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BACTERIAL PEPTIDES AS IMMUNOMODULATORS

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SUMMARY

Mucosal surfaces are habitats for the physiological microflora and are closely related to the mucosal immune compartment (mucosa-associated lymphoid tissue, MALT). Recently, considerable evidence has been accumulated showing that defined members of the physiological microflora express/liberate low molecular weight substances (peptides) which apparently are essential for the adequate immune response of the host. Biochemical analysis of microbial substances (originating from *Propionibacterium avidum* and other microorganisms) revealed reproducible chromatographic fractions with immunopotentiating and antitumour activities.

INTRODUCTION

The importance of the physiological microflora has recently been shown since, apparently, it guarantees the adequate function of organs such as gastrointestinal (GI) tract, skin and immune system (*van der Waaij*, 1985; *Roszkowski* et al., 1988; *Pulverer* et al., 1990a). The attention of many physicians had been focused primarily on the therapy of infections without sufficient notice being given to side effects, e.g. microbial dysbiosis and immunocompromisation, respectively. Data derived from experiments involving the GI tract of humans and animals provide some outline of the immune responses associated with the intestinal mucosal compartment. The mucosa-associated lymphoid tissue (MALT), the primary source of immunological function, extends beyond the intestine and consists of the gut-associated (GALT), bronchial-associated (BALT) and duct-associated lymphoid tissues (DALT). Thus, virtually every mucosal surface of the body has the ability to respond to and to induce effector cells capable of protecting the host from potentially harmful organisms or antigens (*Kagnoff*, 1987; *Kagnoff* et al., 1987; *Sim*, 1995). Our understanding of these defence mechanisms and how they equip the host for its continuing conflict against pathogenic organisms and potentially harmful substances deposited on mucosal surfaces has a wide range of biological and medical applications. For example, studies on mucosal immunity might lead to more effective methods of immunoprophylaxis against infections, neoplastic, and auto-immune diseases (*Araneo* et al., 1996).

PHYSIOLOGICAL MICROFLORA PROVIDES IMMUNOMODULATING SUBSTANCES

Previous studies suggested that the physiological microflora exert a stimulus on certain immune functions, since antibiotic decontamination of experimental animals resulted in immunosuppression and modification of anti-tumour immunity (*van der Waaij*, 1982, 1988; *Gorbach* et al., 1988; *Roszkowski* et al., 1984, 1985, 1993). In the course of investigations certain members of the BALB/c-mouse GI-tract microflora (e.g. *Bacteroides* sp., *Clostridium* sp., *Lactobacillus* sp., and *Propionibacterium* sp.) were found to liberate low molecular weight substances (MW < 6.500 D). To substantiate the assumption that microbial substances might prime basic immune responses, cultivation procedures were established to provide optimal conditions for their generation and release. In BALB/c mice, antibiotic decontamination of the GI-tract reproducibly resulted in considerable immunosuppression, apparently due to the lack of a specific stimulus. The substitution of low molecular weight substances from microorganisms of the GI-tract, such as *Bacteroides* sp. and *Propionobacterium* sp., to digestive-tract-decontaminated animals (route and interval of administration analogue to the antibiotic) reconstituted the cellular function (peritoneal macrophage phagocytic activity) and lymphatic tissue weight (thymus and spleen). To confirm the hypothesis that the human physiological microflora interacts with the immune system, certain bacteria of human origin were tested for their ability to liberate immunomodulating substances. Two species (*P. acnes* and *S. saprophyticus*) could be shown to release considerable amounts. Substitution of those substances (liberated from bacteria of human origin) to antibiotic-decontaminated (and immuno-compromised) BALB/c mice reconstituted the function of their immune system.

Sephadex chromatography revealed a uniform arrangement of peaks for microbial substances of different origin including those liberated from strains of BALB/c mouse GI-tract microflora (*Bacteroides* sp., *Clostridium* sp., *Lactobacillus* sp., *Propionibacterium* sp.) and those from *P. acnes* and *S. saprophyticus* of human sources (*Pulverer* et al., 1990b). Apparently, the generation and release of microbial substances seems to be a unique property of various members of the physiological microflora resulting in a moderate but constant priming of the immune system (mucosa-associated lymphoid tissue, MALT).

