Enhancement of host defense against pathogens by antimicrobial peptides : a new approach to combat microbial drug resistance

Does, A.M. van der

Summary and general discussion

Chapter 7

Introduction

The availability of successful anti-infective drugs has taken away much of the prior threat of infectious diseases in society in the last century. Overconfidence in the accomplishments of antibiotics 'the miracle drugs', however, has now been replaced by apprehension, as antibiotic usage in the human and veterinary field has boomed out of control and boomerangs back at us with the emergence of multi-drug resistant pathogens. Bacteria have developed resistance to most of the existing drugs, making infections difficult and sometimes even impossible to treat. A multi-facetted approach will be needed to constrain the impact of antibiotic resistance on treatment options in infectious diseases. Efforts should include proper education and appropriate antibiotic usage, to both physicians and patients and applying strict infection control measures to prevent transmission. Furthermore, alternatives to antibiotic usage in veterinary practice and agriculture should be identified. In addition to all these measures, it is recognized that there is an urgent need for research into and development of new anti-infective drugs as the pharmaceutical pipeline of new types of antibiotics has dried up and new anti-infective drugs will not become available for some time. Infectious diseases societies have taken up these issues and advocate major investments in research and development of new antibiotics. The development of new antimicrobial agents has mainly focused on variants of current anti-infectives. A recognized disadvantage of this approach is the experience that sooner or later, these new drugs suffer the same limitations as the parental drugs, i.e., in the end emergence of multi-drug resistant pathogens. New long-lasting anti-infective agents are yet to be successfully developed and therefore research into alternative agents is essential (1). Antimicrobial peptides are naturally occurring antimicrobial agents of all living beings and these peptides have been pointed out as potential candidates for drug development (2-4).

In the past, research into these peptides has focused on their antimicrobial activity. Soon it was recognized that some of these peptides, further referred to as host defense peptides, have the potential to strengthen or modulate the hosts' immune response resulting in an enhanced resolution of infection (5,6). As host defense peptides act indirectly, via the very broad armamentarium of the hosts' immune systems, development of drug-resistance against such peptides is unlikely. This makes host defense peptides a promising alternative to current anti-infective agents. Among these peptides, one interesting candidate comprises the first eleven amino acids of the N-terminus of human lactoferrin therefore named hLF1-11. This peptide appears active against a variety of

(multi-drug resistant) pathogens (7-9). However, *in vitro* the antimicrobial activity of this peptide is dependent on factors in its environment, such as salt concentration, e.g. the peptide is highly active against pathogens in low (10 mM) salt concentrations, but hardly active at physiological salt concentrations. Given the natural occurrence of lactoferrin and its degradation product lactoferricin, we hypothesized that in addition to its direct antimicrobial effects the hLF1-11 peptide displays immunomodulatory activities. Since the time-span in the *in vivo* assays was usually set at 48 h maximum, we focused on possible effects of hLF1-11 on cells of the innate immune system, more specifically on monocytes and cells derived thereof such as macrophages and dendritic cells.

Effects of hLF1-11 on murine and human monocytes The antimicrobial activity of hLF1-11 was established in mice first. Therefore, we focused our attention on murine cells and first compared the lipopolysaccharide (LPS)-induced cytokine production of hLF1-11-incubated murine monocytes to that of control monocytes. The findings showed that hLF1-11 enhanced the production of IL-6, IL-10, IL-1β and RANTES by monocytes in response to the immunoreactive component of the Gram-negative bacterial cell wall, LPS (chapter 2). After thus establishing that hLF1-11 has the ability to modulate murine monocyte activity, we next investigated the effect of hLF1-11 on human cells, for two reasons. First, we strive to reduce the number of animal experiments if an alternative approach to the problem is possible, e.g., human blood-derived cells. Secondly, since the ultimate goal would be to assess the feasibility and the development of hLF1-11 as potential anti-infective in humans, next we focused on the interaction of hLF1-11 with human immune cells. We observed that hLF1-11 enhanced the production of almost all cytokines tested and of various chemokines by monocytes in response to LPS (chapter 2). As the N-terminus of lactoferrin is the major binding site in this molecule for a microbial structure like LPS and lipid A (10), we next excluded the possibility that binding of hLF1-11 to LPS was responsible for the enhanced cytokine production by hLF1-11-monocytes. In addition, we found that in the absence of microbial stimuli hLF1-11 did not exert any action on monocytes besides reducing the production of some of the chemokines released by these cells. Taken together, these findings indicate that the actions of hLF1-11 are observed mainly after exposure of the hLF1-11-incubated cells to a second stimulus. We next established that the enhancement of the inflammatory response of monocytes by hLF1-11 was not limited to LPS and also included other microbial structures, like diphosphoryl lipid A, flagellin and CL087. These microbial components are recognized by human monocytes and macrophages through specific cell-surface receptors, collectively designated toll-like

