

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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KEY WORDS

Immunity
Phagocyte
Neutrophil
Defence
Macrophage

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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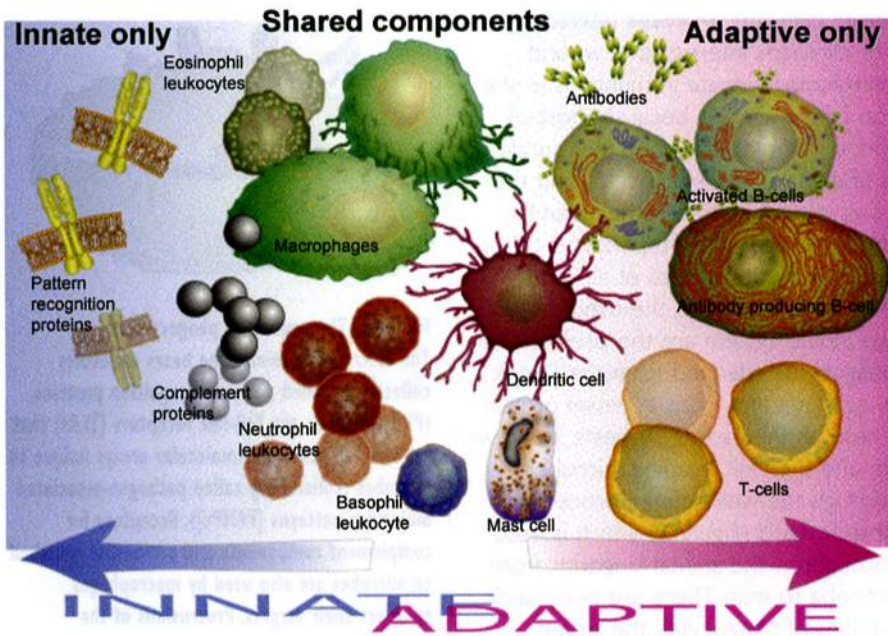


Figure 1. Innate and adaptive compartments of the immune system. The immune system is usefully considered as existing in two compartments, named innate immunity and adaptive immunity. Innate immunity is usually taken to include physical and chemical barriers at the surfaces of the body, although these 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.

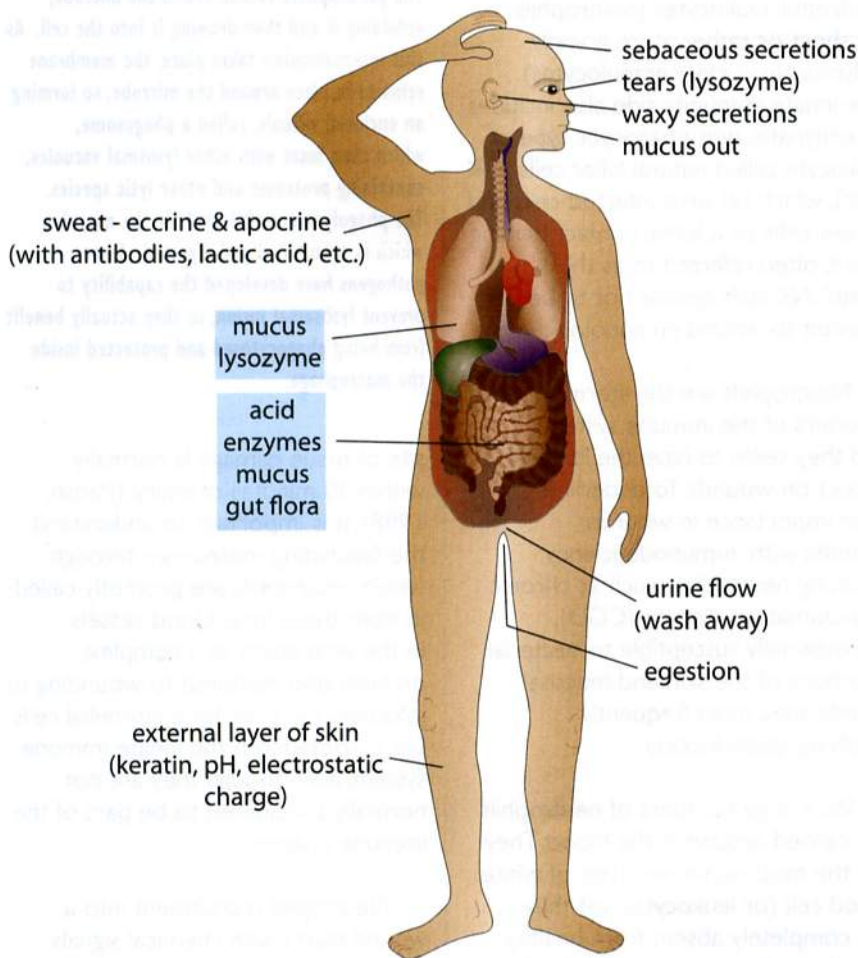


Figure 2. Physical and chemical barriers to entry at the surfaces of the body. The very first line of defence for the human body is formed by the physical and chemical barriers built into the surfaces that have contact with the outside world. Pathogens have to find a way past these barriers before they 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 immunoglobulin M (IgM) interim antibodies are ready to be deployed, and it takes even longer for the really high-efficiency, optimised immunoglobulin 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

