

PROGRAMME 4: Novel antimicrobial agents

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Background

The *Burkholderia cepacia* complex (Bcc) is a diverse group of human pathogens that cause life-threatening lung infections in patients with CF or chronic granulomatous disease, and in patients requiring intensive care. The highly transmissible *B. cenocepacia* lineage ET 12 (e.g. J2315-Edinburgh and K56-2-Toronto) are highly resistant to practically all front-line antibiotics. Large pharmaceutical companies will not work to develop compounds that they envisage to be limited to a “niche” market. The potential benefit of a successful compound to patients with CF is immeasurable. It is unclear why the immune system of CF patients fails to clear these organisms and leads to them being particularly susceptible to infections.

An important component of the human innate immune response to bacterial infection is the family of cationic antimicrobial peptides (cAMPs) known as defensins, of which there are two main subfamilies (α and β) in humans [Zasloff, 2002; Ganz, 2003; Lehrer, 2004]. These small, 3-5 kDa positively-charged peptides are highly potent killers of a range of Gram-positive and -negative pathogens such as *S. aureus* and *P. aeruginosa*. cAMP-based therapies have been explored for their potential to treat lung infections in CF patients [Zhang et al., 2005]. Defensins have a large number of positive amino acids, and it is thought that they bind to negatively-charged components (e.g. lipopolysaccharide, LPS or teichoic acid) of the bacterial membrane and disrupt the lipid bilayer, resulting in bacterial killing via pore formation and/or membrane depolarisation. As well as having potent microbicidal activity, their roles in the stimulation and regulation of the immune response have been revealed over the past decade. The human genome was shown to have >40 different β -defensin genes arranged in large clusters and the exact function of each is unknown [Selsted and Ouellette, 2005]

We have been working on a family of β -defensins which are present primarily in skin and lung epithelial tissues that line surfaces in contact with the environment. They are active as antibiotics at nanomolar concentrations and can also act as chemokines (e.g. human β -defensin 2, HBD2) in the picomolar range, activating the innate and adaptive immune responses by binding to membrane receptors from the GPCR superfamily [Pazgier et al, 2006]. In Edinburgh our multi-disciplinary team has carried out structural and functional studies on a number of natural mammalian defensins, as well as synthetic derivatives. We have recently shown that mice and humans have a repertoire of β -defensin genes that have rapidly evolved by adaptive evolution [Maxwell et al., 2003]. For example, Defr1, one of the mouse specific genes that we have described, is a novel five cysteine containing defensin which has significantly higher antimicrobial activity than its six-cysteine analogue. Using high-resolution mass spectrometry, we have shown that this defensin is a dimer held together by an intermolecular disulfide bond [Morrison et al., 2002; Campopiano et al., 2004]. This dimerisation of peptide monomers has also been observed in other cAMPs and we are exploring the structural and functional consequences of this, with a view to design more potent agents [Clarke and Campopiano, 2006].

cAMP-resistant pathogens

It is well documented that pathogenic bacteria have rapidly evolved resistance to a wide-range of structurally diverse antibiotics acting on many different bacterial targets. Resistance to cAMPs has already been observed in many bacteria –one main mechanism by which this occurs is by the covalent modification of the outer membrane lipids, rendering them less negatively-charged, thus the cAMPs cannot bind [Peschel, 2002; Peschel & Sahl, 2006]. Such modifications include the addition of the sugar, deoxy-amino arabinose (Ara-4N) to the LPS component of the membrane. This is especially important in CF pathogens; it was recently shown that treatment of *P. aeruginosa* with polymyxins led to up-regulation of genes involved in Ara-4N production, LPS attachment and subsequent resistance [McPhee et al., 2003]. Furthermore, various *Burkholderia cenocepacia* strains (including K56-2 and J2315) have been found to be inherently resistant to practically all antibiotics, including cAMPs such as defensins (HBD3) and polymyxins (PMB) (MICs ≥ 1 mg/ml) [Sahly et al., 2003]. Moreover, it was recently shown by Valvano and colleagues that the LPS from *B. cepacia* K56-2 is constitutively modified by Ara-4N in an unusual manner [Ortega et al., 2005]. The unusual chemistry of *B. cepacia* LPS may also explain why it displays highly inflammatory properties even in the presence of PMB [Shimomura et al., 2003; Govan 2003; De Soyza et al., 2004]

Methods of Research

We will seek to explain the chemical and genetic basis for the innate resistance in Bcc to cAMPs such as defensins and PMBs (e.g. polymyxin). It has been shown that Bcc produce a wide variety of proteases which can rapidly degrade cAMPs (see work by Belfast group). The discovery that Bcc also have a constitutively-modified LPS suggests novel mechanisms of self-defense displayed by these pathogens. Our hope is that if we can inactivate the self-defense mechanism using novel agents, these bacteria will be susceptible to not only antibiotics such as polymyxin but also to the host innate immune system.

A recent study by the Valvano group [Loutet et al., (2006)] showed that *B. cenocepacia* SAL1, a mutant strain of K56-2, was sensitive to PMB at 32 ug/ml. The strain was mutated in genes involved in LPS inner core biosynthesis and produced a deep-rough LPS. This breakthrough suggests that it is possible to render *Burkholderia* sensitive to cAMPs. We have begun a collaboration with the Prof. Valvano's group and the structure of the SAL1 LPS is being determined. We will carry out a full cAMP sensitivity analysis of this strain using cAMPs we have in our laboratory (e.g. Defr1, HBD1, 2 and 3). At the same time we are also exploring the Ara-4N biosynthetic pathway within J2315 and K56-2. The biosynthesis of Ara-4N has been studied in *E. coli* and involves >6 enzymes whose genes are encoded on a pmr (polymyxin resistance) locus. We will carry out genetic, biochemical and structural analysis of this locus in *Burkholderia* strains.

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