receptors (TLR), respectively TLR4, TLR5 and TLR7/8. Interestingly, hLF1-11 hardly affected the inflammatory response of human monocytes upon stimulation with lipoteichoic acid (LTA) or PAM2CSK4, both TLR2 ligands. This was confirmed by measuring downstream activation of the transcription factor NF-kB and mRNA production in response to these toll-like receptor ligands (chapter 2). Based on our observation that hLF1-11 inhibits myeloperoxidase (MPO) (chapter 6) in human monocytes we can offer an explanation for the lack of effect of hLF1-11 on TLR2-mediated activation of monocytes. In short, activation of monocytes via the various toll-like receptors triggers an oxidative burst and induces the release of cytokines/chemokines. However, TLR2 activation by LTA and PAM2CSK4 induced a weak cytokine response by human monocytes (chapter 2). It is hypothesized that the oxidative burst induced by TLR2 ligands is only weak and therefore the role of myeloperoxidase may be small. In such cases, inhibition of myeloperoxidase by hLF1-11 might not have enough consequences to result in differences on cytokine level. Unfortunately it is not known at what level of the TLR-signal transduction route toward cytokine production myeloperoxidase is involved.

Effects of hLF1-11 on monocytes are mediated by inhibition of myeloperoxidase activity

After establishing the effect of hLF1-11 on human monocytes, we were intrigued to identify the target of hLF1-11 on/in monocytes that mediates these effects. We first investigated whether hLF1-11 binds to monocytes. Results showed that hLF1-11 binds to monocytes within minutes and that binding is followed by internalization within 60 min after addition of the peptide. Next, we identified myeloperoxidase as the intracellular binding target of hLF1-11 (chapter 6). In addition, we observed that hLF1-11 has the ability to inhibit the enzymatic activity of myeloperoxidase. By use of ABAH, a specific inhibitor of myeloperoxidase activity, we could establish that the inhibition of myeloperoxidase can result in immunomodulating effects, e.g. altered cytokine production, that are comparable to those induced by hLF1-11. The involvement of myeloperoxidase in signal transduction routes leading to cytokine production has not been described previously. There are, however, reports describing the involvement of reactive oxygen species in signal transduction pathways leading to cytokine production. In these publications, enhanced levels of reactive oxygen species (ROS) are necessary to induce cytokine production (11,12). However, based on our data myeloperoxidase appears to be a negative regulator of the production of some cytokines (e.g. IL-6 and IL-10) and chemokines; inhibition of myeloperoxidase enhances the LPS-stimulated cytokine production by monocytes. Either, myeloperoxidase shifts the balance of reactive oxygen species into products less active in

intracellular signaling. Or, MPO is - independently of its ROS-producing activities - involved in a different signaling pathway that leads to cytokine production. Clearly the intracellular pathway involving myeloperoxidase needs to be further deciphered. Interestingly, there are a few diseases involving myeloperoxidase that seem to already hint to its participation in cytokine production and other immune activities. For instance, Wegener's granulomatosis disease comprises the production of anti-neutrophil cytoplasmic autoantibodies (ANCA) with specificity for myeloperoxidase. It has been shown that ANCA enhance phagocytosis, IL-8 production and glucose uptake by neutrophils (13). Also, human monocytes that were incubated with IgG from patients with ANCA-positive, active Wegener's granulomatosis, displayed enhanced expression of CD14 and CD18 on their cell-surface (14).

To establish which amino acids are essential for inhibition of myeloperoxidase enzyme activity we investigated the enzymatic activity of myeloperoxidase in the presence of a set of alanine-substituted hLF1-11 peptides, i.e. peptides that had one (different) amino acid replaced by an alanine. We thus established that the cysteine in the sequence of hLF1-11 is essential for the inhibition of myeloperoxidase activity. Consistent with this notion, the same amino acid was found to be important for enhanced IL-10 induction by hLF1-11-incubated human monocytes in response to LPS (chapter 6). As arginine-rich peptides easily penetrate cellular membranes (15-17), we suggest that the four arginines in hLF1-11 are necessary for cell penetration. Thus, hLF1-11 has two important regions: the arginine-rich penetration site and the cysteine that is responsible for the inhibition of myeloperoxidase activity. The results from this study can be used to facilitate the design of antimicrobial peptides with the optimal qualities for inhibition of myeloperoxidase.