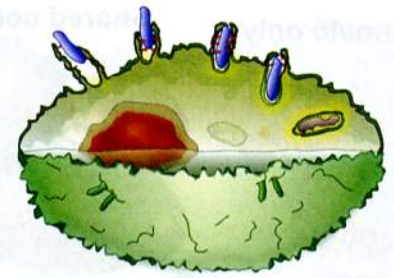


Figure 3. The process of phagocytosis. The macrophage membrane bears molecules collectively called pattern recognition proteins, (PRPs), such as the Toll-like receptors (TLRs) that can bind to particular molecular arrays unique to microbes (collectively called pathogen-associated molecular patterns [PAMPs]). 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 lysosomal 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.

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.

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

Table 1

The main components of the innate immune system — neutrophils, macrophages, mast cells and complement

Innate immunity is based on cells and molecules that can work together to mount a powerful first line of defence. They are present from birth and are ready and waiting for defensive action all the time.

Neutrophils — the most numerous of the blood leukocytes

Neutrophils are generated in the bone marrow and are released into the blood where they remain for several hours. In normal tissues they may last for a few days but in a wound they die quickly after engulfing microbes. Various proteases (e.g. elastase, MMP8, MMP9), other enzymes (e.g. myeloperoxidase, lysozyme), and functional proteins (e.g. lactoferrin, phagocytin) which are carried in their granules are released as they work. Neutrophil-derived proteases and reactive oxygen species (e.g. hydrogen peroxide) cause major local tissue damage. They are recruited from the blood, and are attracted into sites of infection or inflammation by chemical attractants called chemokines. Tissues containing large numbers of neutrophils become severely hypoxic and oedematous. They consume copious amounts of oxygen to carry out phagocytosis and oxygen-dependent antimicrobial activities (an episode of leukocyte oxygen utilisation is known as a respiratory burst).

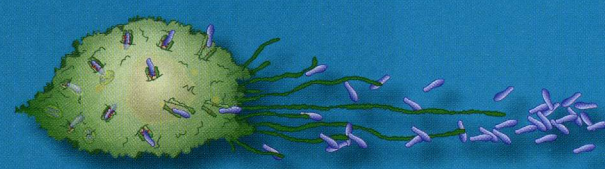


Macrophages — extremely versatile cells that develop from monocytes in the blood

Monocytes stay in the blood for up to 100 hours and then migrate into tissues to become resident macrophages (also known as histiocytes). In normal tissues they may remain for many months. When needed in increased numbers (during infection or certain phases of inflammation) they are called in from the blood (much as neutrophils are) and from surrounding tissues. Once activated, they are very active in killing microbes and consume more oxygen. Macrophages can engulf and kill microbes directly or clear-up dead neutrophils and remaining bacteria after the battle. They secrete a wide variety of enzymes, signaling molecules and active substances, reflecting their multiple roles, including the control of processes beyond inflammation and antimicrobial defence. Cytokine secretion is a major feature of macrophage activity, alongside an ability to process and present antigens to T-cells as a key part of antibody induction and T-cell activation. They have PRPs and receptors for antibodies on their surface. Like neutrophils, their full antimicrobial potential is dependent on oxygen supply.



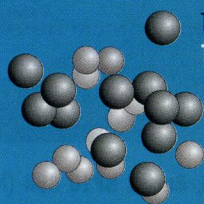
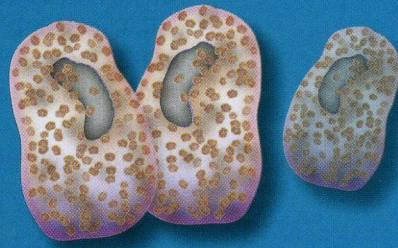
Sessile macrophage



Activated macrophage attacking bacteria

Mast cells — resident in connective tissue, especially in association with blood vessels and nerves

IgE antibodies, best known for anaphylactic allergy and hayfever, are carried on the mast cell surface. They are loaded with pre-formed inflammatory substances, which are stored in densely packed granules in the cytoplasm. On activation the granules swell and move to the cell membrane where they empty into the surrounding tissues an inflammatory cocktail of histamine, heparin and a multifunctional messenger molecule, confusingly called tumour necrosis factor or TNF for short. Several other mediators can also be synthesised afterwards. Activation is achieved by several mechanisms, including allergen binding to IgE or binding of complement fragments C3a and C5a to special receptors. The main consequences of activation are increased vascular permeability and vasodilation in local blood vessels and recruitment of inflammatory cells.



