The pathophysiology of chronic osteomyelitis

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Abstract

Chronic osteomyelitis is a biofilm-based infection of bone where the majority of causative microorganisms are sessile in nature, rendering them less sensitive to systemic antibiotic agents and making routine culture techniques unreliable. Biofilms are the characteristic growth pattern for most bacteria and are now understood to consist of interactive communities with the ability to alter their gene expression in order to ensure survival. Our knowledge of the host's response to infection is also rapidly expanding. The discovery that osteoclastic and osteoblastic cells play a central role in the immune response of bone has resulted in a better understanding of osteo-immunology. This expansion of knowledge has created new opportunities in terms of the development of novel treatment strategies in the management of chronic osteomyelitis and periprosthetic infections.

Key words: osteomyelitis, chronic, pathogenesis, osteo-immunology, biofilm

Introduction

Chronic osteomyelitis remains a daunting challenge to orthopaedic surgeons. It is often described as a disease that can never truly be cured, particularly when the biological characteristics of the causative organism are taken into account.[1]

The two main routes of infection in osteomyelitis are through either haematogenous or contiguous bacterial inoculation. It is estimated that approximately 10 to 30% of acute haematogenous osteomyelitis may become chronic in nature. Chronic haematogenous osteomyelitis is an age-old problem, illustrated by the fact that the palaeopathological analysis of an *Australopithecus africanus* hominid skeleton, from Sterkfontein, South Africa, revealed evidence of chronic infectious disease of the skeleton.[2] The oldest medical text, known as the Edwin Smith Papyrus from the sixteenth century BC also describes cases of 'pus pouring from bone', probably in reference to osteomyeilits.[3] Despite the advent of antibiotic therapy and advances in the management of acute haematogenous osteomyelitis, the incidence of chronic osteomyelitis has steadily climbed, particularly during the past century. This is likely as a result of the increased incidence of high velocity skeletal trauma, as well as the increased use of surgical implants.

Open fractures can lead to the development of contiguous osteomyelitis in 3–50% of cases, depending on the severity of the injury and quality of the subsequent management.[4] The surgical management of closed fractures may result in post-operative osteomyelitis in 1–5% of cases, while the estimated risk of infection complicating an elective primary hip or knee replacement is in the region of 0.5–2%.[5] This risk is, however, significantly higher in revision surgery (5%) and, in the case of second stage revision for periprosthetic infection, the infection rate climbs to approximately 20%. Overall, infectious complications occur in approximately 5% of orthopaedic cases during the life-time of the prosthesis or implant.[5]

Socio-economically underdeveloped regions carry a particularly heavy burden in terms the prevalence of osteomyelitis.[6] This may be attributed to, among other factors, the high incidence of osteomyelitis in childhood, immunosuppression, malnutrition and the high incidence of trauma. The high prevalence of trauma in South Africa is clearly illustrated by the fact that interpersonal violence and road traffic accidents were the second and fourth most common causes of death in South Africa in the year 2000.[7] The road traffic accident fatality rate in South Africa (39.7 per 100 000 population) is higher than for any other WHO region and almost double the world average.[8] This implies a correspondingly high morbidity related to road traffic accidents, which contributes to an increased incidence of post-traumatic osteomyelitis.

In addition to a high trauma load, South Africa is faced with a severe shortage of qualified orthopaedic surgeons. In developed countries, like the USA and Canada, figures range from 4.8–5.6 full-time equivalent orthopaedic surgeons per 100 000 population.[9,10] In contrast, in 2011, public sector medical services in the interior of the KwaZulu-Natal province in South Africa served a population of approximately 3.5 million people with only 0.37 full-time orthopaedic surgeons per 100 000 population. This shortage results in many patients with skeletal trauma, and more specifically compound fractures, not receiving appropriate treatment, contributing to a further increase in the chronic osteomyelitis disease burden.

Although the association between HIV infection and chronic osteomyelitis has not been clearly defined, previous research has shown an increased risk of post-operative infection following surgical fracture fixation in HIV-infected individuals.[11] The prevalence of HIV infection in Southern Africa has reached epidemic proportions. Mid-year estimates for 2011 approximates the national prevalence of HIV infection in adults at 10.6%.[12] In KwaZulu-Natal the situation is worse, with an estimated 21.5% of adults between the ages of 15 and 49 years being infected with HIV.[13]

The problem of high disease prevalence is compounded by the financial implications of the treatment of chronic osteomyelitis. The direct medical cost associated with the management of osteomyelitis, in the USA in 1999, was estimated at $35 000 per episode.[14] In Southern Africa the high cost of treatment is multiplied by the much higher burden of disease, posing a significant challenge to our resource-restricted health systems.