Modulation of the differentiation of monocytes toward macrophages and dendritic cells by hLF1-11

After thus establishing an action of hLF1-11 on monocytes, we next hypothesized how this would affect the differentiation of monocytes into macrophages or dendritic cells. These antigen-presenting cells play a major role in the linking of the innate to the adaptive components of the immune system. We studied whether monocytes that differentiated in the presence of hLF1-11 *in vitro* displayed a different phenotype and if so, whether such an altered phenotype would be potentially beneficial to help clear infection. Results revealed effects of hLF1-11 on GM-CSF-driven macrophages differentiation but not on M-CSF-driven macrophage differentiation (chapter 3). Of interest, GM-CSF was previously reported to be involved in the regulation and enhancement of myeloperoxidase activity in

monocytes and macrophages, while M-CSF was not (18). In view of the above described interaction of hLF1-11 with myeloperoxidase, this might help to explain why the action of hLF1-11 was limited to GM-CSF driven macrophages. Moreover, the presence of hLF1-11 during GM-CSF-driven monocyte-macrophage differentiation resulted in macrophages that displayed enhanced IL-10 production in response to several microbial stimuli and were more responsive toward these microbial stimuli than unstimulated control cells. Also, expression of pathogen recognition receptors like Dectin-1, the mannose receptor and others by hLF1-11-differentiated macrophages as well as the phagocytosis of both *C. albicans* and *S. aureus* by these cells were enhanced as compared to control cells. Strikingly, when we incubated monocytes for 60 min with hLF1-11, washed the peptide away and then induced differentiation toward macrophages for 7 days, these macrophages had a similar phenotype as macrophages that differentiated in the continuous presence of hLF1-11 (chapter 3). Taken together, these findings indicate that hLF1-11 can prime monocytes in such a manner that upon encountering microbial components, these cells differentiate into macrophages with a phenotype that appears to be beneficial for resolution of infection (19).

As hLF1-11 was able to prime these monocytes, we also investigated the effect of this peptide on monocyte-dendritic cell (DC) differentiation. Matured hLF1-11-differentiated dendritic cells displayed enhanced production of IL-10 in response to heat-killed *C. albicans*. In addition, IL-6 production by these cells was enhanced in response to this yeast. These dendritic cells also displayed enhanced expression of Dectin-1, and phagocytosis of *C. albicans* and ROS production in response to *C. albicans* was also elevated (chapter 4). It was noted that hLF1-11-macrophages displayed enhanced activities to several pathogens, while for hLF1-11-dendritic cells this change was restricted to *C. albicans*. What could be the explanation for this difference? At present, this is not clear. One possible explanation touched upon above could be that GM-CSF is involved in regulation and enhancement of myeloperoxidase during monocyte-macrophage differentiation (18). Roy *et al* reported that monocytes incubated with GM-CSF and IL-4 or with IL-4 alone, display diminished myeloperoxidase expression as compared to freshly isolated monocytes (20). As dendritic cells are obtained *in vitro* by incubation of monocytes with both GM-CSF and IL-4, it could be that IL-4 diminished the myeloperoxidase enhancement, thus preventing some of the actions of hLF1-11. Some properties of hLF1-11-differentiated dendritic cells and macrophages might therefore be overlapping between these cell types while others are not.

Differential effects of hLF1-11 and LL-37 on the differentiation of monocytes toward macrophages and dendritic cells

When studying the effects of hLF1-11 on monocyte-macrophage differentiation, we considered the possibility that another antimicrobial peptide i.e. LL-37, similarly affects monocyte-macrophage differentiation. LL-37 also modulates monocyte-macrophage differentiation, although incubation of the cells with this peptide resulted in a completely different macrophage subset as obtained after differentiation in the presence of hLF1-11. While hLF1-11 modulates GM-CSF-driven monocyte-macrophage differentiation, LL-37 modulates M-CSF-driven monocyte-macrophage differentiation resulting in macrophages that displayed striking similarities to the GM-CSF-macrophages (chapter 4). As M-CSF-macrophages display an anti-inflammatory phenotype and GM-CSF-macrophages display a proinflammatory phenotype, LL-37 is thus able to completely redirect the phenotype of M-CSF-differentiated macrophages toward a proinflammatory phenotype. LL-37 is able to induce these effects during differentiation and also upon incubation with already differentiated macrophages.

Investigations on dendritic cell differentiation revealed that in addition to enhanced antimicrobial activities against *C. albicans*, hLF1-11-differentiated dendritic cells were able to induce Th17 polarization upon co-culture with CD4+ T cells (chapter 5). T cells co-cultured with hLF1-11-differentiated dendritic cells produced enhanced levels of IL-17 and diminished Th1 polarization as indicated by reduced levels of IFN-γ. IL-17 is associated with an enhanced anti-fungal and anti-bacterial response (21,22). In addition, IL-17 is involved in the host defense against extracellular pathogens by mediating the recruitment of neutrophils and macrophages to the infected site. Also, IL-17 can induce the production of cytokines/chemokines and antimicrobial peptides by mucosal epithelial cells (23). Others have reported that LL-37 and β-defensins (24) can also modulate monocyte differentiation toward dendritic cells. For example, when CD4+ T cells were co-cultured with LL-37-differentiated dendritic cells these T cells produced enhanced levels of IFN-γ, thereby inducing Th1 polarization (25). Th1 cells drive the protective immune response against intracellular pathogens such as *Mycobacterium tuberculosis*. These data indicate that antimicrobial peptides can modulate immune processes, thereby 'fine tuning' the immune response upon activation. This suggests a possible therapeutic opportunity for employment of antimicrobial peptides, i.e., to (re)direct immune responses to enhance resolution of infection. Figure 1 has depicted the effects that are described in this thesis of hLF1-11 on human immune cells.