The complement system

This is a set of interacting serum proteins that undergo a sequential reaction cascade when the initiating members are triggered by interaction with a microbe or other foreign surface. The cascade results in generation of fragments that act as inflammatory signal molecules (e.g. C3a and C5a) that work by binding to receptors on target effector cells (e.g. mast cells) and assembly of molecular complexes that create holes in microbial membranes.

in attendance, waiting for an event or circumstance that triggers their activation. Activation results in the generation of a flux of small signalling

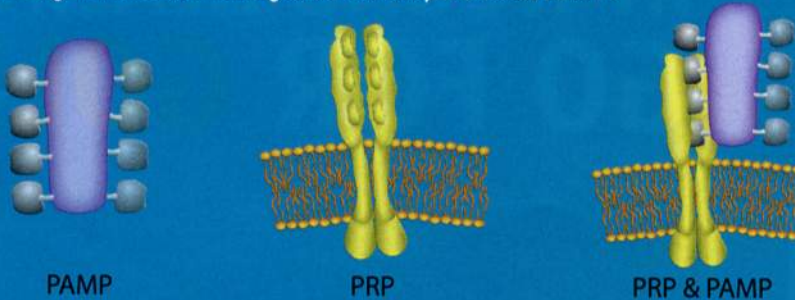
molecules — C3a and C5a — which diffuse away from the site toward the nearest micro-vascular blood vessels, together with other signal substances.

When they reach the blood vessels, these molecules bind to specific receptors on the endothelial cells lining the inner vascular surfaces, so

Table 2

The main components of the innate immune system — pattern recognition proteins (PRPs) and pathogen-associated molecular patterns (PAMPs)

Pattern recognition proteins (PRPs) and pathogen-associated molecular patterns (PAMPs)
Neutrophils and macrophages need to identify and attack microbes as soon as they enter the body, even when there has never been a previous contact with that type of microbe and no antibodies are available. Fortunately, microbes share common molecular signatures in the form of certain molecules (e.g. sugars) arrayed in a particular pattern not found on human cells — the so-called PAMPs. To enable recognition of PAMPs, macrophage surface membranes are equipped with specific binding proteins that bind, not to specific individual molecules but to multiple molecules when they are configured in a particular pattern. These are called the PRPs, of which there are a number of varieties, including toll-like receptors, scavenger receptors and the macrophage mannose receptor. This is an area of great interest to immunologists and is the subject of intense research.



triggering some remarkable localised changes. The most conspicuous change for the neutrophils that are rushing by in the blood is the sudden display of a molecular semaphore system, set-out on the internal surface of capillaries and post-capillary venules. Passing neutrophils can read the semaphore by a process akin to cellular Braille, whereby intimate surface contact allows the cell to 'feel' the displayed molecular shape or sign. If the neutrophils recognise the molecular shape, they slow down by rolling along the capillary wall before locking on to a particular molecular Braille character (a process called margination). Once captured at this stopping point, they haul themselves out of the lumen by clambering between and through the endothelial cells lining the vessel. They then crawl into the interstitial spaces in a process called diapedesis (Figure 4). To achieve this exit, they have to slash their way through the connective tissue barriers by releasing specialist proteases, especially neutrophil elastase and matrix metalloprotease 8 (MMP8, also known as neutrophil collagenase). Once out of the confines of the capillary vessel, like microscopic

bloodhounds, they follow the same chemical trail emanating from the infected site (chemokine-mediated chemotaxis) to throw themselves into the fray.

While this molecular semaphore and guidance system is very conspicuous to the leukocytes, the signs that are apparent to the clinician and patient are all to do with associated changes in blood flow and oedema. So well orchestrated are the responses to injury and infection that the hydrodynamic properties and structure of the micro-blood vessels themselves are changed to ensure the deployment of a speedy defensive strategy. That is why injury or infection is soon followed by visible reddening of the area and a rapid onset of oedema. The chemical signals that cause the molecular semaphore display, together with a pain-triggered neuronal reflex, also trigger blood vessel dilation and cause the endothelial cells of the capillaries and post-capillary venules to retract away from each other so creating tiny leakage points in the endothelial lining. This allows leakage of blood plasma

into the immediately surrounding interstitial tissue spaces, leading to oedema. Together, these effects cause a major slowing of the blood flow, so allowing blood cells to accumulate in the lumen. Having been slowed down, the neutrophils are better able to read the chemical semaphore, and their exit is helped by the gaps in the endothelial lining. In due course, if the infection or inflammatory signals persist, the vessel becomes congested or blocked by backed-up cells, especially the accumulated neutrophils trying to follow the traffic signs pointing to the exit and therefore the route to the wound.

