Correspondence

More research is needed before we can accurately define and understand 'critical colonisation'

Dear Sir,

With the increasing focus on wound microbiology and infection in recent years, the Wound Infection Continuum has been proposed as a model to account for different stages of bioburden and related pathology. Within this continuum is the stage of critical colonisation. This has been widely accepted as a separate entity (Sibbald et al, 2001; Kingsley, 2003; Moore, 2006; Ziegler et al, 2006) but claimed by others to be synonymous with 'local infection', a state of 'pre-infection' or, as a concept that is beyond the realms of reality. What is generally agreed is that critical colonisation has not yet been definitively characterised.

The development of wound infection is known to be dependent upon both microbial and host factors. The number of species and the number of organisms present have been shown to be an important factor in the development of infection (Trengove et al, 1996). However, the host response or immune status of the individual is claimed to be a key factor.

To those who accept it as a distinct entity, critical colonisation is a stage where wound healing is delayed without the overt signs and symptoms of infection and occurs despite the patient being given optimum treatment (Kingsley, 2003). Could this situation arise without the host response playing a part? Certainly, numerous authors have reported delayed ulcer healing influenced by microorganisms (Lookingbill et al, 1978; Daltrey et al, 1981; Halbert et al, 1992; Hansson et al, 1995), without an overt host response.

In terms of wound infection, the host response is recognised by signs and symptoms of inflammation, such as heat, redness, swelling and pain. The occurrence of signs of spreading erythema around the wound is usually indicative of infection such as erysipelas or cellulitis (Eron et al, 2003). However, not all such erythema necessarily has endogenous inflammatory or 'immunological' origins. Recent findings have shown that Morganella species commonly found in chronic wounds, notably *M. morganii*, produce histamine in physiologically significant amounts (Cooper et al, 2004). Thus peri-wound erythema could be attributable to histamine-induced vasodilation from wound colonisation with *M.morganii*. In effect, a 'false positive' sign of wound infection.

Delayed healing is also intimately linked with 'uncontrolled inflammation' (Moore, 1999) or immunopathology (Page et al, 2006). This is not always visually evident in chronic wounds, as it is not always accompanied by the classic signs of inflammation or may be masked, for example, by haemosiderin staining and lipodermatosclerosis in venous leg ulcers. It is, however, histologically evident (Abd-El-Aleem et al, 2005).

The supporters of the concept of critical colonisation believe that delayed healing can often be attributed to microbial factors (Gray et al, 2005) and that diagnosis is often only confirmed retrospectively once antimicrobial measures have been taken and found to be effective. However, our knowledge of wound bacteria and their roles in inflammation — and hence chronicity — is incomplete. We know little of the events that trigger the change from colonisation to infection. Nor do we understand the mechanisms of bacteria-induced inflammation as occurs, for example, through the induction of interleukin-8, tumour necrosis factor-α, matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs), or activation of toll-like receptors, nuclear factor-$\kappa\beta$ and p38 mitogen-activated protein signalling pathways. In short, there is much research to be conducted before we can be categorical.

How can delayed healing be associated with microbial factors and not elicit an obvious host response? There are three or more 'modes of action' whereby bacteria can delay wound healing without any apparent inflammatory, or immunological, response. The first is through the expression of immunoevasion factors (Allen et al, 2005) or immune response modifiers (Schiller et al, 2006); the second, biofilm formation (Serralta et al, 2001); and the third by suppression of cellular wound healing responses (Stephens et al, 2003). These occur when the wound is colonised by certain specific bacteria.

Pseudomonas aeruginosa is an organism commonly found in chronic wounds (Bowler et al, 1999) and is associated with delayed healing (Heggers et al, 1992). It is also associated with chronic infection in other tissues (Lau et al, 2004). It is known to form biofilms (Costerton, 2001) and secrete immunoevasive factors (Usher et al, 2002) that are active against polymorphonuclear leukocytes (PMNs). To this effect, activation of the type III secretion system, an identified virulence determinant of *P. aeruginosa,* has been reported (Dacheux et al, 2002).