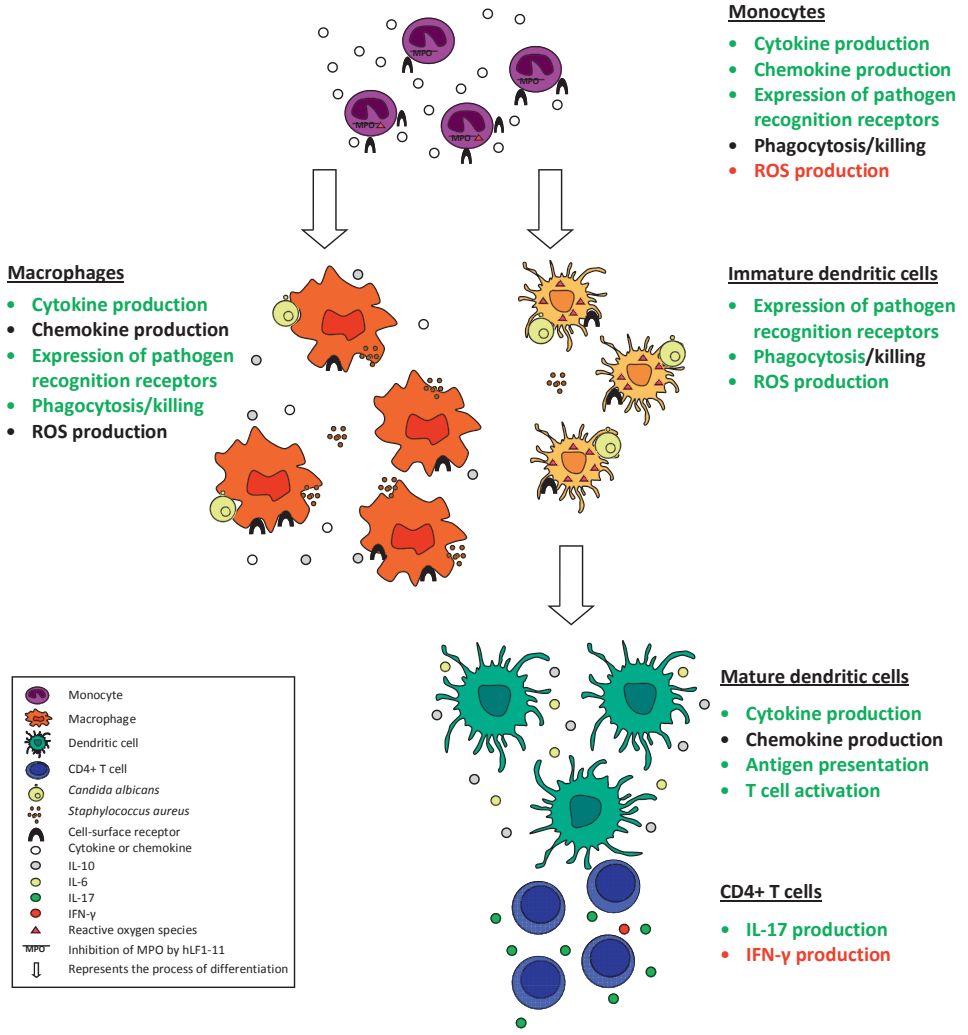

Fig. 1 Simplified representation of the effects of hLF1-11 on the major characteristics of monocytes and monocyte-derived cells *in vitro* When monocytes are incubated with hLF1-11, this peptide is internalized where it binds to and inactivates the enzymatic activities of myeloperoxidase. The hLF1-11-incubated monocytes produce upon activation reduced levels of reactive oxygen species and enhanced levels of cytokines and chemokines as compared to control monocytes. Monocytes differentiated in the presence of both GM-CSF and hLF1-11 become macrophages that display enhanced pathogen recognition receptor expression and phagocytosis and killing of *S. aureus* and *C. albicans* as compared to control (peptide-differentiated) macrophages. In addition, in response LPS, LTA and heat-killed *C. albicans* these macrophages produce enhanced levels of IL-10 as compared to control macrophages. Monocytes differentiated in the presence of GM-CSF, IL-4 and hLF1-11, become immature dendritic cells that display enhanced expression of Dectin-1 and enhanced►

►phagocytosis of *C. albicans*. These hLF1-11-differentiated dendritic cells also produce enhanced levels of reactive oxygen species in response to this pathogen as compared to control (peptide-differentiated) immature dendritic cells. Moreover, upon maturation hLF1-11-differentiated dendritic cells produce enhanced levels of IL-10 and are able to induce Th17 polarization upon co-culture with CD4+ T cells. These T cells produce enhanced amounts of IL-17 and reduced levels of IFN-γ. Represented in green: enhancement, red: reduction, black: untested.