To investigate the immunomodulating potency with another well-established experimental model (Scollay et al., 1984a; Reichert et al., 1986a) substances from *P. acnes* and *S. saprophyticus* were administered to hydrocortisone-treated BALB/c mice. Hydrocortisone-resistant thymocytes have generally been used to investigate the functional maturity since the vast majority of thymocytes surviving the administration of hydrocortisone are of a mature phenotype (*Reichert* et al., 1986a). Intrathymic T-cell differentiation is a process in which immature thymocytes expand and develop by undergoing complicated maturational events leading to the acquisition of immunocompetence and subsequent emigration to the periphery (*Scollay*, 1984; *Scollay* et al., 1984b; *Lefrancois* and *Puddington*, 1995). This thymic microenvironment is thought to exert local influences, which may contribute to the T-cell maturation process (*Reichert* et al., 1986). Quantitative analysis re-

vealed a significantly decreased number of thymocytes after hydrocortisone treatment in BALB/c mice. However, administration of microbial substances apparently stimulated the cell proliferation and maturation, since the number of thymocytes increased significantly compared to non-treated animals.

Administration of microbial substance (released from *S. saprophyticus* or *P. acnes* of human sources) to non-treated BALB/c mice also manifested some immunopotentiation which positively correlated with a remarkable increase of thymus weight. However, weight gain of spleen was less pronounced (*Pulverer* et al., 1990b). It has been shown that T-lymphocyte antigens undergo characteristic changes in their surface density expression as T-cells mature in thymus and lymphoid tissues (*Ledbetter* et al., 1980; *Micklem* et al., 1980; *Reichert* et al., 1986a,b). Quantitative investigations on CD-3 (pan T-cells), CD-8+ (T-cytotoxic/suppressor cells), CD-4+ (T-helper/inducer cells) expression has been facilitated by the use of monoclonal antibodies. Directly fluorescence-conjugated anti CD-3+, anti CD-4+, and anti CD-8+ monoclonal antibodies were each used alone and in combination in FACS (fluorescence-activated cell sorter) staining experiments. The T-cell receptor first appears during thymic ontogeny (Ceredig et al., 1983; Fitch , 1986). Roughly 80% of thymocytes are CD-8+/CD-4+ and a small proportion are CD-8-/CD-4- cells belonging of these thymocyte subsets are thought to be immature (*Micklem* et al., 1980; *Scollay* et al., 1984a,b; *Scollay*, 1984; *Reichert* et al., 1986b; *Lefrancois* and *Puddington*, 1995). In contrast, approximately 15% of thymocytes and nearly all peripheral T-cells express the mature CD-8-/CD-4+ (T-helper/inducer) or CD-8+/CD-4- (T-suppressor/cytotoxic) phenotype (*Ceredig* et al., 1983; *Scollay* et al., 1984a). Administration of microbial substance (liberated from *P. acnes* or *S. saprophyticus*) to BALB/c mice apparently provides a stimulus for the development of lymphoid cells. Accordingly, the numbers of T-helper/inducer cells evidently increased in thymus after injections of microbial substance, whereas T-cytotoxic/suppressor cells did not undergo considerable changes. A calculation of the helper/inducer-suppressor/cytotoxic cell ratio suggested that the administration of microbial substance preferably stimulated the proliferation of T-cells with helper-inducer phenotype (*Pulverer* et al., 1990b). The exact mechanisms for this selection process have not yet been clarified; however, a variety of growth factors and interleukins similarly affect effector tissues (*O'Garra*, 1989; *Heumann* et al., 1994; *Takada* et al., 1995). A further characterisation of the involvement of antigen receptors and/or other cell surface molecules during T-cell development and their activity will provide additional insight into events that determine the T-cell repertoire.

PROPIONIBACTERIA AND ITS COMPONENTS: POTENT IMMUNOMODULATORS

Bacteria (especially *Propionibacterium* species) and their products are known to be highly effective in stimulating the immune system (*Pulverer* et al., 1985). Three species (*Propionibacterium acnes, P. granulosum, P. avidum*) appeared to be of special medical interest, and after evaluating the immuno-active potential of a great number of strains (*Lefrancois* and *Puddington*,

1995). *Propionibacterium avidum* KP-40 and *P. granulosum* KP-45 were selected for further experimental and clinical studies. For practical reasons (e.g. cultivation procedure, biological and immunological standardisation) *P. avidum* KP-40 was preferably introduced for clinical evaluation, although its immuno-active capacity is absolutely identical to *P. granulosum* KP-45.

Recently it was shown that the efficiency of propionibacterial immunomodulation is related not only to the type of tumour involved but also to the bacterial strain used and the route and timing of administration (*Szmigielski* et al., 1982). After optimising these preliminaries, treatment with propionibacteria proved to be of considerable clinical benefit, inducing potent immunostimulation. These data on the obvious efficacy of propionibacterial treatment encouraged the initiation of a prospectively randomised clinical multicentre trial in colorectal carcinoma patients where overall survival, relapse rate, relapse-free interval, metastasis, quality of life, immune response were beneficially modified a single preoperative administration of *Propionibacterium avidum* KP-40 (*Isenberg* et al., 1995).