Macrophages (Table 1) are also phagocytic cells, the 'macro' prefix reflecting their greater size, in comparison with neutrophils (sometimes known as 'microphages'). The term macrophage can be translated as 'big eater', which only describes a very small part of the role they play. There are several different subtypes of macrophage with different names, depending on where they are normally found, such as Kupfer cells which are liver macrophages and histiocytes which are connective tissue macrophages. There is a network of macrophages throughout the body, known as the 'mononuclear phagocyte system'. The job of the mononuclear phagocyte system is multifaceted, and it includes gathering up foreign cells and debris and acting as a primary defensive sentinel network, as well as stimulating and informing the cells of the adaptive immune system. Macrophages have far-reaching effects because they orchestrate various aspects of complex immune responses and secrete numerous growth factors and cytokines that are involved in controlling tissue repair and regeneration (Parish, 1998; Park and Barbul, 2004).

When needed, macrophages and other leukocytes can be called into a wound by the same kind of endothelial semaphore system used by neutrophils (Hart, 2002). Typically, macrophages will be called in when the neutrophils have done their antimicrobial job,

Table 3

The main components of the adaptive immune system

Adaptive immune mechanisms are activated only in response to the ingress of foreign material such as microbes. They are very specific and usually efficient at discriminating between self and non-self material. They provide a substantially more powerful defence than innate immunity. Lymphocytes also provide a memory function by keeping memory-cell records of each immunological response.

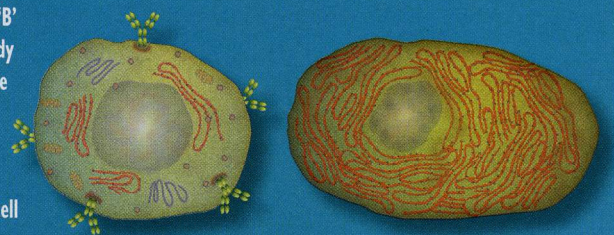
Adaptive immunity is founded on lymphocytes which are deployed in very large numbers in the body. In a normal individual there are about a trillion lymphocytes at any one time. All resting lymphocytes look virtually uniform, yet there are several different types with distinct roles. It is the T-lymphocytes and the B-lymphocytes that are the most important to our understanding of adaptive immunology.

T-lymphocytes (or T-cells) are schooled through the thymus (hence the 'T' prefix) and have the job of recognising and responding to foreign proteins by means of their amazingly versatile molecular recognition receptors — the T-cell receptors (TCRs). In effect, they 'see' the universe of foreign proteins through their TCRs, but TCRs can only 'see' proteins that have been cleaved into small peptides and displayed on the surface of specialised antigen presenting cells (usually dendritic cells). TCRs are not secreted, so T-cells can only attack or control target cells by close combat, or by influencing other immune cells (especially macrophages) to attack for them. Depending on the other surface receptors they express alongside the TCRs, and on the profile of the cytokines they secrete, they adopt different roles. Some T-cells help B-cells to make antibodies while others become lethal cytotoxic cells, capable of killing virus-infected cells and pathogens hiding inside host cells. T-cells can activate macrophages and help them to kill intracellular parasites. They have a central role in controlling and directing the adaptive immune responses and some subsets (e.g. T-helper or Th cells) can even direct responses either towards allergic-type responses (Th2 type) or towards the cellular immunity responses (Th1 type).



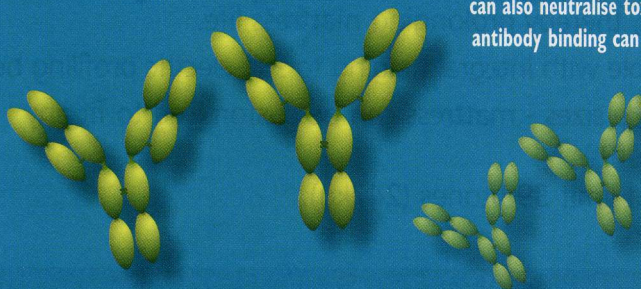
Resting B- and T-lymphocytes

B-lymphocytes (or B-cells) are schooled through the bone marrow in adults (hence the 'B' prefix), and are concerned with recognising and defending against pathogens in the body fluids, rather than those inside cells. Their job is to make antibodies which they secrete in great quantities (about 2,000 molecules/minute/cell) from fully matured, activated B-cells (antibody producing cells [APC] or plasma cells). Although they start life looking indistinguishable from T-cells, they undergo major changes when activated and as they mature towards their ultimate destiny of becoming an antibody factory. Each B-cell is capable of making only one specificity of antibody, which it permanently displays on its surface membrane while it waits for the opportunity to become activated. It can only become activated to start antibody production when it receives two signals — one caused by the foreign substance binding to its antibody and the other by particular cytokines released from helper T-cells. In effect B-cells need permission from T-helper cells to make antibodies, because antibodies are so powerful they would cause devastating damage if one was produced that attacked self molecules (that is what happens in an auto-immune disease like rheumatoid arthritis). Plasma cells (APCs) usually congregate in the lymph nodes or the spleen to set up multi-cell antibody factories, causing the symptom of hard, swollen lymph nodes.