Future research

The research laid down in this thesis concerns *in vitro* experiments only. It should be realized that this is an important limitation because extrapolation of the present findings to the *in vivo* situation clearly is not possible without further studies. Thus, future research in animal models should shed further light on the contribution of the immunomodulatory effect of hLF1-11 to its antimicrobial effects previously described *in vitro*. For instance, experiments in myeloperoxidase knock-out mice could be instrumental in investigating whether myeloperoxidase is the main intracellular target mediating the actions of hLF1-11 in monocytes and monocyte-macrophage differentiation. In addition, cytokine and chemokine responses could be measured in the blood of these mice. An alternative approach would be to assess that action of hLF1-11 on monocytes of individuals with myeloperoxidase-deficiency or monocytes in which myeloperoxidase is silenced. Recently, it has been reported that enhanced levels of IL-10 were present in infected mice that were treated with hLF1-11 (7). Moreover, based on our *in vitro* findings, it is expected that in mice that are depleted of monocytes the anti-infective activity of hLF1-11 is reduced (26). In addition, a longer *in vivo* infection set-up could be instrumental in investigating the subsequent involvement of components of the adaptive immune system that hLF1-11 is known to modulate *in vitro*. Again, it should be realized that our experiments mainly concerned human blood-derived mononuclear cells, and findings may not necessarily be identical in the murine model.

As safety of hLF1-11 in humans has been established (27), the goal of the studies in patients should be to obtain proof of principle for the usefulness of hLF1-11 as an anti-infective agent in a human patient population. Besides experiments that would further elucidate the mechanism of action of hLF1-11, one could also research the involvement of myeloperoxidase in signal transduction routes that lead to cytokine production. Currently, it is not known whether these effects are mediated through the reactive oxygen species production route or that myeloperoxidase displays an additional mechanism of action by being a component of another signaling pathway.

Therapeutical applications

As a future perspective, we suggest that antimicrobial/host defense peptides like hLF1-11 and LL-37 might prove useful in several distinct therapeutic applications. Obviously such applications are at the end of a long road of development still to come, and some general comments and thoughts on possible applications should suffice here. First, LL-37, as well as hLF1-11, could act as a single anti-infective agent; either to prevent infection when used in a prophylactic approach or to treat infection. As LL-37 and hLF1-11 displayed differential effects on the immune system (chapters 3-5), several infectious diseases might be treated with different antimicrobial/host defense peptides. For example, transplantation recipients are susceptible for infections and need an alternative when current antibiotics can no longer perform adequately. hLF1-11 could be involved in an alternative treatment for these infections. Host defense peptides like LL-37 and hLF1-11 can also be applied locally, for example like Stallmann *et al.* have shown (28). They used hLF1-11 as a prophylactic agent in treatment of osteomyelitis (29-31). hLF1-11 in cement was injected into the femoral canal of rabbits earlier infected with MRSA. The continuous release of the peptide by the cement significantly reduced the bacterial load as compared to the control group. In over 75% of the hLF1-11 treated rabbits no growth at all of bacteria was detected (32). Also, hLF1-11 could be used in combination with conventional antibiotics making use of a synergistic antimicrobial activity. Lupetti *et al* showed *in vitro* that hLF1-11 at non-candidacidal concentrations exerted synergistical effects with fluconazole, results showed this combination to be highly effective against fluconazole-resistant *C. albicans* (33). hLF1-11 was necessary to initiate this effect as the observed synergistic effect was only induced when *C. albicans* were pre-incubated with (non-candidacidal concentrations of) hLF1-11 followed by exposure to fluconazole in comparison to no killing effect when the experiment was performed in the opposite order. Second, an alternative approach would be to focus on ways to induce endogenous antimicrobial peptide synthesis, thereby modulating the host response, and such an approach could be employed to treat infections of any kind (34). This could also be induced locally resulting in specific targeting of the infection. For instance, experiments with butyrate -an inducer of endogenous LL-37 synthesis- have shown that it promoted elimination of *Shigella* (35). Moreover, it is known that vitamin D metabolites regulate the expression of LL-37. Vitamin D insufficiency is common still in both industrialized and developing nations (36). Recent studies have shown that vitamin D insufficiency is associated with a somewhat higher risk of active tuberculosis (37-39). Of note, LL-37 shows activity against *M. tuberculosis* and therefore induction of LL-37 transcription by

