinal proteases) have recently been shown to be potential activators of microbial autolysis [16,30].

Testing the hypothesis

The main question that needs to be answered in order to verify the hypothesis is whether oral proteases are able (and to what extent) to lyse/mediate lysis of intestinal microorganisms *in situ*. Methods based on up-to-date molecular biology techniques to allow investigation of the influence of exogenous proteases on microbial lysis processes *in vivo* (in the intestines) need to be developed. Research testing of this hypothesis may have an important impact in development of novel preparations for the systemic enzyme ther-

apy. In this regard, it is quite believable that a future list of most efficient preparations will include acid-protected bacterial protease ones as some representatives thereof have already revealed promising features [31,32]. We can also expect that the research will bring the long-lasting dispute over the question of where does the borderline between 'proteases', 'lytic proteases', and 'lytic enzymes' lie to an end.

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