An activated but not yet mature B-cell (left) and a fully mature antibody-producing B-cell, full of antibody-producing structures (right)

Antibodies are large, modular, multi-domain globular proteins. They come in various forms and sizes, but the most abundant type is the IgG class, with a general architecture as depicted here. They are modular in that each one consists of 12 domains, each with a similar basic structure, assembled together approximately in a Y-shape. Distinct activities are carried out in particular regions. The two tips at the top of the Y are where the antibody attaches to its target. These tips are very precisely shaped to fit only one unique molecular shape on the surface of the target — just like the blade of a key is shaped to fit only one lock. The tips usually bind very tightly indeed. Within the stem of the Y there are sites at which the phagocytes and complement can recognise and bind to the antibody, thus enabling it to become a powerful weapon-guidance system. While antibodies work very well with phagocytes and complement, they can also neutralise toxins just by binding tightly to them on their own. Likewise with viruses, antibody binding can strongly inhibit their infectivity. The potential repertoire of antibody binding specificity is truly amazing — many millions of different binding structures (at the tips of the Y) are possible, allowing them to cover every new infection eventually.



where they clear up the battleground by engulfing and digesting any remains of neutrophils or bacteria.

Continued neutrophil domination of a wound is a sign that a wound is in trouble, while macrophage domination indicates that progress is being made and recovery is under way (Diegelmann and Evans, 2004). It is worth remembering that while neutrophils are an essential and important component of defence, inviting them into a wound is a dangerous step to take (Sansone et al, 2006) as they can outstay their welcome and cause a self-perpetuating state of chronic inflammation and hypoxia, dominated by excessive, destructive proteases (Dovi et al, 2004).

Despite the apparently clear distinction between innate and adaptive immunity, it soon becomes apparent to a student of immunology that it is a distinction of convenience, as many of the cells and molecules are closely involved with both forms of immunity (Akira et al, 2001). For example, neutrophil leukocytes are the first defenders at the scene of bacterial invasion, yet they carry receptors for antibodies even though antibodies only turn up several days after the start of infection. Antibodies are, of course, part and parcel of the adaptive arm. Macrophages are clearly part of the innate system, as they carry special recognition molecules that can selectively bind to pathogenic microbes, but they are also central to the adaptive system where they participate in the process of commissioning specific antibodies and specific T-lymphocytes. The enigmatic complement proteins are part and parcel of non-specific innate immunity, yet they can be activated and deployed with fine specificity and power by the antibodies of the adaptive system.

The complement system (Table 1) is a collective term for a hugely important group of more than 25 interacting proteins found throughout the body which play a core role in immune defence. This makes

complement seem impossibly complex, but its main roles and function are fairly easy to appreciate without going into tedious detail. Essentially, the complement system recognises and attacks foreign intruder cells, especially bacteria, by direct and indirect means, from the moment the infection starts to the moment it has cleared. Complement can act directly on its own or it can team-up with phagocytes and/or antibodies to give an enhanced effect. There are three main functions of complement. The first is to cause the lysis of foreign cells, especially bacteria and enveloped viruses. The second function is to coat foreign cells or particles with specific complement protein fragments that can be recognised by receptors on the cell membranes of neutrophils and macrophages, thus helping these phagocytes to engulf and kill them. This is termed opsonisation, which is derived from Greek and means to 'prepare to eat'. In other words, complement is acting as a kind of molecular relish, encouraging phagocytes to get on with the job of clearing infection as fast as possible. The third main function has been described above, wherein active signalling molecular fragments (especially C5a and C3a fragments) help to orchestrate the calling-up of phagocytes from the blood.

Complement is such a powerful trigger of inflammation that it has to be equipped with restraining mechanisms. Its potentially devastating power can be seen in the clinical condition of hereditary angioedema, in which there is a deficiency of the inhibitor of the first component of complement (C1q). Patients with this condition suffer from recurrent episodes of severe inflammatory oedema (typically non-itchy), usually localised to the skin, larynx and/or gut. If there is a severe attack involving the larynx, the oedema can block the airways leading to collapse or death. On the other hand, a deficiency that prevents complement from unleashing its full power against invading microbes can leave a patient unable to fight infection (e.g. deficiency of C3),

causing them to suffer recurrent and sometimes life-threatening infections.

