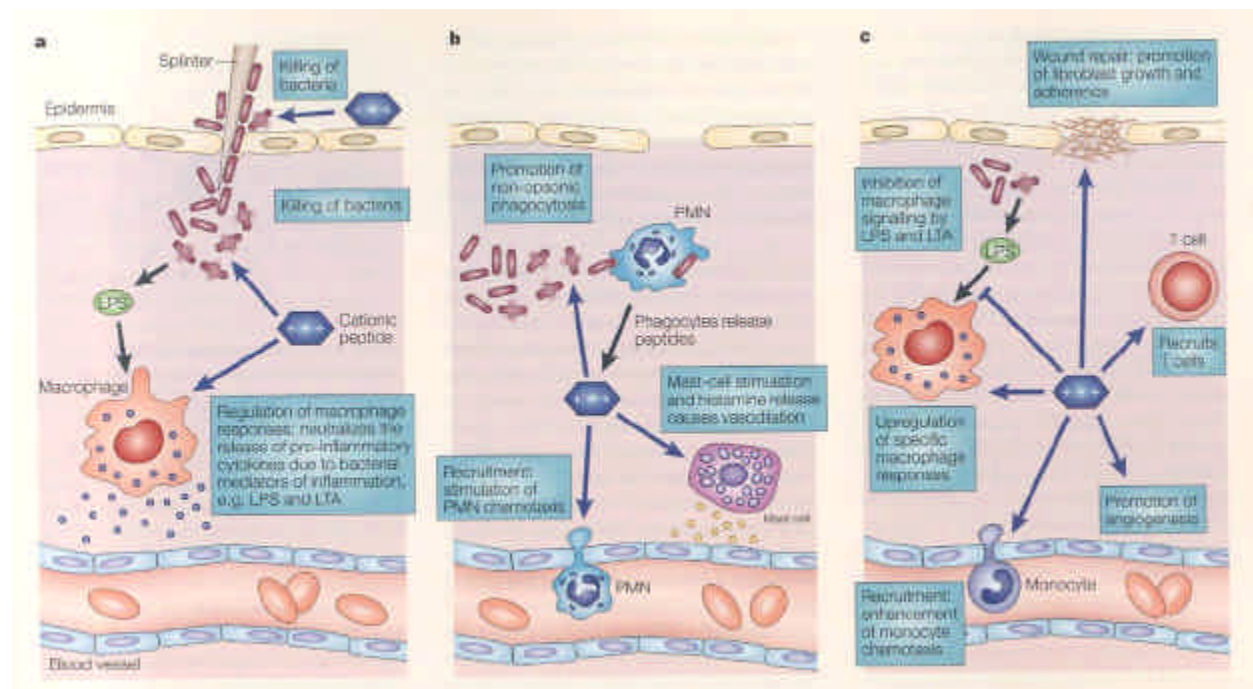


## Host Defence Peptides and Innate Immunity

We are exposed daily to tens of thousands of potential bacterial pathogens, through dermal contact, ingestion and inhalation. The system of innate immunity prevents these pathogens, in small to modest doses, from colonizing and growing to a point where they can cause life-threatening infections. The case for a primary role for antimicrobial peptides in innate host defences is becoming increasingly convincing (1,2). In *Drosophila*, gene knockouts influencing the expression of antibacterial (*imd*) or antifungal (*dif*, insect NF $\kappa$ B) peptide expression render these fruit flies susceptible to subsequent infection (3). Similarly genetic inactivation of matrilysin, which is involved in processing 20 prepro-defensins to their active forms, renders mice susceptible to gastrointestinal infections (4), while chemical inhibition of neutrophil elastase, that prevents activation of cathelicidins (most of the non-defensin peptides) in pig skin wounds, substantially impairs clearance of both Gram-negative and Gram-positive bacteria (5). In humans, a rare specific granule deficiency blocks  $\alpha$ -defensin production and leads to frequent and severe bacterial infections (6). Conversely, delivery of excess peptide, either through adenovirus mediated overexpression of a cathelicidin gene (human LL-37) in the mouse airways (7) or active administration of peptide (2,6,8) protects against infection and endotoxaemia.