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Definition

Osteomyelitis is characterised by the progressive inflammatory destruction of bone followed by the apposition of new bone as part of the reparative process. Classically chronic osteomyelitis was therefore defined by the presence of either sequestrum or involucrum as a result of an infective process involving bone. This definition originated from the observation that acute haematogenous osteomyelitis, if left untreated, may result in the formation of necrotic segments of bone, which would then serve as a source of on-going or chronic infection.

As orthopaedics evolved into a primarily surgical field and the use of surgical implants increased, the incidence of contiguous post-operative osteomyelitis dramatically increased, necessitating revision of our definition. No longer was haematogenous spread considered to be the major cause and the emphasis shifted towards the duration of the disease. The duration of infection that defined chronicity gradually decreased over time and in 1997 it was defined as symptoms remaining for longer than ten days.[15] An alternative, more philosophical approach was to define chronic osteomyelitis according to the response to therapy, where chronicity was defined as infection unresponsive to multiple therapeutic attempts to eradicate infection.[16]

As our understanding of the pathophysiology has grown, the definition of chronic osteomyelitis has been refined even further. Cierny proposed a definition more appropriate to the setting of contemporary orthopaedics.[17] He defined chronic osteomyelitis as a biofilm-based infection where only a minor fraction of the causative microorganisms are planktonic (free-swimming). The majority of pathogens are sessile-based, resiliently attached to necrotic bone, surgical implants or foreign material and embedded within a glycocalyx slime (biofilm). This renders them less sensitive to systemic antibiotic agents and makes routine culture techniques less reliable. With time the bacterial toxins and by-products of the host's immune system accumulate to result in the local and systemic manifestations of chronic osteomyelitis. Cierny's definition is not restricted to the cause, the presence of surgical implants, nor to the anatomic nature or duration of the disease, but rather defines it by the presence of a universally applicable pathogenesis.

The pathogen

Normal bone is highly resistant to infection. Osteomyelitis typically occurs in the setting of a large bacterial inoculation in combination with trauma, necrosis or ischaemia of tissue and/or the presence of foreign material. Large strides have been made over the past few decades in our understanding of the disease process underlying chronic infections of bone. Central to this understanding lies the concept of bacterial biofilm. In 1987 Gristina *et al*. coined the phrase 'the race for the surface'.[18] The host cells strive to establish an integrated protective cellular layer with functional defence mechanisms (including opsonification, phagocytosis and complement mediated lysis), while the invading bacteria enter their default growth pattern and establish a biofilm. This is a layer-like aggregation of microbial cells and extracellular polymeric substances attached to a substrate which provides an environment for the exchange of genetic material between bacterial cells.[19]

The presence of a foreign body has been shown to significantly increase susceptibility to infection. For example, the minimal infecting dose of *Staphylococcus aureus* is more than 100 000-fold lower in the vicinity of subcutaneous devices than in skin without an implant.[20] This increased susceptibility to infection is partially due to a locally acquired granulocyte defect.[21]

Biofilm formation occurs in five stages, namely adhesion, production of the extra-cellular matrix, colonisation, maturation and finally dispersion of bacteria. The first stage involves adhesion of the bacteria to the substrate through specific and non-specific mechanisms.[22] Specific mechanisms involve the expression of adhesion molecules known as adhesins or MSCRAMM (microbial surface components recognising adhesive matrix molecules) specific to certain host proteins like fibronectin, laminin, sialoglycoproteins, fibrinogen and collagen. Non-specific mechanisms involve surface tension gradients, hydrophobicity and electrostatic forces. Once contact is made with the substrate, bacteria migrate (with the aid of flagella) until other bacteria are encountered, thus establishing micro-colonies. Once the microbial density reaches a critical point, the volume of cell-to-cell signal molecules released is sufficient to activate genes involved in the production of an exocellular polysaccharide or glycocalyx. The ability of a microbial colony to sense its size and respond by altering its gene expression is referred to as quorum sensing. This phase of biofilm formation is being investigated as a target for the prevention of biofilm formation on orthopaedic implants. Animal models have shown that the quorum-sensing inhibitor RNA III-inhibiting peptide can help prevent staphylococcal biofilm formation and infection.[23]