vitamin D could possibly be helpful by treatment of mycobacterial infections (40-42). Obviously, the safety and consequences of administration of butyrate and vitamin D should be further investigated before application in humans (34). Moreover, additional compounds that induce endogenous production of antimicrobial/host defense peptides need to be identified, but these preliminary results already seem to indicate the potency of this application. Third, as hLF1-11 is a potent inhibitor of myeloperoxidase one can think of applications of hLF1-11 as an agent for diseases in which myeloperoxidase plays an unfavourable role. Myeloperoxidase has emerged as a potential participant in the promotion and/or propagation of atherosclerosis (43). Liu *et al* showed that pre-treatment of rabbits that were subjected to myocardial ischaemia and reperfusion with ABAH significantly reduced cardiac caspase-3 activity, providing direct evidence that myeloperoxidase is a significant contributor to post-ischaemic cardiomyocyte apoptosis (44). These results can have implications as they suggest therapeutic interventions, for example hLF1-11 may (in part) exert cardioprotective effects by inhibition of myeloperoxidase. Lastly, antimicrobial/host defense peptides have been mentioned as potent components of vaccine formulations (5,45). The presence of these peptides could (re)direct the development of antigen presentating cells toward a type driving a favorable immune response to the presented antigen.

Conclusion

In conclusion, the future of antimicrobial peptides with immune modulating activity appears bright as these peptides might be developed further into a novel class of anti-infectives to which microbial drug-resistance is unlikely to develop quickly. Obviously, research on antimicrobial/host defense peptides (including hLF1-11) has to be extended before these peptides can be safely introduced in medical practice. It is encouraging to see that the safety of hLF1-11 has not been a concern in clinical trial phase I and IIa. Together, our findings strongly support the notion that the therapeutic potential of hLF1-11 and other small host defense peptides should be investigated further.

Reference list

1. **Norrby, S. R., C. E. Nord, and R. Finch.** 2005. Lack of development of new antimicrobial drugs: a potential serious threat to public health. *Lancet Infect. Dis.* 5: 115-119.
2. **Guani-Guerra, E., T. Santos-Mendoza, S. O. Lugo-Reyes, and L. M. Teran.** 2010. Antimicrobial peptides: general overview and clinical implications in human health and disease. *Clin. Immunol.* 135: 1-11.
3. **Radek, K., and R. Gallo.** 2007. Antimicrobial peptides: natural effectors of the innate immune system. *Semin. Immunopathol.* 29: 27-43.
4. **Gordon, Y. J., E. G. Romanowski, and A. M. McDermott.** 2005. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. *Curr. Eye Res.* 30: 505-515.
5. **Easton, D. M., A. Nijnik, M. L. Mayer, and R. E. Hancock.** 2009. Potential of immunomodulatory host defense peptides as novel anti-infectives. *Trends Biotechnol.* 27: 582-590.
6. **Hamill, P., K. Brown, H. Jenssen, and R. E. Hancock.** 2008. Novel anti-infectives: is host defence the answer? *Curr. Opin. Biotechnol.* 19: 628-636.
7. **Lupetti, A., C. P. Brouwer, S. J. Bogaards, M. M. Welling, E. de Heer, M. Campa, J. T. Van Dissel, R. H. Friesen, and P. H. Nibbering.** 2007. Human lactoferrin-derived peptide's antifungal activities against disseminated Candida albicans infection. *J. Infect. Dis.* 196: 1416-1424.
8. **Lupetti, A., J. T. Van Dissel, C. P. Brouwer, and P. H. Nibbering.** 2008. Human antimicrobial peptides' antifungal activity against Aspergillus fumigatus. *Eur. J. Clin. Microbiol. Infect. Dis.* 27: 1125-1129.
9. **Nibbering, P. H., E. Ravensbergen, M. M. Welling, L. A. van Berkel, P. H. van Berkel, E. K. Pauwels, and J. H. Nuijens.** 2001. Human lactoferrin and peptides derived from its N terminus are highly effective against infections with antibiotic-resistant bacteria. *Infect. Immun.* 69: 1469-1476.
10. **van Berkel, P. H., M. E. Geerts, H. A. van Veen, M. Mericskay, H. A. de Boer, and J. H. Nuijens.** 1997. N-terminal stretch Arg2, Arg3, Arg4 and Arg5 of human lactoferrin is essential for binding to heparin, bacterial lipopolysaccharide, human lysozyme and DNA. *Biochem. J.* 328 (Pt 1): 145-151.
11. **Knight, J. A.** 2000. Review: Free radicals, antioxidants, and the immune system. *Ann. Clin. Lab Sci.* 30: 145-158.
12. **Hsu, H. Y., and M. H. Wen.** 2002. Lipopolysaccharide-mediated reactive oxygen species and signal transduction in the regulation of interleukin-1 gene expression. *J. Biol. Chem.* 277: 22131-22139.
13. **Hsieh, S. C., H. S. Yu, S. H. Cheng, K. J. Li, M. C. Lu, C. H. Wu, C. Y. Tsai, and C. L. Yu.** 2007. Anti-myeloperoxidase antibodies enhance phagocytosis, IL-8 production, and glucose uptake of polymorphonuclear neutrophils rather than anti-proteinase 3 antibodies leading to activation-induced cell death of the neutrophils. *Clin. Rheumatol.* 26: 216-224.
14. **Nowack, R., K. Schwalbe, L. F. Flores-Suarez, B. Yard, and F. J. van der Woude.** 2000. Upregulation of CD14 and CD18 on monocytes In vitro by antineutrophil cytoplasmic autoantibodies. *J. Am. Soc. Nephrol.* 11: 1639-1646.