Although these results might be explained in part by the known antimicrobial nature of such peptides, this is by no means the only explanation. The major evidence that there is another general role of these peptides in inflammation is their known ability to protect against high doses of the bacterial signalling molecule LPS, which otherwise would kill galactosamine-sensitized mice (8), as well as the very high concentrations of peptides found at sites of inflammation (13-300  $\mu$ g/ml) in cystic fibrosis sputum, inflamed dorsal tongue, the plasma of septic individuals, etc (see 8,9 for review). There is now a growing body of evidence, including work from our laboratory for an impressive variety of activities of cationic antimicrobial peptides other than direct killing, whereby these peptides act directly on cells of the immune system (10). Such

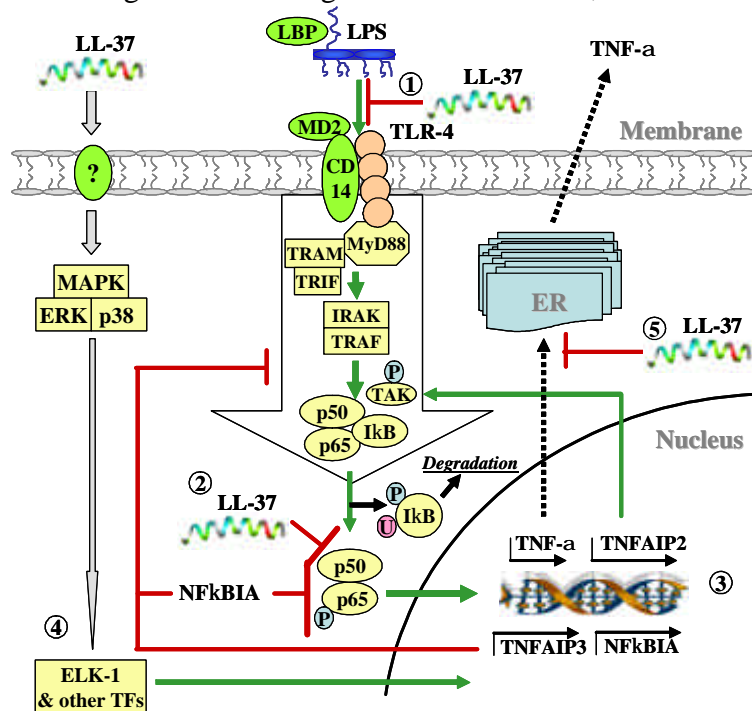


activities would be expected to impact on the quality and effectiveness of innate immune responses and inflammation (1). These activities are summarized in a Figure from a recent (1) review that describes these activities schematically.

With these activities in mind we have proposed that cationic peptides represent potential agents for boosting helpful innate immune responses to assist in the resolution of infections, without the co-incident up-regulation of potentially harmful inflammatory responses (indeed, as mentioned above such pro-inflammatory responses are suppressed). Based on this hypothesis, we discovered peptides that have no antibacterial activity but through selective boosting of innate immunity are active therapeutically in resolving both Gram-negative (*Salmonella Typhimurium*) and Gram-positive (*Staphylococcus aureus*) infections and sepsis in mouse models (2). Considerable effort was made towards understanding these novel peptides and particular host peptides (e.g. human LL-37) during the course of the [FPMI project](#) and based on this the company partner and in-licensor of the Hancock-Finlay technology, [Inimex Pharmaceuticals](#) received significant venture capital financing to permit them to move towards the clinic.

### Anti-endotoxin/ anti-sepsis activity

We have demonstrated that LL-37 is a potent anti-sepsis molecule that can protect against endotoxaemia in mouse models. To understand the mechanisms we have utilized tissue culture models. Low, physiological concentrations of LL-37 ( $\leq 1 \mu\text{g/ml}$ ) were able to modulate inflammatory responses by inhibiting the release of the pro-inflammatory cytokine TNF $\alpha$  in LPS-stimulated human monocytic cells. Microarray studies established a temporal transcriptional profile, and identified differentially expressed genes in LPS-stimulated monocytes in the presence or absence of LL-37. LL-37 significantly inhibited the expression of specific pro-inflammatory genes upregulated by NF $\kappa$ B in the presence of LPS, including NF $\kappa$ B1 (p105/p50) and TNF $\alpha$ -induced protein 2 (TNFAIP2). In contrast, LL-37 did not significantly inhibit LPS-induced genes that antagonize inflammation, such as TNF $\alpha$ -induced protein 3 (TNFAIP3) and