Adaptive immunity — wound interactions

The adaptive immune system (Table 3) should be seen as a mechanism that specifically beefs up the innate immune system, providing a quantum leap in specific potency. The big advantage of the adaptive system is that it tailors its responses very specifically to the foreign invaders, be they toxins, viruses, bacteria, fungi or eukaryotic parasites (e.g. malaria). By tailoring its responses, the adaptive system can deliver much more powerful attacks against its foes, partly because there is so much less collateral damage. There is a problem, however, for it takes several days for the specific, tailored preparations to be assembled by the lymphocytes. The lymphocytes are the custodians and drivers of adaptive immunity. The active agents they turn out are, principally, antibodies (made by B-lymphocytes) and cytotoxic cells of the T-lymphocyte lineage, complete with their own recognition molecules (T-cell receptors) and signalling molecules (e.g. interleukins). With a few exceptions, B-cells can only make antibodies with the assistance of 'helper T-cells', who have the job of co-recognising foreign material and triggering a second signal. It also turns out that macrophages get involved with making antibodies too. When they engulf microbes, the macrophage digestive machinery dismembers them, breaking their bodies down into small molecular fragments. The macrophage then presents these incriminating pieces of molecular evidence to receptive T-lymphocytes in order to direct the adaptive immune attack onto the right targets. This task is also carried out by another type of cell found in skin, called the Langerhans cells. These are similar in many ways to macrophages, but they specialise in taking up, processing and presenting any antigens they come across. Their collective name is that of dendritic cells.

Powerful adaptive immunity is essential to life because many microbial attacks will overcome the

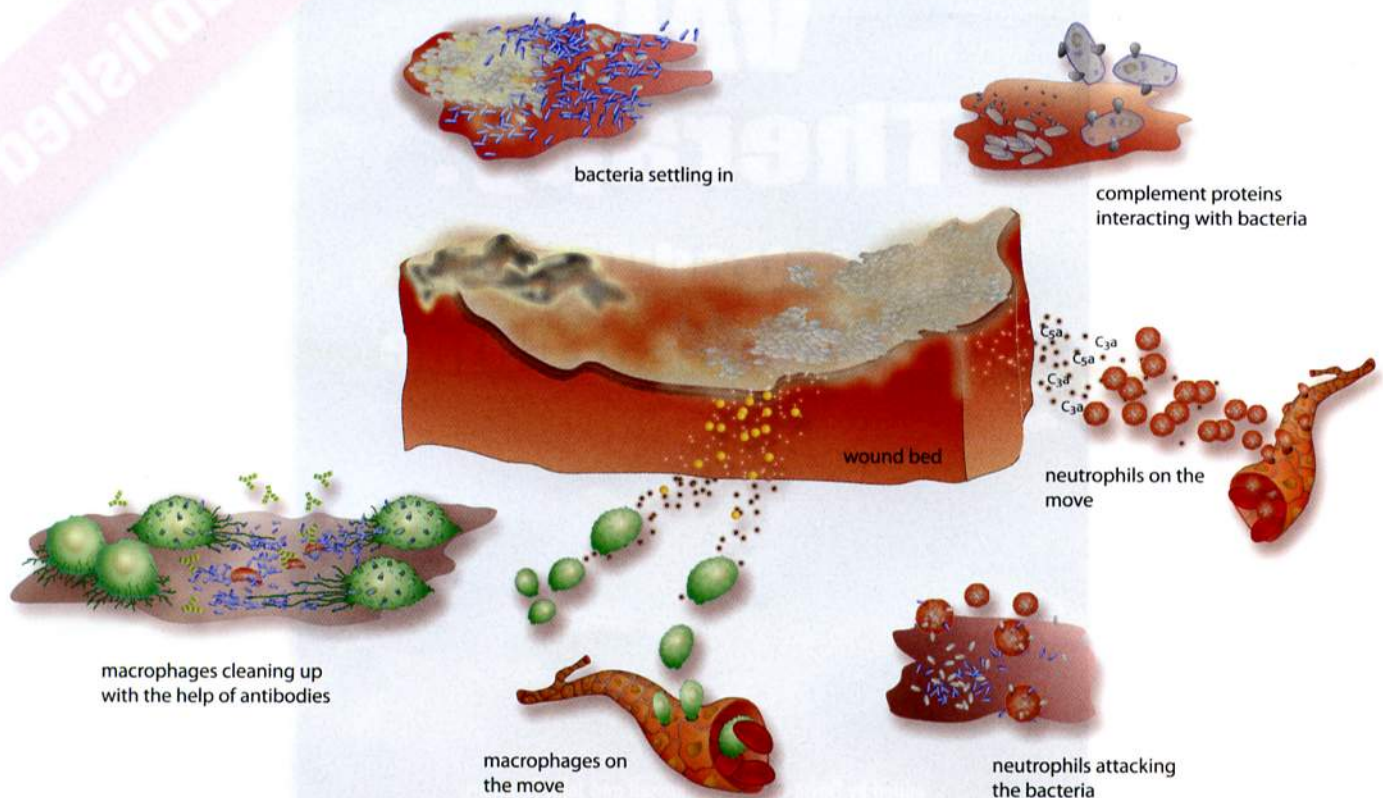


Figure 4. Battle plan: the immune defence of the wound — an overview of the immunological events taking place in a wound as it becomes chronic and then progresses towards healing.

