

## Nutrition Discussion Forum

### How food-borne peptides may give rise to their immunostimulatory activities: a look through the microbiologist's window into the immunologist's garden (hypothesis)

Food proteins supply organisms not only with the energy needed for survival, but also with bioactive compounds, including food-borne peptides, which are beneficial to health. The beneficial effects of food-borne peptides have led to their proposal as potential nutraceuticals for food and pharmaceutical applications. Peptides with antimicrobial and immunostimulatory activities are among the possible nutraceuticals, although the mechanisms of their action and the link between the two activities (if it exists) are not well understood. A possible mechanism for the antimicrobial action of food-borne peptides is stimulation of the microbial autolytic system, as determined by *in vitro* studies. I propose a hypothesis that at least some food-borne peptides activate the autolytic processes of the intestinal microflora *in situ* and thus cause the release of microbial lysis products; these lysis products are well-known immunoenhancers and may account for the immunostimulatory effects of consumption of food proteins.

*'Medicine and food have a common origin.'*

Ancient Chinese saying (see Arai *et al.* 2002)

*'Let food be thy medicine and medicine be thy food.'*

Hippocrates (see Milner, 2002)

The high nutritional value of protein-rich foods has always attracted living beings. Recent advances indicate that food proteins supply the organism not only with the energy needed for survival, but also with bioactive compounds beneficial to health.

Bioactive peptides encrypted in food protein molecules remain inactive until liberated in the digestive tract by proteolytic enzymes. Once liberated these peptides carry out a wide range of functions. Many food-derived peptides possess bioactive properties (antimicrobial, immunostimulatory, antihypertensive, mineral binding, opioid, etc.) and are often multifunctional, i.e. have two or more different actions (Matin & Otani, 2001; Clare *et al.* 2003; Floris *et al.* 2003; Meisel & FitzGerald, 2003; Pellegrini, 2003; Yamamoto *et al.* 2003; Yoshikawa *et al.* 2003). The number of published papers on the topic is increasing every year.

Because of their bioactive properties, some peptides have been claimed to be potential nutraceuticals for food and pharmaceutical applications, and some of them have already found their place in Foods for Specified Health Use products (Arai *et al.* 2002; Ashwell, 2002). Although opportunities for designing foods on the basis of food-derived bioactive peptides seem to be scientifically and technologically sound (Arai *et al.* 2002; Milner, 2002; Kitts & Weiler,

2003; Korhonen & Pihlanto, 2003), much work needs to be done before the idea is put into practice successfully; this work includes better evaluation of health benefits, as well as broadening our understanding of the molecular events by which peptides influence biological processes. As a personal contribution in this regard as a result of 20 research years spent studying microbial lysis processes and another 10 years devoted to the search for potential immunostimulants, I propose a hypothesis for a possible link between the antimicrobial and immunostimulatory effects of food-borne peptides, the former effect being thought to be expressed through a mechanism involving activation of the microbial autolytic system.

### Food-borne immunostimulatory and antimicrobial peptides

Theoretically any plant or animal protein that enters the digestive tract is capable of generating the appearance (and ensuing effects) of immunostimulatory and antimicrobial peptides. At present, milk is considered to be the richest (or at least the best studied) source of these bioactive compounds. The active peptides in milk may originate from its various constituents (casein, including its various fractions:  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, lactoferrin, etc.). Moreover, the peptides may form in some fermented milk products (e.g. cheese) as a consequence of food processing or storage (Korhonen & Pihlanto, 2003).

Properties of food-derived immunostimulatory and antimicrobial peptides are described in reviews by Matin & Otani (2001), Clare *et al.* (2003), Floris *et al.* (2003), Korhonen & Pihlanto (2003), Meisel & FitzGerald (2003) and Pellegrini (2003). Because of space limitations, I will stress only the main points regarding the peptides that I consider as being of importance to grasp the meaning of the hypothesis.

Bioactive peptides usually contain 3–20 amino acid residues per molecule. The peptides that express only one of the effects (immunostimulatory or antimicrobial), or both together, are known, but the relationship between these two activities (if any) still remains without an explanation. Moreover, although the roles of immunocompetent cells (macrophages, lymphocytes, Peyer's patch cells, etc.) in the immunostimulatory activities of peptides have been intensively studied for several years, the true mechanisms by which the immune system is affected remain to be elucidated. Similarly, the mechanisms of actions of the antimicrobial peptides are still unclear, although their basicity, hydrophobicity and structural characteristics are thought to play a crucial role. There is some correlation between the mode of action of cationic food-derived antimicrobial peptides (not all of them are cationic) and that of the peptides naturally occurring in the organism

as components of the immune system (Scott & Hancock, 2000; Powers & Hancock, 2003; Zhang & Falla, 2004). The involvement of the microbial autolytic system into the antimicrobial activities of food-derived peptides has also been highlighted (Biziulevičius *et al.* 2002).

### The microbial autolytic system and its stimulation

The microbial autolytic system is composed of a set of enzymes (called autolysins) that cause damage to the essential components responsible for the integrity of the cell wall. In bacteria these are peptidoglycan (murein) hydrolases, with five classes of enzymes differing in their specificity for the various covalent linkages within the peptidoglycan (Shockman & Höltje, 1994); in fungi, lytic endopeptidases, endoglucanases and chitinases are considered to be the main autolytic enzymes (White *et al.* 2002). Autolysins are essential for the growth and division of the microbial cell wall. Moreover, the integrity of a microbe depends entirely on the precise control of its autolytic system. On the other hand, as the control of autolysin activity still remains a mystery (Smith *et al.* 2000), strategies for lysing microbes by direct activation of their autolytic system are among possible approaches for antimicrobial drug development (Goldman & Branstrom, 1999; Koch, 2000).