15. **Futaki, S., I. Nakase, A. Tadokoro, T. Takeuchi, and A. T. Jones.** 2007. Arginine-rich peptides and their internalization mechanisms. *Biochem. Soc. Trans.* 35: 784-787.
16. **Futaki, S.** 2005. Membrane-permeable arginine-rich peptides and the translocation mechanisms. *Adv. Drug Deliv. Rev.* 57: 547-558.
17. **Futaki, S., S. Goto, and Y. Sugiura.** 2003. Membrane permeability commonly shared among arginine-rich peptides. *J. Mol. Recognit.* 16: 260-264.
18. **Sugiyama, S., Y. Okada, G. K. Sukhova, R. Virmani, J. W. Heinecke, and P. Libby.** 2001. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. *Am. J. Pathol.* 158: 879-891.
19. **Bowdish, D. M., M. S. Loffredo, S. Mukhopadhyay, A. Mantovani, and S. Gordon.** 2007. Macrophage receptors implicated in the "adaptive" form of innate immunity. *Microbes. Infect.* 9: 1680-1687.
20. **Roy, K. C., G. Bandyopadhyay, S. Rakshit, M. Ray, and S. Bandyopadhyay.** 2004. IL-4 alone without the involvement of GM-CSF transforms human peripheral blood monocytes to a CD1a(dim), CD83(+) myeloid dendritic cell subset. *J. Cell Sci.* 117: 3435-3445.
21. **Cho, J. S., E. M. Pietras, N. C. Garcia, R. I. Ramos, D. M. Farzam, H. R. Monroe, J. E. Magorien, A. Blauvelt, J. K. Kolls, A. L. Cheung, G. Cheng, R. L. Modlin, and L. S. Miller.** 2010. IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. *J. Clin. Invest* 120: 1762-1773.
22. **Iwakura, Y., S. Nakae, S. Saijo, and H. Ishigame.** 2008. The roles of IL-17A in inflammatory immune responses and host defense against pathogens. *Immunol. Rev.* 226: 57-79.
23. **Onishi, R. M., and S. L. Gaffen.** 2010. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology* 129: 311-321.
24. **Biragyn, A., P. A. Ruffini, C. A. Leifer, E. Klyushnenkova, A. Shakhov, O. Chertov, A. K. Shirakawa, J. M. Farber, D. M. Segal, J. J. Oppenheim, and L. W. Kwak.** 2002. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. *Science* 298: 1025-1029.
25. **Davidson, D. J., A. J. Currie, G. S. Reid, D. M. Bowdish, K. L. MacDonald, R. C. Ma, R. E. Hancock, and D. P. Speert.** 2004. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. *J. Immunol.* 172: 1146-1156.
26. **Scott, M. G., E. Dullaghan, N. Mookherjee, N. Glavas, M. Waldbrook, A. Thompson, A. Wang, K. Lee, S. Doria, P. Hamill, J. J. Yu, Y. Li, O. Donini, M. M. Guarna, B. B. Finlay, J. R. North, and R. E. Hancock.** 2007. An anti-infective peptide that selectively modulates the innate immune response. *Nat. Biotechnol.* 25: 465-472.
27. **Velden, W. J., T. M. van Iersel, N. M. Blijlevens, and J. P. Donnelly.** 2009. Safety and tolerability of the antimicrobial peptide human lactoferrin 1-11 (hLF1-11). *BMC. Med.* 7: 44.
28. **Stallmann, H. P., C. Faber, A. V. Nieuw Amerongen, and P. I. Wuisman.** 2006. Antimicrobial peptides: review of their application in musculoskeletal infections. *Injury* 37 Suppl 2: S34-S40.