initial onslaught of the defences. It may seem surprising that any microbes can survive in the face of such unbridled innate aggression but, in real life, that is what happens. We can expect only that the innate system will be able to provide a holding action, keeping the infection at bay long enough to deploy the adaptive cavalry. When the cavalry eventually arrives it unleashes powerful, targeted adaptive weapons designed to transform the offensive capabilities of the phagocytes and complement proteins that had failed the first time round. Although the adaptive responses start virtually immediately, and the first adaptive defences are ready within a few days, the system continues refining and strengthening them for as long as the target adversary remains undefeated.

Both the neutrophil phagocytes and the macrophage phagocytes have receptors on their surface membranes for certain classes of antibodies, allowing them to go microbe hunting, armed to the teeth with these

powerful, specific molecules designed to latch on, terrier-like, to their quarry. The integration between the two arms of immunity goes still further, for some antibodies also bear binding sites for complement. Microbes unlucky enough to have such antibodies locked onto their outer surface are immediately in extreme danger, for the complement protein C1q can attach to those antibodies and trigger a massive local activation of the whole cascade. The end point of this activation is the formation of complement 'membrane attack complexes', which punch holes in the microbial membrane, leading rapidly to the death of the hapless cell.

All of these immunological processes are deployed against each microbial attack but, in the peculiar situation of a chronic wound, it seems that in most cases the adaptive immune system has little extra impact. The situation typically continues as a stand-off between the immune system and the entrenched microbes. There is no prominent accumulation

of lymphocytes while the wound is open and the ever-improving antibody response that must be going on in the background has little perceivable effect. Cytotoxic T-lymphocytes have activities that generally are not relevant to the defence of most wounds, as their particular role is to kill virally-infected cells and intracellular pathogens. Certain subpopulations of helper T-lymphocytes (Th cells) are involved in helping B-lymphocytes to make the right antibodies so, if antibodies are made in the wound, these Th cells are needed there, too. There is another type of Th-cell that has an impact on wounds. These are most prominent after wound closure, when microbial invasion is no longer a significant factor, suggesting that they play a role in the post-closure tissue remodelling phase (Eming et al, 2007). The role of Th1- and Th2-cell subsets in wound repair is not well understood and more research is needed, though it is likely that they regulate the wound micro-environment at particular times and in particular ways by secreting

their own distinctive cytokine profiles with which we are familiar in different circumstances (Martin and Muir, 1990; Hart, 2002).

Another type of T-cell has a particular association with the skin — the so-called $\gamma\delta$ T-cells. They are also known as dendritic epidermal T cells (DETC) and they are found in the epidermis in association with damaged, stressed or transformed keratinocytes. Studies in mouse wound models have shown that healing is delayed if DETC are deficient, and it is evident that they provide crucial signalling molecules controlling keratinocyte actions and macrophage infiltration (Jameson and Havran, 2007).

It is likely that the underlying factors responsible for causing and perpetuating the wound, such as vascular insufficiency, chronic hypoxia and oedema all combine to hinder most of the adaptive mechanisms, although a robust set of antibodies against particular species of microbes will do much to prevent reinfection by that particular species, but not by other species of pathogens.

The immune system in action defending a wound — an active battleground

With a basic understanding of the mechanisms of immunity, it is possible to piece together a typical course of immunological events based on a hypothetical wound (Figure 4). While the wound is present, vulnerable sub-epidermal tissues are exposed and open to bacterial colonisation. Some chemical signals are released from blood platelets and mast cells, causing vasodilation and endothelial cell retraction in the nearest blood vessels, thus changing their flow dynamics and allowing fluid leakage to trigger local oedema. Newly arrived bacteria, most likely staphylococci from adjacent skin, settle in and immediately encounter complement proteins that become activated by molecules on the microbial surface (Jones et al, 2004). Active fragments of complement and other signalling molecules (chemokines) continue to diffuse inwards from the wound, setting up a

concentration gradient. In response to the chemical signals, the endothelial cells display special semaphore molecules that can be recognised by circulating phagocytes as an instruction to leave the blood and follow a chemical trail into the wound. Before long, neutrophils begin arriving in the wound where they encounter the proliferating microbes, many of which are coated with activated complement proteins. The neutrophils recognise the complement coating, and also use their own pattern recognition proteins (PRPs) to sense the pathogen-associated molecular patterns (PAMPs) on any uncoated microbes (Robinson et al, 2006). Recognition through either of these mechanisms causes the neutrophils to engulf and kill the microbes, simultaneously discharging destructive enzymes and reactive oxygen species into the microenvironment — a process which kills adjacent microbes but causes immense damage to the tissues of the wound bed. Dead neutrophils and microbes, together with the debris of battle accumulate within the wound as pus and slough.

