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Short Communication

Leukocytic antimicrobial peptides kill autoimmune T cells

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Summary

Small antimicrobial peptides are abundantly produced by leukocytes. These peptides are active against a broad range of pathogens, notably bacteria, fungi and enveloped viruses, but hardly anything is known about their physiological and pathophysiological relevance. We observed that indolicidin, and to a lesser extent bactenecin, are strongly cytotoxic to rat and human T lymphocytes, while a variety of other cell lines are not affected by these endogenous antibiotics. The defensins HNP-1, HNP-2 and HNP-3, the structurally related but not bactericidal corticostatin, or cecropin P1 did not affect T lymphocyte viability or proliferation. Thus, indolicidin and bactenecin might function as local regulators inhibiting clonal expansion of T lymphocytes during ongoing immune responses. As immunosuppressive agents in the treatment of autoimmune disease, these peptides appear to be of limited potential, as systemic activity of such peptides is low, and we did not observe significant immunosuppressive effects in experimental autoimmune neuritis or encephalomyelitis.

Introduction

Antibiotic peptides are released in large quantities by activated leukocytes. Much interest has focussed on the human defensins HNP-1, HNP-2 and HNP-3. Defensins are 29–34 amino acids long cyclic peptides that share a strictly conserved framework of six cysteines (Ganz et al., 1985; Daher et al., 1988; Lehrer et al., 1991). They jointly make up about 10% of the total cellular protein of neutrophils and 1–2% of alveolar macrophages. Some less well-known members of mammalian anti-microbial peptides are indolicidin, bactenecin, or cecropin which are structurally unrelated to the defensins but share very similar antibiotic activity (Romeo et al., 1988; Lee et al., 1989; Selsted et al., 1992). While their potential role in host defense against bacteria, fungi and viruses is a matter of intense research, they might well serve for other functions in the immune system. This hypothesis is strengthened by the observations of chemotactic activity of such peptides and by their ability to kill some tumor cells in vitro (Sheu et al., 1985; Territo et al., 1989). Antimicrobial peptides are thought to be released at sites of infection and inflammation. Due to their non-specific activity on membranes (Lichtenstein et al., 1988; Kagan et al., 1990) they could most probably interact with a variety of immune and parenchymal cells. We therefore studied the effects of antimicrobial peptides on autoimmune T cells and in rat autoimmune disease of the central and peripheral nervous system.

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Materials and Methods

Peptides

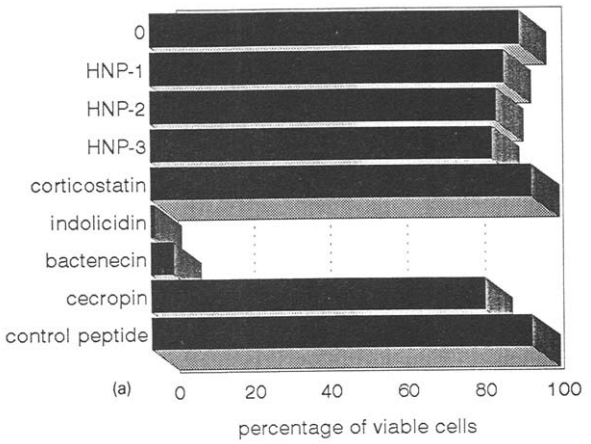
Defensins HNP-1, HNP-2, and HNP-3, bactenecin, and corticostatin were from Bachem, Heidelberg, FRG. Indolicidin was synthesized by Bachem and Multiple Peptide Systems (San Diego, CA) and cecropin P1 was purchased from Peninsula Lab. (Belmont, CA). A control peptide of irrelevant sequence (GSGSSDYNG-SELKTA) was synthesized by Multiple Peptide Systems.