P. aeruginosa is likely to be of far greater significance to wound chronicity and infection than has been hitherto recognised. Experienced wound clinicians have long noticed green colouration in chronic wounds and have associated it with the presence of *P. aeruginosa* and with delayed healing (Hamilton Jakobsen and Danielsen, 1997). The green pigment is pyocyanin, a phenazine which is a highly-diffusable exotoxic metabolite (Denning et al, 2003). This has been shown to inhibit many cell functions (Lau et al, 2004) and even impair host defences through apoptosis. Many pathogens, including *P.aeruginosa,* are known to induce inappropriate or premature apoptosis of immune cells such as macrophages and neutrophils (Zychlinsky and Sansonetti, 1997a) and that this can promote inflammation (Zychlinsky and Sansonetti, 1997b).

P. aeruginosa has evolved immunoevasive strategies by which it affects host immunity (Buret and Cripps, 1993). Indeed, pyocyanin and other, similar phenazines have been shown to be pro-apoptotic upon human neutrophils (Usher et al, 2002). This is postulated to be a clinically important mechanism of persistence of *P.aeruginosa* in human tissue.

Staphyloccocus aureus is also an important human wound pathogen that interferes with host-cell functions. Impaired healing is often observed in *S. aureus* infected wounds where the extracellular adherence protein (EAP) has been implicated (Athanasopoulos et al, 2006). EAP is a potent anti-inflammatory (Chavakis et al, 2002) and antiangiogenic agent, preventing recruitment of inflammatory cells to the wound site as well as inhibiting neovascularisation (Haggar et al, 2004).

Correspondence

A similar phenomenon is that of wound malodour. This is a regular feature of chronic wounds and is linked to short chain fatty acids (SCFA) (Bowler et al, 1999). These volatile compounds are the metabolic by-products of anaerobic bacterial metabolism (Reed and Sanderson, 1979). Malodour is associated with organisms known to generate SCFA such as *Bacteroides* spp and anaerobic cocci (Bowler, 1998). In an in vitro study, Stephens et al (2003) demonstrated that SCFA generated by *Peptostreptococci* inhibited the growth of key cells responsible for wound healing – keratinocytes, fibroblasts and endothelial cells. This, if translated to the in vivo situation, would give rise to delayed healing without infection. It is reasonable, therefore, that SCFA contribute to delayed healing in many, if not all, malodorous wounds.

A chronic wound colonised, but not infected, with *Morganella spp*, *Pseudomonas,* and *Peptostreptococci* among others may exhibit erythema and delayed healing, without an otherwise evident host response. This situation, involving any or all of these organisms is possibly what we recognise as critical colonisation. Local infection, on the other hand, is generally accepted by clinicians as being more easily diagnosed. Clearly, this is an area for research before mechanisms can be defined.

Abd-El-Aleem SA, Morgan C, Ferguson MW et al (2005) Spatial distribution of mast cells in chronic venous leg ulcers. *Eur J Histochem* **49(3):** 265–72

Allen L, Dockrell DH, Pattery T et al (2005) Pyocyanin production by Pseudomonas aeruginosa induces neutrophil apoptosis and impairs neutrophil-mediated host defences in vivo. *J Immunol* **174(6):** 3643–49

Athanasopoulos AN, Economopoulos M, Orlova V, et al (2006) The extracellular adherence protein (Eap) of Staphylococcus aureus inhibits wound healing by interfering with host defense and repair mechanisms. *Blood* **107(7):** 2720–27

Bowler PG (1998) The aerobic and anaerobic microbiology of wounds: a review. *Wounds* **10(6):** 170–8

Bowler PG, Davies BJ, Jones SA (1999) Microbial involvement in chronic wound malodour. *J Wound Care* **8(5):** 216–8

Buret A, Cripps AW (1993) The immunoevasive activities of Pseudomonas aeruginosa. *Am Rev Respir Dis* **148(3):** 793–805

Chavakis T, Hussain M, Kanse SM, et al (2002) Staphylococcus aureus extracellular adherence protein serves as an anti-inflammatory factor by inhibiting the recruitment of host leukocytes. *Nat Med* **8(7):** 687–93

Cooper RA, Morwood S, Burton N (2004) Histamine production by bacteria isolated from wounds. *J Infect* **49:** 39

Costerton JW (2001) Cystic fibrosis pathogenesis and the role of biofilms in persistent infection. *Trends Microbiol* **9(2):** 50–52

Dacheux D, Epaulard O, de Groot A et al (2002) Activation of Pseudomonas aeruginosa type III secretion sytem. *Infect Immun* **70(7):** 3973–7

