The human immune system exists to protect against pathogens including bacteria. For many aspects of immunity bacteria have evolved counter measures to evade the immune system, enabling them to survive within a host body. One successful organism is Pseudomonas Aeruginosa (PA), a nosocomial pathogen which possesses a number of mechanisms to evade the human immune system. As a multiple drug-resistant organism Pseudomonas Aeruginosa mechanisms of immune evasion have huge potential as drug targets.

Introduction

Immune evasion of the immune system is essential for survival of invading bacteria, enabling proliferation and further spread infection. One bacterium that is successfully able to avoid immune destruction is Pseudomonas aeruginosa. As a hospital-acquired pathogen Pseudomonas aeruginosa is often present as a secondary infection, where the immune system is already active, causing selective pressure, stimulating evolution of evasion techniques to avoid pre-mounted responses.

As Pseudomonas Aeruginosa is a multiple drug-resistant organism, which can cause chronic infection, particularly in cystic fibrosis patients it is of huge importance that new treatment strategies are developed. Immune evasion techniques of Pseudomonas aeruginosa have been well-identified, and provide an excellent knowledge base for drug development. Identification and manipulation of one or more of these mechanisms could not only provide a novel treatment, but also enable the host immune system to better handle the infection.

The human immune system

Humans are constantly exposed to environmental bacteria, the vast majority of which cause no harm, in part due to the immune system, which protects the host by preventing colonisation of human tissue by normal flora, and through destruction of potentially pathogenic non-indigenous bacteria. The human immune system can be divided into two branches, the innate and adaptive. These branches work cohesively to eliminate pathogens from the host body preventing harm and disease. The key components of the immune system are varied, having soluble and insoluble receptors which act in all host tissues. These varied responses have developed in response to pathogens.

Pattern associated molecular patterns are common identifiable structures on pathogenic cells, which can be detected by complementary pattern recognition receptors on host cells. Thus preventing initiation of a host immune response. PA possess type four pili, which are fundamental in the bacterium adhering to the host cell, as well as in biofilm formation as they enable aggregation. PA is able to secrete an alkaline metalloprotease, AprA, via a type one secretion system, which inhibits TLR3 activation by degrading flagellin, preventing IL-8 production.

Antimicrobials, such as beta-defensin hBD-2 are vital in clearing a PA infection. Pseudomonas aeruginosa secretes rhamnolipids which prevent the host hBD-2 response in epidermal cells, by impairing calcium regulated pathways and deactivating kinase C, preventing hBD-2 induction in response to bacterial flagellin. The presence of rhamnolipids also caused inhibition of IL-1β, a hBD-2 mediator.

There are five key mechanisms of immune evasion employed by Pseudomonas aeruginosa. The first is formation of biofilms, these structures prevent immune cells and antibiotic molecules from accessing cells, providing protection against the host immune defences. Quorum sensing is a mechanism of communication employed in biofilms, the release of small molecules known as AHLs and protein ndvB stimulate cells to produce cyclic glucons, a key component of biofilm matrix, stimulating further accumulation of bacteria.

Evasion of destruction by the complement system is crucial, and can be achieved by several mechanisms. Including expression of enzymes, such as elastase, which damages C3b, preventing it from activating by binding to the bacterial cell wall, and deactivates cytokines IL-1a and beta. Evidence shows that Pseudomonas aeruginosa produces modified versions of LPS which are known to limit the capability of C3b to associate with the cell surface.

PA secretes a protein known as exotoxin A, an ADP-riboseyl transferase which is able to inhibit the production of cytokines IL-1, lymphotxin, IFN gamma and TNFα, all of which are key aspects of the immune response. Exotoxin A also causes inhibition of protein synthesis, by suppressing human elongation factor 2, which is essential for translation. As the toxin is unable to be secreted a great distance from the bacterium, a high proportion of affected cells are cells involved in immune response.

Immune evasion

PA evasion

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Potential drug targets

As quorum sensing allows biofilms to form more readily and includes production of immunosuppressant molecules, it has real potential as a drug target to combat PA infections. One potential method for this would be to use small molecules, analogous to AHLs, to competitively bind. Alternatively a potential target would be ndvB protein, which catalyses formation of cyclic glucons, a key component of the biofilm matrix.

When considering complement evasion the deactivation of PA elastase is not a desirable drug target, as PA elastase has a similar structure to neutrophil elastase, with 78% similarity, meaning any compound able to cleave PA elastase may also cleave neutrophil elastase, harming the immune response. LPS is difficult to disrupt in vivo, although it is possible through a laborious and extreme procedure, meaning LPS removal is not an ideal method of PA treatment. It is notable that as certain strains of PA are more susceptible to complement mediated destruction that this may not be as widely effective as other targets.

The action of Exotoxin A delivery is not understood to a level where this could be a potential drug target. Although it has been found in the plasma of infected humans, suggesting that Exotoxin A moves extracellularly to reach host cells, providing an ideal opportunity to target the toxin.

Rhamnolipids work extracellularly, meaning there is huge potential to target them in a drug treatment. The key rhamnolipid in PA is rhaA gene which is expressed in 43% of PA strains, as its frequency is so low this is not an ideal drug target.

As alkaline metalloprotease is the key protein in pili degradation interfering with it would prevent loss of flagellin. Metalloprotease inhibitors already exist and are commercially available which work on common beta-barrel structures, such as that which exists in alkaline metalloprotease. Although currently not utilised for PA infections, there is huge potential for this application. Due to the nature of human digestion this would most likely be ineffective if taken orally, with topical treatment being ideal.

Conclusion

When considering PA infections targeting of biofilm formation is a promising method of controlling infection as it would enhance the action of currently available antibiotics, as well as leave the pathogen more susceptible to the host immune system.

Biofilms in particular pose a good opportunity as the extracellular ndvB protein is easily accessible to antibiotic molecules. Degradation of this protein can prevent formation of biofilms, as well as limiting growth of existing biofilms. As there is no similar host protein there should be no undesirable side effects.

Key references