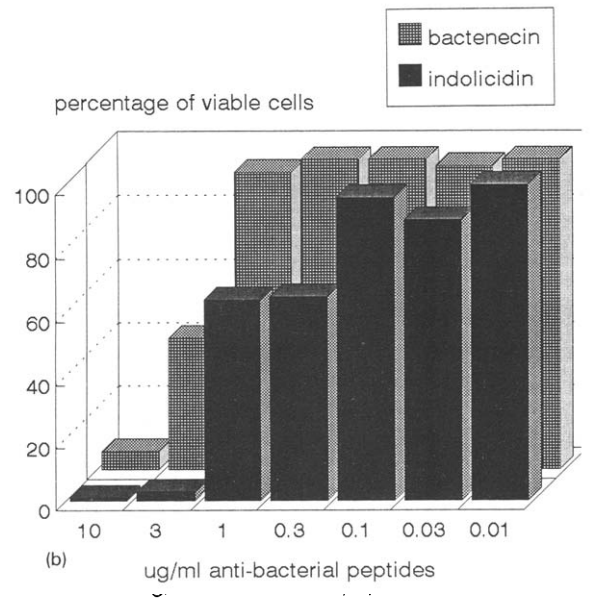
Cell culture

Establishment and culture of T cell lines has been described (Schluesener et al., 1989; Melms et al., 1989; Jung et al., 1991). Human glioblastoma and rat astrocyte cell lines have been characterized previously (Schluesener, 1991; Schluesener and Meyermann, 1991) and all other cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD). Peptides were diluted in PBS and were used in cell cultures up to a final concentration of 10 μg ml^{-1}. Higher concentrations were not used, as massive precipitation was observed with one of the peptides in RPMI medium. Viability of cells was determined by trypan blue dye exclusion (Mishell and Shigii, 1980).

Induction of autoimmune disease and histology

EAN and EAE was induced in 8-week-old Lewis rats as described (Schluesener et al., 1986; Jung et al., 1991, 1992). When signs of neurological disease were becoming evident (Day 11 or Day 12 after immunization) rats received daily i.p. injections of antimicrobial peptides (1 mg kg^{-1} in 1 ml PBS) for a period of 5 days. On Day 17, some EAE rats were killed for





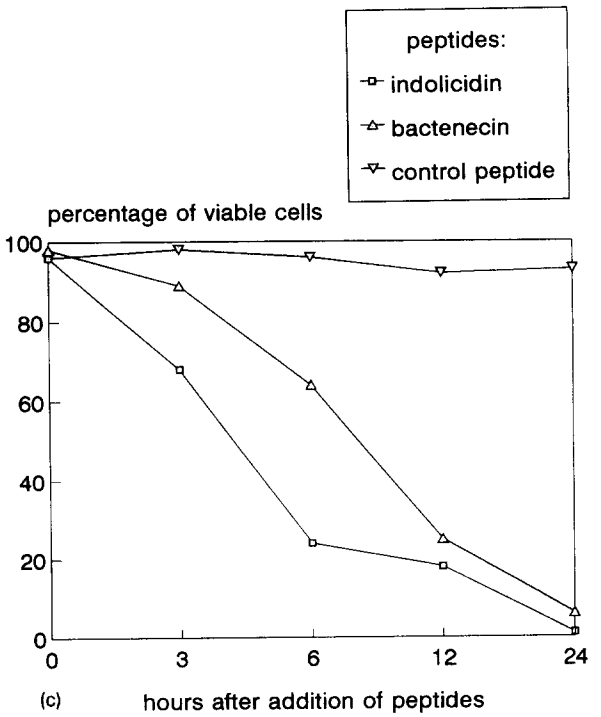


Fig. 1. Cytotoxicity of antimicrobial peptides was analyzed with rat encephalitogenic T cell line S179.3 (Schluesener and Lider, 1989). Line cells were freshly restimulated as described and cultured in flat-bottomed microtiter plates at a final density of 5×10^4 cells/well in 200 μl serum-free RPMI 1640. Antimicrobial and control peptides were added at various concentrations. Viability of cells was analyzed 24 h. later by standard trypan blue dye exclusion (Mishell and Shigii, 1980). Data are displayed as arithmetic means of four replicate experiments; SD was less than 12% of means. (**a**) Cell viability was determined 24 h after addition of peptides (final concentration: 10 μg ml^{-1}). The first bar (0) shows cell viability in the absence of any peptides, the last bar shows viability in the presence of an irrelevant control peptide. Only indolicidin and bactenecin were cytotoxic to line cells, while the defensins HNP-1, HNP-2 and HNP-3, the related corticostatin, and cecropin had no effect on cell viability. Fig. (**b**) Loss of cell viability after addition indolicidin and bactenecin. Final concentration of peptides: 10 μg ml^{-1}. (**c**) Dose–response curve showing cytotoxicity of indolicidin and bactenecin. Cells were cultured in the presence of peptides for 24 h.

histological analysis as described (Schluesener et al., 1986).

