The immunology of wound healing: the body as a battlefield

Open wounds are a nightmare for the immune system. With the external barrier of the skin breached, the exposed tissues are vulnerable to attack by the hordes of microbes rampant in the external environment. The immune system rallies to the defence of the body by sending in the cells and molecules of the innate immune division which throw themselves into close combat at the front line. The battle can go either way, but in chronic wounds it usually develops into a lengthy stand-off. This article describes the details of the battle looking at the combined defensive forces of the innate and the adaptive immune systems.

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A wound is an immunological crisis. An open wound is an open invitation to bacteria, welcoming them to a rich life on an ideal growth medium — the human body (Brown et al, 2008). Even under normal circumstances, human life is dependent on the protection of a healthy immune system that can quickly and effectively identify and eradicate disease-causing microbes, and that dependence is even greater in the presence of a wound. When the protective layer of the skin is breached, the only thing that stands between the hordes of invading microbes and the vulnerable tissues and fluids of the body is the immune system. An open wound sets the immunological alarm bells ringing, and all available defensive resources are quickly called upon to form a first line of defence in the wound bed. In an acute wound with a normal healing trajectory, the defenses will act quickly and the system can return to normal once the skin barrier is re-formed. But in a non-healing wound the grim prospect is of sustained 'trench warfare' where both combatants (microbes vs. the human immune system) dig in for a drawn-out conflict, laying waste to the landscape over which they are fighting.

The ebb and flow of battle leads to complex reactions and variable symptoms, immediately apparent to wound care clinicians in the visual appearance of the wound's surface and the surrounding skin. The pain, heat, swelling and redness of inflammation are the primary macroscopic signs of the immune system's activity. A range of other variable symptoms also result from the conflict in the wound, including visible slough and pus or the tell-tale signs of infection breakthrough such as friable granulation, foul odour, wound breakdown, cellulitis or even the febrile response of systemic spread. These indirect, observable signs give clues about what is going on, but the real, direct action is at the cellular and molecular levels, where the drama is intense and the action fast and furious. If we had a deeper understanding of these cellular and molecular dramas within the wound's micro-environment, we would be better equipped to come up with new diagnostic techniques, new types of therapy and new approaches to caring for wounds.

As the drama of wound defence plays out, a variety of adversaries are drawn into the fray. On the immune system's side there are various types of active cells and interacting molecules. On the microbial side there are over a hundred commonly encountered bacterial species. Infecting bacteria can form all sorts of unholy alliances of mixed species (called microbial consortia) with enhanced menace, although individually and as consortium members *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common invaders of wounds.

There is scarcely a mention of chronic wound care in any of the mainstream medical textbooks on microbiology and immunology. Although this area is a relatively neglected subject as a specialty, steady progress is being made — but there is still a lot more to discover. At first sight, it all seems dauntingly complicated. But, although it is undeniably complex, our present knowledge allows us to comprehend

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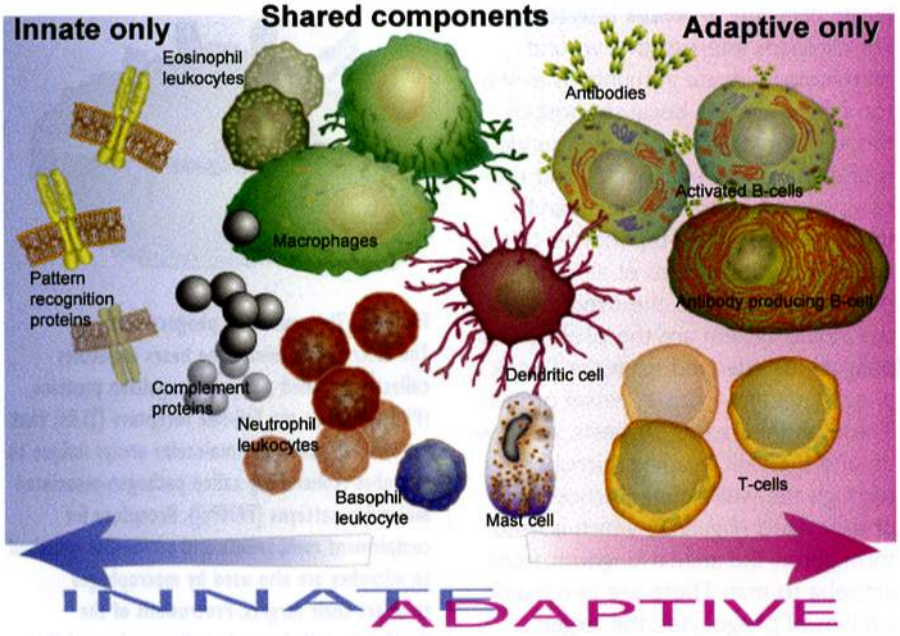

Figure 1. Innate and adaptive compartments of the immune system. The immune system is usefully considered as existing in two compartments, named innate immunity and adaptive immunity. Innate immunity is usually taken to include physical and chemical barriers at the surfaces of the body, although these components do not usually react or respond. As can be seen from this diagram, many of the most important components are actually shared by both compartments. Some components are only considered to be adaptive, while others are only innate. Innate immune responses seem to be the most significant immune defences for chronic wounds.

