

INFLUENCE OF STAPHYLOCOCCAL LEUKOCIDINS ON PHAGOCYTE AND LYMPHOCYTE ACTIVITY – A COMPARATIVE STUDY

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Key words: leukocidins, lymphocyte activity, phagocyte activity

Leukocidins belong to a group of bicomponent leukotoxins produced by certain *Staphylococcus aureus* strains. Some *in vitro* and *in vivo* studies performed on immunocompetent cell populations in different animal species proved the modulatory properties of leukocidins on the phagocyte and lymphocyte activities.

INTRODUCTION

Staphylococci are gram positive bacteria widespread in many ecosystems. Some species like *Staphylococcus aureus* are responsible for severe animal and human diseases like certain types of skin infections (impetigo, furuncles abscesses), pneumonia, osteomyelitis and cellulitis [Lina, 1999].

Many *Staphylococcus aureus* strains produce a great variety of toxins like cytolytins (alpha-, beta-, gamma- and delta hemolysins and leukocidins), exfoliatins (ETA, ETB, ETC) and superantigenic toxins (toxic shock syndrome toxin, enterotoxins). Leukocidins (Luk) and gamma-hemolysins (Hlg) are bicomponent beta-barrel exotoxins possessing different biological activity [Jeljaszewicz, 2000]. Both toxins are water-soluble and consist of two S class and F class protein components. The two subunits are necessary for biological activity of the toxins. Various *S. aureus* strains produce the following classes:

- S class (molecular weight about 32 kDa) – HlgA, HlgC, LukE, LukS, LukS-PV, LukS-R;
- F class (molecular weight about 34 kDa) – HlgB, LukD, LukF, LukF-R, LukF-PV, LukM [Supersac *et al.*, 1993; Choorit *et al.*, 1995; Gravet *et al.*, 1998; Jeljaszewicz, 2000].

Staphylococcus intermedius produces LukS-I and LukF-I fractions [Prevost *et al.*, 1995; Jeljaszewicz, 2000].

Leukocidin Pantone-Valentine (PVL) is secreted by various staphylococcal strains responsible for dermonecrosis [König, 1995], skin infections, necrotic pneumonia [Gillet *et al.*, 2000, 2002], septicemia [Prevost *et al.*, 1995; Couppie *et al.*, 1997; Lina *et al.*, 1999]. Frequent isolation of *S. aureus* strains secreting leukocidins suggests that together with other virulent factors these toxins could play an important role in acute bacterial infections. Nowadays, a great atten-

tion is being paid to leukocidins in the studies on determination of their structure, mechanism of action and influence on the immunocompetent cells in different animal species.

The molecular structure of leukocidin is not entirely known but some biochemical studies have shown that the toxin molecule is a hexamer. On the other hand some authors have demonstrated that leukocidin could probably have an octameric form [Miles *et al.*, 2001, 2002].

Staphylococcal leukocidins are leukolytic toxins forming cation-selective transmembrane pores in leukocytes and macrophages. The S subunit of the toxin binds to the specific receptor present in the cell membrane and conformational changes allow the F subunit to bind to the formed S subunit-receptor complex. Three S class molecules and three F class molecules form a hexameric (or octameric) pore. Influx of divalent cations to the cell starts enzymatic stimulation and subsequent secretion of inflammatory mediators followed by the cell lysis [König *et al.*, 1994; Siqueira *et al.*, 1997]. Simultaneous secretion of various leukocidins and hemolysins enables bacteria to spread the infection by inhibition of phagocyte and macrophage activity. It is believed that staphylococcal leukocidins possess leukotoxic properties towards human and rabbit polymorphonuclear cells, monocytes and macrophages [König *et al.*, 1997]. No other cell populations were described to be susceptible to the toxins except for human myelotic leukemia cells – HL-60.