29. **Stallmann, H. P., C. Faber, A. L. Bronckers, A. V. Nieuw Amerongen, and P. I. Wuisman.** 2004. Osteomyelitis prevention in rabbits using antimicrobial peptide hLF1-11- or gentamicin-containing calcium phosphate cement. *J. Antimicrob. Chemother.* 54: 472-476.
30. **Stallmann, H. P., C. Faber, E. T. Slotema, D. M. Lyaruu, A. L. Bronckers, A. V. Amerongen, and P. I. Wuisman.** 2003. Continuous-release or burst-release of the antimicrobial peptide human lactoferrin 1-11 (hLF1-11) from calcium phosphate bone substitutes. *J. Antimicrob. Chemother.* 52: 853-855.
31. **Stallmann, H. P., R. R. de, C. Faber, A. V. Amerongen, and P. I. Wuisman.** 2008. In vivo release of the antimicrobial peptide hLF1-11 from calcium phosphate cement. *J. Orthop. Res.* 26: 531-538.
32. **Faber, C., H. P. Stallmann, D. M. Lyaruu, U. Joosten, C. von Eiff, A. A. van Nieuw, and P. I. Wuisman.** 2005. Comparable efficacies of the antimicrobial peptide human lactoferrin 1-11 and gentamicin in a chronic methicillin-resistant Staphylococcus aureus osteomyelitis model. *Antimicrob. Agents Chemother.* 49: 2438-2444.
33. **Lupetti, A., A. Paulusma-Annema, M. M. Welling, H. Dogterom-Ballering, C. P. Brouwer, S. Senesi, J. T. Van Dissel, and P. H. Nibbering.** 2003. Synergistic activity of the N-terminal peptide of human lactoferrin and fluconazole against Candida species. *Antimicrob. Agents Chemother.* 47: 262-267.
34. **Zasloff, M.** 2006. Inducing endogenous antimicrobial peptides to battle infections. *Proc. Natl. Acad. Sci. U. S. A* 103: 8913-8914.
35. **Raqib, R., P. Sarker, P. Bergman, G. Ara, M. Lindh, D. A. Sack, K. M. Nasirul Islam, G. H. Gudmundsson, J. Andersson, and B. Agerberth.** 2006. Improved outcome in shigellosis associated with butyrate induction of an endogenous peptide antibiotic. *Proc. Natl. Acad. Sci. U. S. A* 103: 9178-9183.
36. **Holick, M. F.** 2007. Vitamin D deficiency. *N. Engl. J. Med.* 357: 266-281.
37. **Nnoaham, K. E., and A. Clarke.** 2008. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. *Int. J. Epidemiol.* 37: 113-119.
38. **Martineau, A. R., R. J. Wilkinson, K. A. Wilkinson, S. M. Newton, B. Kampmann, B. M. Hall, G. E. Packe, R. N. Davidson, S. M. Eldridge, Z. J. Maunsell, S. J. Rainbow, J. L. Berry, and C. J. Griffiths.** 2007. A single dose of vitamin D enhances immunity to mycobacteria. *Am. J. Respir. Crit Care Med.* 176: 208-213.
39. **Wilkinson, R. J., M. Llewelyn, Z. Toossi, P. Patel, G. Pasvol, A. Lalvani, D. Wright, M. Latif, and R. N. Davidson.** 2000. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet* 355: 618-621.
40. **Yamshchikov, A. V., E. V. Kurbatova, M. Kumari, H. M. Blumberg, T. R. Ziegler, S. M. Ray, and V. Tangpricha.** 2010. Vitamin D status and antimicrobial peptide cathelicidin (LL-37) concentrations in patients with active pulmonary tuberculosis. *Am. J. Clin. Nutr.*
41. **Liu, P. T., S. Stenger, D. H. Tang, and R. L. Modlin.** 2007. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *J. Immunol.* 179: 2060-2063.
42. **Zasloff, M.** 2006. Fighting infections with vitamin D. *Nat. Med.* 12: 388-390.

43. **Nicholls, S. J., and S. L. Hazen.** 2005. Myeloperoxidase and cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.* 25: 1102-1111.
44. **Liu, H. R., L. Tao, E. Gao, Y. Qu, W. B. Lau, B. L. Lopez, T. A. Christopher, W. Koch, T. L. Yue, and X. L. Ma.** 2009. Rosiglitazone inhibits hypercholesterolaemia-induced myeloperoxidase upregulation--a novel mechanism for the cardioprotective effects of PPAR agonists. *Cardiovasc. Res.* 81: 344-352.
45. **Biragyn, A. 2005.** Defensins--non-antibiotic use for vaccine development. *Curr. Protein Pept. Sci.* 6: 53-60.