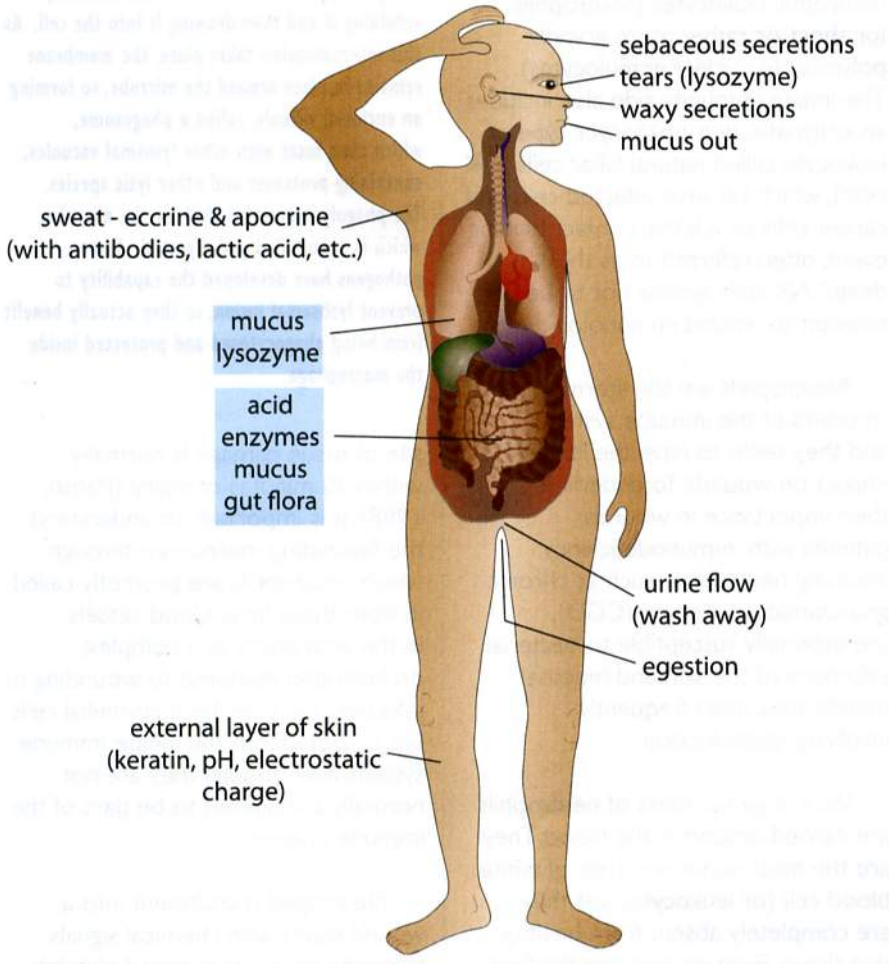

Figure 2. Physical and chemical barriers to entry at the surfaces of the body. The very first line of defence for the human body is formed by the physical and chemical barriers built into the surfaces that have contact with the outside world. Pathogens have to find a way past these barriers before they can cause a problem. A wound is, in effect, a very open door for pathogens because the first lines of defence have already been breached by the event that caused the injury.

the main themes and overall shape of the key immune responses, the roles of the most prominent cells and molecules and their essential interactions.

Overview of the immune system

The immune system consists of specialised cells and proteins that are present and active throughout the whole body (Friedl and Weigelin, 2008). There are some discrete organs, such as the spleen and lymph nodes, and tissue sites (e.g. gut lamina propria, skin) in which certain immunological cells are located, often with a particular structure and organisation with which to accomplish essential interactions and functions. The common, grand purpose of all these components is that of protecting the individual against invasion by foreign organisms, viruses and toxins. In addition, the system undertakes a number of subsidiary, related roles, including removal of dead or spent cells, control of certain physiological processes and the elimination of cancerous cells (Park and Barbul, 2004).

It is helpful to view the immune system as consisting of two broad, interacting divisions, called innate immunity and adaptive immunity (*Figure 1*). There is a very good reason for this arrangement. When a wound occurs, there is an urgent need for

immediate defence mechanisms that can be deployed without the delay incurred by making specific preparations. But the problem with ready-to-go defences is that they have to be generalist — capable of recognising and attacking any kind of foreign invader of whatever molecular complexion — which goes hand-in-hand with a relatively muted response and lots of collateral damage. The innate immune system is defined as all the ready-to-go defences and, usually, the definition includes the physical and chemical barriers to entry at the various body surfaces (*Figure 2*).