Several *in vitro* studies showed the modulatory effect of leukocidins on human phagocytes. Szmigielski *et al.* [1998], using the method of ⁵¹Cr and ⁸⁶Rb release, found cytotoxic effect of leukocidin Pantone-Valentine (PVL) on human peritoneal phagocytes *in vitro*. The toxin induced cell lysis at cytolytic concentrations of 1000–5000 ng/mL. Gravet *et al.* [1998] observed a slight cytolytic effect of leukocidin

LukE+LukD on human phagocytes. The toxin had less destructive potency towards phagocytic cells in comparison to other leukotoxins produced by *Staphylococcus aureus*. Staphylococcal leukocidin subunits combined with other bicomponent toxin proteins like gamma-hemolysins (HlgA+LukD and LukE+HlgB) can form transmembrane pores in human phagocytes. The complexes formed possess different biological activities.

Leukocidin PVL stimulates inflammatory mediator release from human leukocytes *in vitro*. LukS-PVL+LukF-PVL subunits start an increased secretion of IL-8 from human monocytes and macrophages, stimulate the release of oxygen metabolites by neutrophils and release of histamine by basophils [Köller *et al.*, 1993; König *et al.*, 1994]. An increased release of beta-glucuronidase from human polymorphonuclear cells was also noted. Incubation of human neutrophils with leukocidins evokes an increased release of LTB₄ from these cells [Hensler *et al.*, 1994]. Leukocidin Panton-Valentine and leukocidin E/D exert a modulatory influence on the activity of blood phagocytes isolated from dogs. The toxins diminish the metabolic activity and potential killing activity of phagocytes at a cytolytic range of concentrations (1 000–25 000 ng/mL medium). The stimulatory effect on the metabolic and potential killing activity of dog phagocytes is observed at subcytolytic concentrations of the two leukocidins [Siwicki *et al.*, 2001a, 2002b].

The *in vitro* modulatory effect of staphylococcal leukocidin on rabbit phagocytes was described by Szmigielski *et al.* [1966]. A single intravenous injection of leukocidin PVL induced decreased, and subsequently, increased number of peripheral blood granulocytes. The elevated number of myeloid cells in the bone marrow and stimulation of ATP-ase and Na-K-ATP-ase activity in granulocytes was noted. Grójec *et al.* [1981] observed *in vivo* a stimulatory effect on the immune system of mouse and granulocyte proliferation in the bone marrow. Those findings were confirmed later in the histopathological studies. Staphylococcal leukocidin was found to stimulate the granulopoiesis in rabbit after suppression induced by cytostatic drugs. Daily injection of the toxin during the myelosuppression phase caused faster regeneration of the blood system and augmented granulopoiesis manifested by the increased number of nitroblue tetrazolium-reducing granulocytes and the increased level of serum muramidase [Szmigielski *et al.*, 1976]. Staphylococcal leukocidins can influence the activity of fish polymorphonuclear (PMN) and mononuclear (MN) cells. The *in vitro* modulatory influence of leukocidin Panton-Valentine (PVL) and *Staphylococcus intermedius* (Luk-SI) on phagocytes isolated from carp (*Cyprinus carpio* L.) was found recently. Both complete toxins and their S subunits induced statistically significant suppression of respiratory burst activity (RBA) and potential killing activity (RBA) of polymorphonuclear (PMN) and mononuclear (MN) cells at the cytolytic concentrations but the difference between inhibitory potency of the two toxins was visible. Luk-SI expressed its phagocyte-inhibiting activity at lower concentrations than PVL. Suppression of the RBA and PKA could be probably the result of cell membrane damage. Slight but statistically significant stimulation of the RBA and PKA was noted at subcytolytic concentrations of the complete toxin as a result of the unknown enzymatic pathway stimulation.

The biological activity of bicomponent toxin can be modified by prior incubation of phagocytes with some cytokines. Human polymorphonuclear cells turned out to be less toxin-sensitive after their incubation with TNF-alpha, interleukin 3 and interleukin 6 [König *et al.*, 1995].

Leukocidin PVL exhibits cytolytic properties towards human promyelotic leukaemia cells HL-60. When incubated *in vitro* with organic compounds like dimethyl sulphoxide (DMSO) or dimethyl formamide the cells differentiate to myelocytes and metamyelocytes. HL-60 cells exposed to DMSO are six times more sensitive to leukocidin than the not treated HL-60 cells. It was also proved that leukocidin PVL is more toxic to mature myelocytes than promyelocytes [Morinaga *et al.*, 1988]. The results of those studies suggest the possibility of using leukocidins as biotherapeutic factors in myelotic leukaemia treatment.

