Concerns regarding resistance to self-proteins

In their review '*Arming the enemy: the evolution of resistance to self-proteins*', Bell & Gouyon (2003) present an interesting perspective. They suggest that the introduction of cationic antimicrobial peptide antibiotics into general (clinical) use '*may provoke the evolution of resistance to our own defence proteins and thus compromise our natural defences against infection*'. I feel it is very appropriate to raise this argument, but would like to introduce another perspective to this discussion.

Peptides of the innate immune system (also called cationic antimicrobial or cationic host defence peptides) are produced by virtually all organisms, ranging from plants and insects to humans, as a major part of their immediately effective, non-specific, defence against infections (Hancock, 2001; Zasloff, 2002). These peptides vary in length from 12 to around 50 residues and have a net positive charge conferred by lysine and arginine residues and usually greater than 50 % hydrophobic amino acid residues. Biochemical and animal model studies have demonstrated that these peptides have potential as stand-alone, broad-spectrum antibiotics, and late clinical trials of efficacy of topically applied peptides against infections are under way. Bell & Gouyon (2003) rightfully point out that I and other reviewers have claimed that '*it is also very difficult to raise mutants resistant to these cationic peptides, and there are very few naturally resistant bacteria*'. However, this is not the same as stating that such resistance development will never occur. In my opinion, bacteria will eventually develop resistance, and we recently reviewed these resistance mechanisms (Devine & Hancock, 2002), as did Bell & Gouyon (2003). However, I know of no data that support the authors' claims that resistance to cationic peptides '*is widespread in nature and readily induced in the laboratory*'. Indeed, there is clear published evidence that resistance to antimicrobial peptides develops at rather low frequencies [see study of Steinberg *et al.* (1997), where they demonstrate that resistance to pig protegrin is more difficult to select than even vancomycin resistance]. Another claim that bears scrutiny is the idea that since therapeutically utilized peptides share physical properties with host defence peptides, they will give rise to cross-resistance to these (host defence) peptides. In fact, there are, to my knowledge, no universal mechanisms of resistance to such peptides. For example, the lantibiotics nisin and epidermin, which are discussed in detail by Bell & Gouyon (2003), are clearly different from human antimicrobial peptides as Gram-negative bacteria are resistant to the lantibiotics, but are in fact preferred target species for many host defence peptides. Cationic peptides do not all act by a generalized mechanism of action (i.e. attacking an 'Achilles heel') (Devine & Hancock, 2002; Hancock & Rozek, 2002; Kobayashi *et al.*, 2000). Indeed, we have proposed that each bacterium has multiple potential targets for such peptides, making resistance difficult (not 'impossible') (Hancock & Rozek, 2002). If one accepts this, then the evolution of resistance to a given therapeutic peptide will not necessarily result in resistance to host defence peptides and thus constitute a '*serious and unprecedented threat*'. Consistent with this, there is no known universal mechanism of resistance. I do accept, however, there is a potential threat that should be explored on a case-by-case basis with cationic peptides that are proposed for use in the clinic.

What are the reasons for believing that such cross-resistance may not occur? First, Bell & Gouyon (2003) quote Zasloff (2002), who pointed out that such host defence peptides have remained effective against bacterial infections for millions of years. Indeed, despite the continual presence of peptides in all host environments, generalized or high level resistance is very rare (largely limited to *Burkholderia*, *Proteus* and *Serratia* spp.), particularly amongst the normal flora (which includes many of the more important nosocomial pathogens) (Devine & Hancock, 2002). If Bell & Gouyon's arguments really are correct, why has resistance not developed in the normal flora over evolutionary time? Why are the above-named resistant species not dominant in human medicine? Second, despite the use of nisin in foods in Europe since 1969, and the known movement of antibiotic resistance from the food chain into humans, there has been no obvious impact on the level of virulence of bacterial species, as indeed Bell & Gouyon (2003) mention. And yet from the authors' discussion, this would be a field test for their population biology arguments. Similarly, derepression of the two-component regulator PhoPQ leads to cross-resistance to polymyxin B and some (but not all; Macfarlane *et al.*, 2000) cationic peptides (about a fourfold increase in MIC;

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Chris Thomas, Editor-in-Chief

Macfarlane *et al.*, 2000; Fields *et al.*, 1989). Since polymyxin B is found in most over-the-counter topical skin, wound, eye and ear ointments, surely this also represents a potential danger. And yet this danger has not really materialized and polymyxin resistance remains fairly rare, and does not appear to have impacted on virulence. Finally, defensins and other peptides are found at concentrations of 25 mg ml^{-1} or greater in the granules of phagocytic cells (e.g. neutrophils) and in intestinal crypts (Devine & Hancock, 2002), concentrations that are high enough to overwhelm resistance.

Other host defence peptides, and possibly the majority, are found at most (human) body sites at concentrations below their MICs for most bacteria. It has become clear that peptides have non-antibiotic activities, i.e. they regulate immunity (Hancock, 2001). Peptide resistance would not affect such activities, as bacterial killing would occur by immune mechanisms. Significantly, host defence (antimicrobial) peptides are only one element of an arsenal of host immune defences. Resistance to a given antimicrobial therapeutic peptide will not affect most phagocytic killing mechanisms (e.g. toxic oxygen radicals), or complement-mediated killing, or mechanisms of adaptive immunity. Thus, to state that resistance to peptides will '*compromise our natural defences against infection*' (Bell & Gouyon, 2003) is perhaps a bit extreme. The authors' perspective on this topic is, however, worthy of discussion and hopefully will alert scientists to the potential concerns with this group of molecules, and stimulate research on this topic in their own laboratories and those of other scientists.

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