The obvious therapeutical benefit of *Propionibacterium avidum* KP-40 treatment in neoplastic disease induced further experimental studies. Recently, we investigated and confirmed its stimulating effects on the non-specific immune system (*Pulverer* et al., 1985). During these investigations we were able to determine the effect of *Propionibacterium avidum* KP-40 on thymocyte proliferation, maturation and emigration into peripheral blood using a murine model. Single intraperitoneal administration of the optimal immunomodulating dose of *Propionibacterium avidum* KP-40 (1 mg per mouse, as determined in preceding studies) to BALB/c mice resulted in enhanced thymus weight and accelerated thymocyte maturation (generally leading to emigration of these cells into peripheral blood), followed by enhanced proliferation of immature cells. Furthermore, we found that absolute counts of peripheral blood lymphocytes (PBL) and monocytes (PBM) were significantly enhanced as well as the expression of activation markers (e.g. interleukin (IL)-2 receptors on PBL; MAC-3 antigens on PBM) with peak values 6 days after *Propionibacterium avidum* KP-40 injection.

To evaluate the anti-tumour/anti-metastatic effect of *P. avidum* KP-40 induced immunomodulation, BALB/c-mice were intravenously challenged with RAW 117-H 10 lymphosarcoma cells and checked for liver tumour colonisation as described elsewhere (*Scollay*, 1984). Compared to control group the number of liver colonies was significantly lower in *Propionibacterium avidum* KP-40 treated mice. The above mentioned experiments were analogously performed with chromatographic purified components (peptide fractions 1-3 and 10) of *P. avidum* KP-40 and yielded comparable results.

The ability of bacteria to modulate the immune response to non-related antigens is well documented. Propionibacteria are amongst the most potent immunomodulators stimulating cell populations involved in (non-) specific resistance. Generally, the activated immune system provides protection from infectious pathogens and spread/growth of malignant cells through mechanisms of recognition and elimination. Accordingly, propionibacteria and its defined low molecular weight substances could be shown to be effective in the treatment of infections and neoplastic diseases in experimental and clinical settings. Further studies are currently being performed to confirm these promising data

CONCLUSION AND FUTURE ASPECTS

Mucosal surfaces are habitats of the physiological microflora and are closely related to the mucosal immune compartment (mucosa-associated lymphoid tissue, MALT) which interacts with the systemic immune compartment on separate levels of host defences (*Tomasi* et al., 1980; *Bienenstock* and *Befus*, 1984). It is the first line of defence and has the ability to block antigen-access to the systemic compartment of the host by producing local responses (*Walker* and *Isselbacher*, 1977; *Challacombe* and *Tomasi*, 1980). However, antigens (e.g. microbial substances) can gain access to the MALT and trigger (local) immune responses. In addition, some antigens are able to produce systemic tolerance (*Challacombe* and *Tomasi*, 1980). If these particular antigens gain access to the local immune system, a suppression of the systemic immune response may be induced by suppressor cells which were activated in the MALT and then translocated to the systemic immune compartment (*Walker* and *Isselbacher*, 1977; *Richman* et al., 1981).

Recently, considerable evidence has been accumulated showing that the physiological microflora liberates/expresses low molecular weight substances which, apparently, prime certain immune responses. Investigations on suppression and reconstitution of immune functions depending on the presence of the physiological microflora (respectively on microbial substances liberated from members of the physiological microflora) favoured the hypothesis that symbiotic microorganisms (respectively defined microbial substances) are essential for adequate immune functions. Since those events are generally stimulated and regulated by T-helper/inducer cells, this activity may be explained by the production of growth and differentiation factors. These properties of interleukins and related molecules (e.g. microbial substances) indicate a key role in the positive and negative regulation of antigen-specific cellular and humoral immune responses and in the ontogeny of the immune system. Preliminary investigations suggested that microbial substances may be considered to be potential growth factors (e.g. for fibroblasts, epithelial cells, bone marrow cells, tumour cells) as well as differentiation factors (e.g. for lymphoid cells, bone marrow cells). Accordingly, a wide range of biological and medical applications of these substances should be considered, e.g. (1) specific immunomodulation (with special emphasis on anti-infectious and anti-neoplastic immunity, (2) therapeutical administration of growth and differentiation factors (interleukin-like molecules) (3) specific adjuvant in patients treated with decontaminating antimicrobial drugs (omnispectrum therapy) and, last but not least, (4) a contribution to current knowledge on interactions of the physiological microflora and immune responses.

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