Morinaga *et al.* [1993] noted that HL-60 cells incubated with 12-O-tetradecanoylphorbol 13-acetate (TPA) are more sensitive to staphylococcal leukocidin than the not treated HL-60 cells. The differentiated HL-60 cells after 18-h incubation with TPA to macrophages, are more susceptible to the leukolytic activity of leukocidin.

There is a general believe that lymphocytes isolated from higher vertebrates are not susceptible to leukocidins but a few *in vitro* studies have shown that those toxin could induce some effects on the lymphocyte activity. Pfanneberg *et al.* [1975] showed that partially-purified leukocidin produced by bovine P83 strain of *Staphylococcus aureus* caused statistically significant destruction of bovine lymphocytes. However, the examined cells did not show morphological changes during contrast-phase observation. Loeffler *et al.* [1986], using ⁵¹Cr release assay, described a slight but significant cytotoxic influence of leukocidin produced by P83 *Staphylococcus aureus* strain on bovine lymphocytes. The toxic activity towards lymphocytes observed in the studies probably could not be the result of the direct influence of leukocidin itself but it was rather an effect of some other virulent factors like alpha-hemolysins that coexisted in the toxin samples used. On the other hand some authors that used highly purified leukocidin described that the toxin affects lymphocyte activity. Siwicki *et al.* [2001b, 2002a] reported on the *in vitro* immunosuppressive influence of cytolytic concentrations of leukocidin Panton-Valentine and leukocidin LukE/LukD on T and B lymphocyte proliferation in dogs. A slight stimulatory effect on T and B lymphocytes was observed at subcytolytic concentrations of the toxins. Fish lymphocytes also turned out to be sensitive to leukocidin. Bownik *et al.* [2000] described the modulatory influence of leukocidins: PVL and Luk-SI on the proliferative ability of T and B lymphocytes isolated from carp (*Cyprinus carpio* L.) *in vitro*. The highest suppression of both T and B lymphocyte proliferation was noted at the cytolytic concentrations of the complete toxins used in the experiment with PVL expressing higher inhibitory activity than Luk-SI. The cytolytic concentrations of the S components also induced statistically significant inhibition of T and B lymphocyte proliferation. Subcytolytic concentrations of the complete leukocidin (1.6 and 0.32 ng/mL) distinctly increased T and B lymphocyte proliferative ability. The F subunit did not seem to exert the immunomodulatory influence at any concentration used in the studies. This finding supports the hypothesis that S component is necessary for cytolytic action of bicomponent toxins.

Staphylococcal leukocidins were described to exert stimulatory influence on gamma-immunoglobulin levels in serum. High titres of antileukocidin were observed in patients with staphylococcal infections like osteomyelitis [Butler *et al.*, 1943; Towers *et al.*, 1958].

The toxoid of leukocidin (anatoxin) was used for the first time about 50 years ago in the therapy against staphylococcal infections. The anatoxin did not possess cytolytic properties but induced the immune response to leukocidin in human, mouse guinea pig and rabbit [Gladstone *et al.*, 1957]. Sebek *et al.* [1959] observed the reduction of staphylococcal infections after administration of a toxoid consisting of staphylococcal leukocidin PVL and alpha-hemolysin to pregnant women. Moreover, much smaller number of staphylococcal infections was noted in infants of the treated mothers. Loeffler *et al.* [1986] showed that immunization of cattle with a toxoid of leukocidin produced by P83 strain of *Staphylococcus aureus* enhanced the production of antileukocidin IgG1 detected in serum and milk samples. Specific antibodies isolated from serum of the immunized animals possessed the ability to reduce the cytolytic action of leukocidin towards bovine neutrophils *in vitro*.

There is a need for *in vitro* and *in vivo* comparative studies on the effects of leukocidins on different animal species. The future research would help us to enrich the knowledge on bacterial virulence factors and mechanisms of staphylococcal pathogenesis. Moreover, recombinant mutants of bicomponent toxins or immunotoxins could be used in therapy against staphylococcal diseases and medical treatment in some types of leukaemia.

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