Contrary to popular belief bacteria do not differentiate during the colonisation phase of biofilm formation but rather they alter their pattern of gene expression and should therefore be seen as interactive communities, rather than a multicellular organism.[19] A sub-population of bacteria may evolve into a phenotypically resistant state and express biofilm-specific antimicrobial resistance genes. Other bacteria within the biofilm may produce hydrolase enzymes and exotoxins, resulting in local tissue invasion. Biofilm-based bacteria have up to a 1000 times greater resistance against antimicrobials and host immune defences. This derives from a combination of phenotypic, mechanical and metabolic mechanisms. Antibiotics face mechanical and osmotic challenges in penetrating a biofilm, while the reduced growth rate of bacteria due to incomplete penetration of metabolic substrates and accumulation of waste product, makes the biofilm-based bacteria even more resilient.[24] These so-called small colony variants are characterised by slow growth, decreased pigment formation, low coagulase activity, reduced haemolytic activity, and resistance to antibiotics.[25] Small colony variant bacteria are able to persist within host cells and it has been suggested that the intracellular location of this subpopulation might shield them from host defences and antibiotics, thus providing one explanation why chronic osteomyelitis is able to reactivate years after the initial infection.[26] The final stage in the evolution of a biofilm involves the dispersion of planktonic bacteria. Through quorum sensing, gene expression may alter the bacterial phenotype from colonising to invasive and as environmental conditions deteriorate within the biofilm, bacteria disperse to find a surface with a more favourable environment.

The host response

The innate immune response is critical in the early phase of bacterial colonisation. It is triggered at the site of bacterial infection by the production of cytokines like interleukin-1 (IL-1), IL-6 and tumour necrosis factor (TNF). These cytokines recruit and activate phagocytic cells such as polymorphonuclear (PMN) leukocytes and macrophages to produce bacteriolytic free radicals.[27] Neutrophils, which engulf bacteria, die at the site of infection and comprise much of the material we see as pus draining from a sinus. Macrophages are critical for the phagocytosis of planktonic bacteria and necrotic material. This process is facilitated by opsonisation (the binding of an antibody to a bacterial antigen), which anchors the bacteria to the Fc-receptors on phagocytic cells and activates intracellular signalling pathways to produce free radicals like superoxide and nitrous oxide.

Antibiotics face mechanical and osmotic challenges in penetrating a biofilm

Acquired or adaptive immunity is responsible for the eradication of chronic or persistent infections and also plays an important role in the prevention of recurrence. The first component of the adaptive immune response is the cellular response in which cytotoxic CD8^{+} T cells lyse infected host cells. The second component is the humoral response involving the production of antibodies by B lymphocytes. Centrally positioned within the adaptive immune response are macrophages which produce TH1 lymphokines (IL-12 and interferon-γ) which drive cell-mediated immunity, as well as TH2 lymphokines (IL-3 and -4) regulating the humoral response. Most cases of chronic osteomyelitis involve extracellular organisms and therefore the humeral immune response, incorporating antibody opsonisation and phagocytosis of bacteria, plays a central role. Animal studies have identified several bacterial antigenic proteins in antibody-mediated immunity in *Staphylococcus aureus* biofilm-based infections, including cell-surface-associated beta-lactamase, lipoprotein, lipase, autolysin and ABC transporter lipoprotein.[28] Some of these antigens are currently being investigated as possible targets for vaccination.[29] Anti-autolysin monoclonal antibodies (mAbs), for example, may have a protective effect through the inhibition of adhesion and growth of *Staphylococcus sp.*