While the innate responses attempt to hold the line (or win the day on their own), the formidable adaptive system takes time out to prepare a killer blow for the pathogens, with carefully crafted guided weapons (antibodies and activated T-lymphocytes) that home-in on their targets with great specificity and minimal collateral damage. Adaptive immune responses are much more powerful and effective than innate responses, but it can be several days before the first immunoglobin M (IgM) interim antibodies are ready to be deployed, and it takes even longer for the really high-efficiency, optimised immunoglobin G (IgG) antibodies to be perfected by the system. The familiar process of vaccination prepares adaptive responses well in advance of microbial or viral attack by administration of inactivated or attenuated microbes or viruses (in a state in which they cannot cause disease). This stimulates the production of antibodies that are able to attack the full strength pathogen with unabated ferocity, even though they were brought into being in response to inactivated forms.

Although there are distinct activities and roles attributable to the innate and the adaptive (often also called 'acquired') compartments of the immune system, it is also important to appreciate that they are closely linked and they interact with each other (Iwasaki and Medzhitov, 2004; Hoebel et al, 2004).

Innate immunity — wound interactions

For clinicians interested in wound immunology, innate immunity is at the top of the agenda, because most of the immunological action in wounds is undertaken by phagocytes and the complement system, rather than by the antibodies and lymphocytes of the adaptive system (Jones et al, 2004). As shown in *Table 2*, the main cells of the innate system are the 'professional phagocytes' (derived from the Greek phag = eat and kytos = vessel or cell). These, as their name suggests, swallow-up and then kill invading microbes through the well defined process of phagocytosis (*Figure 3*) which is used throughout the animal kingdom, from amoeba to man. There are two major families of phagocytes: the larger variety, typified by the macrophages and the smaller, aggressive neutrophil leukocytes (neutrophils for short or, rather more grandly, polymorphonuclear granulocytes). The innate immunity side also includes an enigmatic, non-phagocytic type of leukocyte called natural killer cells (NK cells), which kill virus-infected cells and cancer cells by a lethal contact binding event, often referred to as the 'kiss of death'. NK cells appear not to be very relevant to wound immunology.

Neutrophils are the storm troopers of the immune system, and they seem to have the largest impact on wounds. To underline their importance in wounds, patients with immunodeficiency involving neutrophils, such as chronic granulomatous disease (CGD), are especially susceptible to bacterial infections of the skin and mucosal membranes, most frequently involving staphylococci.

Very large numbers of neutrophils are carried around in the blood. They are the most numerous type of white blood cell (or leukocyte), yet they are completely absent from healthy skin tissue. Even so, they are the first defensive cells to reach the scene of any microbial infection or tissue injury, rushing into the affected site from nearby capillary blood vessels or post capillary venules. Their arrival at the site of tissue damage is normally within 30 minutes of injury (Parish, 1998). It is important to understand the fascinating mechanism through which neutrophils are promptly called in from these local blood vessels. In this enactment of a complex, orchestrated response to wounding or infection, the vascular endothelial cells are co-opted into the innate immune system, even though they are not normally considered to be part of the immune system.

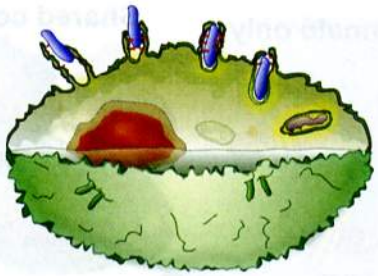

Figure 3. The process of phagocytosis. The macrophage membrane bears molecules collectively called pattern recognition proteins, (PRPs), such as the Toll-like receptors (TLRs) that can bind to particular molecular arrays unique to microbes (collectively called pathogen-associated molecular patterns [PAMPs]). Receptors for complement components and antibodies attached to microbes are also used by macrophages to select their targets. Protrusions of the membrane, called pseudopodia, are formed that can attach to a microbe via PRP-PAMP binding. The pseudopodia extend round the microbe, enfolding it and then drawing it into the cell. As this internalisation takes place, the membrane remains in place around the microbe, so forming an enclosed vacuole, called a phagosome, which then fuses with other lysosmal vacuoles, containing proteases and other lytic species. The phagolysosome is lethal for the microbe, which is soon killed and digested. Some pathogens have developed the capability to prevent lysosomal fusion, so they actually benefit from being phagocytosed and protected inside the macrophage.

Neutrophil recruitment into a wound starts with chemical signals triggered either from blood platelets or tissue mast cells, such as histamine or prostaglandin PGD2, and/or through a special set of sentinel proteins called 'complement'. The complement proteins are always