The autolytic enzyme system can be stimulated by pH, temperature and pressure, as well as chemicals such as mineral salts, EDTA, organic solvents or surfactants, with dissipation of the proton motive force being the most reliable mechanism of microbial autolysis induction (Smith *et al.* 2000). However, it is difficult to imagine how these measures applied *in vitro* can be safely implemented *in vivo*.

About 20 years ago it was declared that some food protein hydrolysates are capable of stimulating the microbial autolytic system (Kislukhina *et al.* 1981), but it was only recently that this was supported by data from the experimental studies with tryptic casein hydrolysate (Biziulevičius *et al.* 2002). The autolytic enzyme systems of twenty-four microbial (bacterial and fungal) strains were stimulated *in vitro*. Tryptic casein hydrolysate was effective as an antimicrobial agent when investigated in a newborn-calf colibacillosis model. There is no evidence to suggest that therapeutic, prophylactic and non-specific immunity-enhancing effects (Biziulevičius *et al.* 2003) were due to *in vivo* microbial autolysis induction by tryptic casein hydrolysate. However, it is presumed that an increased resistance of animals to infection might result from a synergy between its effect on microbial autolysis and the immunostimulatory effect of cell wall fragments from lysed microbial cells.

### Microbial lysis products as immunostimulants

Microbial lysis products (muropeptides and other peptidoglycan fragments, lipopolysaccharides, fungal cell wall glucans, etc.) are well known for their immunostimulatory actions (Werner & Jollès, 1996; Alexander & Rietschel, 2001; Brown & Gordon, 2003; Beutler, 2004). Without going into details of the immunopharmacological

significance of these compounds, it should be added that a great amount of work by scientists and clinicians (not to mention the contribution of the pharmaceutical industry) has meant that at least a half of the immunostimulants being applied in clinical practice are based on microbial cell-derived substances (Werner & Jollès, 1996).

Man hosts about 1.2 kg bacteria located mainly in the gut lumen, which according to Bocci (1992) represents 'the neglected organ having a crucial immunostimulatory role'. Thus, there is a possibility of *in vivo* induction of the release of microbial lysis products with the aim of achieving health benefits, in addition to the lysis products resulting from naturally occurring microbial autolysis (Bocci, 1992). In fact, the far-sighted hypothesis of Jollès (from as long ago as 1976) on the immunostimulatory effects of products originating from microbial cells solubilized by lysozyme *in situ* in the intestines has been confirmed (Sava, 1996). Moreover, although direct hydrolysis of the peptidoglycan has always been considered to be the main mechanism of its bactericidal action, the possibility of the triggering of microbial autolysis by lysozymes has also been shown (Ibrahim *et al.* 2001); this effect may be attributed to other cationic proteins present in the human body (Ginsburg, 2001, 2004). The potential of food-borne peptides in this regard was discussed on p. 1010.

### The hypothesis

The evidence cited earlier strongly suggests to me the hypothesis that at least some food-borne peptides may activate the autolytic processes of the intestinal microflora *in situ* and thus result in the release of microbial lysis products, these being finally accountable for the immunostimulatory effects achieved from consumption of food proteins. By submitting this hypothesis for consideration, I do not intend to convince the reader in any way that all food-borne peptides are acting on intestinal microflora in the proposed manner or that all immunity-enhancing effects resulting from consumption of food proteins are expressed through the cascade of events as proposed. Rather I want to show that existence of such a phenomenon is quite realistic.

### Testing the hypothesis

The main question that needs to be answered in order to verify the hypothesis is whether the stimulation of the microbial autolytic system by the food-borne peptides *in vitro* is expressed *in vivo* as well. Methods based on up-to-date molecular biology techniques to allow investigation of the influence of food-borne peptides on the microbial autolysis processes *in vivo* (in the gastrointestinal tract) need to be developed.

There are other areas to be addressed in the hypothesis. Do the food-borne peptides ascribed as antimicrobial possess immunostimulatory properties and, conversely, do peptides known for their immunoenhancing properties act as antimicrobials? Are all the antimicrobial food-borne peptides capable of stimulating the microbial

autolytic system and which peptides are the most active? Are the antimicrobial food-borne peptides of value in the treatment or prophylaxis of intestinal infections? Are there any prospects for a diet-based approach to management of microbial infections? What about the enhancement of immunity? The answers to these and similar questions are already appearing in the scientific literature, and provide indirect support for the hypothesis. Questions of more general interest that have been emphasized earlier, such as, for example, the elucidation of the mechanisms of microbial autolysis induction, are being successfully answered in several laboratories all over the world. On the other hand, as the content of the hypothesis is multidisciplinary in nature, it is obvious that successful testing of the hypothesis can only be achieved by cooperation among a wide range of specialists (microbiologists, immunologists, nutritionists, physicians, etc.).

According to Chandra (1996), 'the era of nutritional manipulation of the immune system has finally dawned and it brings with it the promise of using diet and nutrition as innovative powerful tools to reduce illness and death caused by infection.' We may have come to the time when any new ideas in this regard are worth being discussed.

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