Once the bacterial threat has been contained, the signals for neutrophil recruitment are down-regulated, unless the wound remains hypoxic. Continued hypoxia maintains the neutrophil signals in the nearby microvasculature, so perpetuating a state of inflammation. The chronic state that ensues, allows the wound to become stuck in a condition dominated by hypoxia, oedema (contributing to ischaemia) and proteolytic damage to the extracellular matrix and growth factor profile (Hopf and Rollins, 2007). When neutrophils outstay their welcome the outlook for healing is very bleak.

In a wound on a healing trajectory, macrophages are also called into the wound from the blood and by migration in from the surrounding subcutaneous tissues. When, or if, the neutrophils have done their job and the microbial proliferation is brought under control and other local factors remain in balance, the macrophages

begin to dominate the wound, engulfing and digesting the remains of the neutrophils and any surviving bacteria. The macrophages remain in action to keep infection at bay and to maintain the healing environment, achieving this by secretion of the appropriate cytokines and other signalling molecules (Park and Barbul, 2004). Fragments of digested bacteria are displayed by macrophages for scrutiny by appropriate T-lymphocytes as the starting point in the process of antibody induction, either within the wound or, more likely, in the nearest lymph node (Brady et al, 2006). Bacteria and bacterial metabolic or degradation products that escape into the tissues, blood or lymphatic system are captured by other antigen-presenting cells. This happens most efficiently in local lymph nodes (lymphocyte meeting points) of the draining lymph vessels and, more distantly, within the spleen. These items of molecular debris are used by the immune system as stimuli to make specific antibodies, memory cells and activated T-lymphocytes. Memory cells are the immune system's essential archive. Each time there is a significant immune response to a foreign invader, some of the lymphocytes involved are programmed to become inactive bystanders with the sole function of holding the molecular memory of the invasion. That is why an individual who has recovered from an infectious disease remains immune to further attacks for many decades. If the same species of microbe attacks again, there is an army of memory cells carrying the essential information needed to mount an instant and irresistible attack. Memory cells do harbour grudges!

The new antibodies circulating in the blood and lymph may find their way into the wound, where they attach to any recognisable bacteria, so helping neutrophils, macrophages and complement to unleash further powerful attacks. More importantly, they act systemically to eliminate any living bacteria that escape from the wound site, thus containing the infection and reducing the chances of systemic infection.

Overall, as the drama of a wound healing (or non-healing) process plays out, we can see a number of key features that could be used to improve treatments or provide clear diagnosis. Perhaps one of the clearest rules of thumb is that the presence of a significant neutrophil infiltration denotes a wound in trouble, while the ascendancy of the macrophages denotes a wound in which healing is progressing (Diegelmann and Evans, 2004). If only clinicians could see them!

Conclusion

In the ugly world of chronic wounds, the healing process is complex and variable. Among all of the cellular and molecular interactions, the immunological activities are a major, defining component of the normal healing process. Immunological activities go well beyond antimicrobial defence, into the realms of debridement, homeostasis and cellular control. While the whole topic is of enormous academic interest, it is also highly relevant to the everyday practice of wound care. Knowledge of the essentials of wound immunology can help to ensure that clinicians make the right decisions at the right time, especially by helping them to accurately interpret the available clues and symptoms observed in the wounds they are treating. For the future, a greater understanding of the immunology of wound healing will, undoubtedly, lead to improved therapies and new diagnostic tests. Despite the obvious promise in this area of research and the many unanswered questions that remain, relatively little background research is currently being conducted on the specific topic of wound immunology. As with so many areas of wound science 'more research is urgently needed'. **WUK**

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Key Points

- ▶ Visual and physical signs of inflammation (swelling, redness, pain, heat) within or around wounds are primarily caused by neutrophil leukocytes, together with the complement proteins, aided and abetted by tissue mast cells.
- ▶ Most of the immunological activities taking place in a chronic wound are mediated by the innate arm (or division) of the immune system.
- ▶ Neutrophil infiltration is the most prominent feature of the innate response, but neutrophils as a weapon of defence are a double edged sword. They are aggressive against microbes, but they cause major collateral damage by releasing a corrosive cocktail of protease enzymes and active oxygen species.
- ▶ Even though crucial for antibacterial defence, neutrophils can become an unwelcome, damaging presence in a wound, if they stay in residence for too long. Excessive proteases and/or chronic hypoxia can be root causes of these problems. New healing technologies will re-instate healing by dealing with these local causes.
- ▶ As a general rule, wounds dominated by neutrophils are in trouble, either because of infection or chronic inflammation, while macrophage domination is usually a sign that a wound is progressing well. But these are signs that clinicians cannot see. New diagnostic tests will reveal these changes to give crucial guidance to care strategies.