Daltrey DC, Rhodes B, Chattwood JG (1981) Investigations into the microbial flora of healing and non-healing decubitus ulcers. *J Clin Path* **34(7):** 701–5

Eron LJ, Lipsky B, Low D et al (2003) Managing skin and soft tissue infections. *J Antimicrob Chemother* **52(Suppl S1):** 3–17

Gray D, White RJ, Kingsley A, Cooper P (2005). Using the wound infection continuum to assess wound bioburden. *Wounds UK 1(2Suppl):*15–21

Haggar A, Ehrnfelt C, Holgersson J (2004) The extracellular adherence protein from *Staphylococcus aureus* inhibits neutrophil binding to endothelial cells. Infect Immun 72(10): 6164–7

Halbert AR, Stacey MC, Rohr JB, et al (1992) The effect of bacterial colonization on venous ulcer healing. *Austral J Dermatol* **33(2):** 75–80.

Hamilton Jakobsen B, Danielsen L (1997) Venous leg ulcer. *Ugeskr Laeger* **159(19):** 2836–40

Hansson C (1995) The microbial flora in venous leg ulcers without clinical signs of infection. *Acta Derm Venereol (Stockh)* **75:** 24–30

Heggers JP, Haydon S, Ko F, Hayward PG, Carp S, Robson MC (1992) Pseudomonas aeruginosa exotoxin A: its role in retardation of wound healing: the 1992 Linberg Award. *J Burn Care Rehabil* **13(5):** 512–8

Kingsley AR (2003) The wound infection continuum and its application to clinical practice. *Ostomy Wound Manag* **49(Suppl 7a):** 1–7

Lau GW, Hassett DJ et al (2004) The role of pyocyanin in Pseudomonas aeruginosa infection. *Trends Mol Med* **10(12):** 599–606

Lookingbill D, Miller S, Knowles R, et al (1978) Bacteriology of chronic leg ulcers. *Arch Dermatol* **114(12):** 1765–8

Moore K (2006) A new silver dressing for management of wounds with delayed healing. *Wounds UK* **2(2):** 74–81

Page KR, Scott AL, Manabe YC (2006) The expanding realm of heterologous immunity: friend or foe? *Cell Microbiol* **8(2):** 185–96

Reed PJ, Sanderson P (1979) Detection of anaerobic wound infection. *J Clin Path* **32(12):** 1203–05

Schiller M, Metze D, Luger TA et al (2006) Immune response modifiers – mode of action. *Exp Dermatol* **15(5):** 331–41

Serralta VW, Harrison-Balestra C (2001) Lifestyles of bacteria in wounds: presence of biofilms? *Wounds* **13(1):** 29–34

Sibbald RG, Browne AC, Coutts P, Queen D (2001) Screening evaluation of an ionized nanocrystalline silver dressing in chronic wound care. *Ostomy Wound Manage* **47(10):** 38–43

Stephens P, Wall IB, Wilson MJ et al (2003) Anaerobic cocci populating the deep tissues of chronic wounds impair cellular wound healing responses in vitro. *Br J Dermatol* **148(3):** 456–66

Trengove N, Stacey M, McGechie D, et al (1996) Qualitative bacteria and leg ulcer healing. *J Wound Care* **5(6):** 277–80

Usher LR, Lawson RA, Geary I, et al (2002) Induction of neutrophil apoptosis by Pseudomonas aeruginosa exotoxin pyocyanin; a potential mechanism of persistent infection. *J Immunol* **168(4):** 1861–8

Ziegler K, Gorl R, Effing J, et al (2006) Reduced cellular toxicity of a new silver-containing antimicrobial dressing and clinical performance in non-healing wounds. *Skin Pharmacol Physiol* **19(3):** 140–6

Zychlinsky A, Sansonetti P (1997a) Perspective series: host/pathogen interactions. Apoptosis in bacterial pathogensis. *J Clin Invest* **100(3):** 493–5

Zychlinsky A, Sansonetti P (1997b) Apoptosis as a pro-inflammatory event: what can we learn from bacteria-induced cell death? *Trends Microbiol* **5(5):** 201–4

Richard White, Scientific Editor, Wounds UK, and Senior Research Fellow, Aberdeen Royal Infirmary

Correspondence

If you would like to comment on the articles published in Wounds UK or on an issue concerning wound care in general, please send a letter of no more than 400 words to:
Nicola Rusling, Managing Editor (nicola@wounds-uk.com).