Osteo-immunology

Chronic osteomyelitis is characterised by osteolysis in combination with reparative osteosclerosis, which aims to confine the inflammatory process. Bacterial components and toxins have a strong stimulatory effect on osteoclastic activity through indirect (RANKL and other osteoclastogenic factors) and direct mechanisms.[30] As is the case with TNF, bacterial surface-associated material (SAM) can induce the formation of osteoclasts from monocytes independent of the RANKL mechanism. Other bacterial products such as lipopolysaccharide (LPS) and endotoxin induce the expression of osteoclastogenic cytokines including RANKL, TNF, IL-1, and IL-6 by osteoblasts and

other cells.[31] These cytokines, not only stimulate osteoclasts, but also inhibit bone formation through impairment of osteoblast differentiation, proliferation, activity and survival, resulting in net resorption of bone at the site of chronic infection. Although it is well known that bacteria that cause chronic osteomyelitis can be found intracellularly, within osteoblasts, it is not known if these bacteria have a direct inhibitory effect on osteoblasts or inhibit bone formation through mechanisms involving the known inhibitory cytokines like sclerostin, DKK1 or noggin.[27]

The role of osteoclasts

Receptor activator of nuclear factor kappa-B ligand (RANKL) is a potent activator of osteoclasts and is produced by bone marrow stromal cells under normal conditions. However, in osteomyelitis certain bacterial components, such as lipopolysaccharide (LPS), result in the production of RANKL by a variety of cells (including activated T-cells) ultimately causing abnormal bone loss.[32] Dendritic cells (DCs) are monocyte-derived antigen-presenting cells which play an important role in both innate and adaptive immunity. Migrating dendritic cells (mo-DCs) transport antigens from the site of infection to lymphoid organs in order to initiate T-cell responses, including CD4+ activation. Dendritic cells also affect osteoclasts through the stimulation of RANKL production by T cells.[33] A subset of resident dendritic cells, called Tip-DCs, present at the site of infection also have strong anti-microbial effects through the production of TNF-alpha and inducible nitric oxide synthase (iNOS). Although these products are beneficial in terms of eliminating pathogens they also contribute to tissue damage.

Staphylococcus aureus directly activates dendritic cells through the production of an exotoxin called leukocidin which triggers a Toll-like receptor (TLR-4) dependent signalling pathway.[34] *Staphylococcus aureus* enterotoxin B induces maturation of dendritic cells and stimulates them to produce high levels of IL-2. A third mechanism whereby *Staphylococcus aureus* up-regulates dendritic cell function is through the production of the protease staphopain B, which, through a complex mechanism results in the formation of chemerin. Chemerin, in turn, has been suggested to act as a potent chemoattractant to immune-regulatory dendritic cells. It is also interesting to note that upon activation by microbial antigens, CD11c+ dendritic cells can differentiate into functional osteoclasts in the presence of macrophage colony-stimulating factor (M-CSF) and RANKL expressed by activated CD4+ T cells.[35]

The role of osteoblasts

Although our understanding is still cursory, osteoblasts are seen as the last line of defence in the fight against bacterial infection and biofilm formation. They express a wide array of immune-stimulatory cytokines in response to ligation of bacterial products, like LPS and DNA, to the Toll-like receptors (TLR-2, 4 and 9) on their surface. These cytokines include anti-microbial peptides (beta-defensin-3), chemokines (CCL-2, CCL-5, CXCL-8, CXCL-10), nflammatory cytokines (IL-6), co-stimulatory molecules (CD40) and MHC II.[27,36,37] The secretion of these chemokines suggests that osteoblasts do not only play an important role in the innate immune response but also in the cellular immune response. Cells expressing MHC II molecules typically present exogenous antigens to T-helper cells. The fact that osteoblasts express MHC II may explain why osteoblasts internalise bacteria like *Staphyloccus aureus*. This process has been investigated further and *Staphylococcus aureus* sigma B regulon has been shown to be the key mediator of the internalisation of bacteria by osteoblasts, and is thus a possible target for therapeutic intervention.[38]

Conclusion

The physiological status of the host determines not only the clinical extent of the disease, but also the treating physician's ability to effect cure. Without a competent immune response from the host, any attempt at surgical eradication of the infection may well be futile. The importance of the host's ability to launch an effective immune response is clearly illustrated in the principles behind the Cierny and Mader classification, which incorporates assessment of local and systemic factors affecting the hosts immune competency.[39] Studies using the Cierny and Mader classification have confirmed that the host status is the most important predictor of treatment failure.[40]

The discovery that osteoclastic and osteoblastic cells play a central role in the immune response of bone has resulted in better understanding in the relatively new field of osteo-immunology. As is the case with bacterial biofilms, our knowledge of the host's response to infection is also rapidly expanding. This knowledge creates new opportunities in terms of the development of novel treatment strategies in the management of chronic osteomyelitis and periprosthetic infections.

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