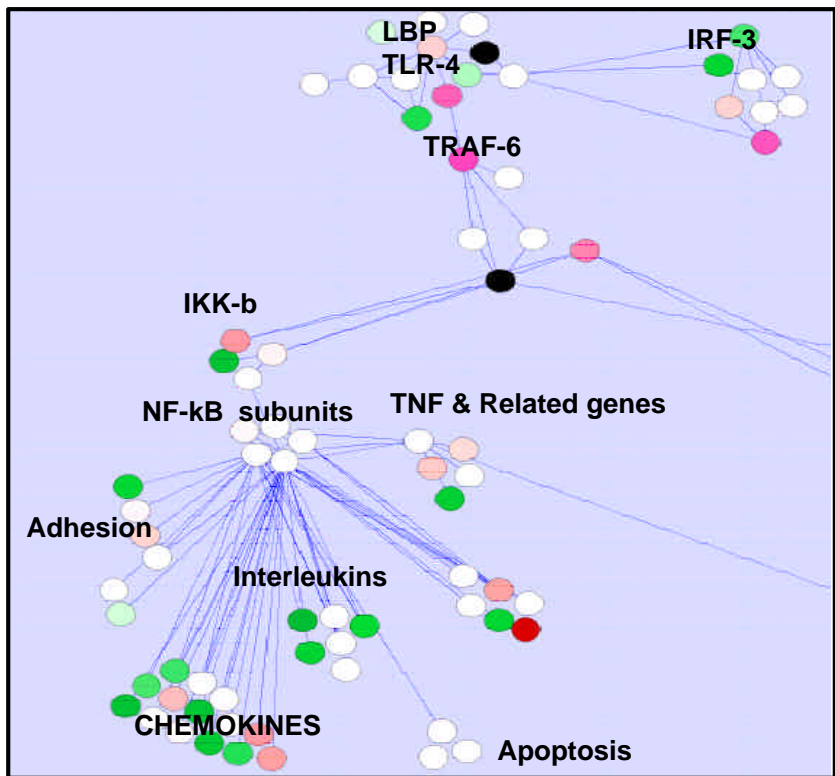
the NF $\kappa$ B inhibitor, NF $\kappa$ BIA, or certain chemokine genes that are classically considered pro-inflammatory.

Nuclear translocation, in LPS-treated cells, of the NF $\kappa$ B subunits p50 and p65 was reduced  $\geq 50\%$  in the presence of LL-37, demonstrating that the peptide altered gene expression in part by acting directly on the TLR to NF $\kappa$ B pathway. LL-37 almost completely prevented the release of TNF $\alpha$  and other cytokines by human PBMC following stimulation with LPS and other TLR2/4 and TLR9 agonists, but not with cytokines TNF $\alpha$  or IL-1 $\beta$ . Biochemical and inhibitor studies were consistent with a model

whereby LL-37 modulated the inflammatory response to LPS/endotoxin and other agonists of TLR by a complex mechanism involving multiple points of intervention. We have proposed that the natural human host defence peptide LL-37 plays roles in the delicate balancing of inflammatory responses in homeostasis as well as in combating sepsis induced by certain TLR agonists.

### Functional Genomic Studies of Innate Immunity

Through Genome Canada funding in Competition II, the Functional Pathogenomics of Mucosal Immunity Program developed the concept that it was possible to stimulate an innate immune response that assisted in resolution of infection, while dampening or at least not exciting harmful inflammation. New insights were obtained through a combination of comparative functional genomic studies in man, cattle and chickens, genomic studies of economically-relevant food animal infections, a highly effective new bioinformatics platform, and investigation of mechanistically unique components of innate immunity, particularly host defence peptides and



CpG oligonucleotides. With respect to the peptides, evidence was obtained in animal infection studies for their potential use in anti-infective therapy through the boosting of innate immunity with the co-incident suppression of potentially harmful inflammatory responses. CpG on the other hand boosted adaptive immunity (adjuvant activity) in animals without excessive inflammation. Using advanced bioinformatics approaches we are mapping such responses to Networks built using the bioinformatic program [Cytoscape](#) as shown on the left.

Recently our genomics research program was renewed as the Pathogenomics of Innate Immunity (PI<sup>2</sup>) program and this project involves a collaborative team from UBC, VIDO in Saskatoon, University of Alberta, SFU, Sanger Centre in Cambridge England, Trinity College and National University of Singapore. We are proposing to investigate the functioning of a variety of gene products that were identified through our previous functional genomics studies by using, as a primary tool, mouse gene knockouts developed in collaboration with [Sanger Centre](#). Stable ES cell lines and the mice derived therefrom will be subjected to detailed infection models using *Salmonella* as the model pathogen. The responses to infection, including gene and protein expression profiling, will be utilized to infer function. A significant number of genes, carefully

selected to represent key pathways and decision points identified in our previous functional genomics studies, will be knocked out. The relevance of these genes in human and animal infections are also being assessed by knocking out, using siRNA methods, the equivalent genes in human and cattle cells. These data will add to our knowledge of important infection-fighting mechanisms of immunity and provide the basis for novel methods of fighting infections. Together with our colleagues we are building a bioinformatics database, termed InnateDB, of the genes and biomolecular interactions involved in innate immunity,

Two other large projects in this area in which the lab is engaged are funded through the Gates [Grand Challenge](#) program. Thus in one project led by [Brett Finlay](#) we are pursuing the potential of peptides to selectively boost innate immunity for developing country applications. In a second led by [Lorne Babiuk](#), we are attempting to develop peptide adjuvants that will enable current pertussis vaccines to be used as single dose neonatal vaccines.

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