Results

Modulatory activity of antimicrobial peptides on T cell proliferation was first tested with rat autoimmune T cell lines specific for myelin basic protein (MBP) or P2 protein.

Maximal inhibition of [^{3}H]thymidine incorporation was observed at doses of 10 μg ml^{-1} of indolicidin. Bactenecin was less inhibitory, and cecropin, the defensins HNP-1, HNP-2, HNP-3, and the structurally related corticostatin did not show any inhibitory activity. Similar results were obtained with T cells grown in IL-2 medium (data not shown). In contrast to inhibition mediated by other immunosuppressive factors like TGF-β (Schluesener et al., 1989), indolicidin and to a lesser extent bactenecin were found to be cytotoxic, with indolicidin killing all T cells at a dose of 10 μg ml^{-1} (Fig. 1). Cytotoxicity of indolicidin was becoming apparent within 3 h after addition of peptide to cell cultures (Fig. 1c). Again, bactenecin was less active and other peptides (see Fig. 1a) were not cytotoxic.

As the effects of antimicrobial peptides are not mediated by specific receptors, but are due to a direct action of the peptides on cellular membranes, cytotoxic activity of the peptides is not restricted to a certain species. Consequently, we observed that a variety of other cells, like human T line cells, human leukemic T cells (Jurkat cell line), EBV-transformed B-lymphoblastoid cell lines, and cells from promonocytic cell line U397 are killed by indolicidin, but are only weakly effected by bactenecin and not at all by cecropin, corticostatin or the defensins. However, overt cytotoxicity is not seen with all cells, as rat astrocytes, human neuroblastoma, astrocytoma, glioblastoma, fibroblast, fibrosarcoma, and melanoma cell lines were not killed by indolicidin in doses of up to 10 μg ml^{-1}.

Such non-specific cytotoxic, immunosuppressive effects on T-lymphocytes might be of importance in the local regulation of the immune response. In addition, such peptides could be of therapeutical use to combat infection and inflammation. We therefore tested whether systemic injections of indolicidin or bactenecin modulate the clinical disease course of experimental autoimmune encephalomyelitis or neuritis. Rats were immunized with MBP or P2 protein and antimicrobial peptides were injected as soon as clinical signs of disease became apparent (Day 11 or Day 12 after immunization). Peptides were daily i.p. injected at a dose of 1 mg per kg rat. However, we did not observe significant suppression of autoimmune disease. This observation is in agreement with reports describing rapid inactivation of such peptides by serum components.

Discussion

Antimicrobial peptides are prominent secretory products of leukocytic cells. While their potential ability to combat bacterial, fungal and viral infections received much interest, other functions of these massively produced peptides are unrecognized so far. Considering their way of action not by signalling via receptors but by non-specifically interacting with cellular membranes, forming damaging pores, it can be anticipated that a variety of other cells in the inflammatory lesion might be affected by these peptides.

It is surprising that these antimicrobial peptides share similar mechanism of action despite their structural diversity. We observed that one of the peptides, indolicidin, is highly cytotoxic to T-line cells. Thus, indolicidin might be structuring the immune response by limiting clonal expansion of certain lymphocytes. In addition, indolicidin or the other antibiotic peptides might modulate function of parenchymal or endothelial cells. Our notion that antimicrobial peptides are modulators — most possibly acting strictly locally — of immune function is supported by the observation that defensins induce chemotactic activity in neutrophils and chemokinetic activity in macrophages (Territo et al., 1989). While such effects have not been seen so far with indolicidin or bactenecin, these peptides might as well induce monocyte and neutrophil recruitment into the lesion.

Notably in autoimmune disease such pore-forming molecules could be of importance to the pathogenic processes as electrochemical signalling used by neurons might be particularly sensitive to the formation of the typical voltage-dependent, weakly ion-selective channels in cellular membranes.

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