# THE NERVOUS SYSTEM AND IMMUNITY OF THE SKIN

MICHAEL ZASLOFF

MedStar Georgetown Transplant Institute, Georgetown University Hospital,

## INTRODUCTION

One might imagine that the central nervous system (CNS) could play an important role in the defence of the skin. The skin and the underlying dermis are densely innervated by afferent and efferent neurons. The CNS can detect and map a site of injury on the skin surface through its afferent circuits, and deliver a neutrally mediated response to that site through its efferent arms. Furthermore, once an injury is perceived by the CNS anywhere on the skin surface, the nervous system could activate defensive signals throughout the body, including uninjured skin in anticipation of potential downstream consequences of microbial spread from the site of injury. Finally, the nervous system can act within seconds, faster than a microbe can propagate.

I believe the role played by the nervous system in the immunity of the skin can be appreciated through a careful analysis of two human diseases, infected ulcers on the feet of the diabetic, and erysipelas, superficial skin infection caused by Group A *Streptococcus*. In diabetes, the nervous system fails to protect the skin, while in erysipelas, the nervous system is being put on high alert. Here, I will tease out the role of the nervous system in the pathophysiology of each of these conditions, highlighting unappreciated immune functions of the nervous system, some of which are amenable to therapeutic intervention in these and other human diseases.

## NEURAL DISCHARGE OF ANTIMICROBIAL PEPTIDES FROM GRANULAR GLANDS ON THE FROG'S SKIN

The skin of most species of frog contains granular glands, secretory structures that release a cocktail of defensive substances onto the surface of the animal's skin (*Zasloff*, 1987, 2002). The substances include antimicrobial peptides that constrain the skin microbiome, along with compounds that dissuade macroscopic predators. The glands are innervated by adrenergic nerves, which when stimulated by pain (or an electrical discharge) will cause the granular glands to empty their contents onto the skin surface. Indeed, in these animals the application of either adrenaline or nor-adrenaline directly onto the skin surface results in an almost immediate covering of the skin surface by a viscous hydrophobic secretion containing concentrations of antimicrobial peptides sufficiently high to kill almost any microorganism the frog would face at the site of an open skin wound. Thus, in the frog we see a straightforward example of how the nervous system can be engaged to provide immune defence of the skin.

# THE INFECTED DIABETIC FOOT ULCERS

The diabetic is at risk of developing life threatening infected wounds on the skin of the lower extremities. The wounds appear initially as clinically unremarkable erythematous lesions, progress to superficial non-healing ulcers, and then can progress to deep, soft tissue wounds that penetrate the underlying bones. Gangrene and subsequent amputation of portions of the foot occur in about 50,000 diabetics annually, despite our awareness of the prevalence of the condition, and adequate management of the metabolic aspects of diabetes.

Why do wounds such as these occur in the diabetic foot? The "textbook" explanation places the proximate cause on the loss of sensory nerve functions that occurs as the diabetic ages. Normal levels of pain are not perceived in the lower extremities and, as a consequence, injury to the skin and soft tissues are not recognized and are untreated. In addition, the diabetic loses the usual moment-to-moment positional redistribution of weight bearing on the surfaces of the feet that occurs subconsciously in response to sensory input, leading to pressure-induced impaired vascular perfusion ("pressure sores") in certain areas of the foot that bear weight chronically. Accordingly, physicians advise individuals with diabetes to regularly examine their feet and to wear footwear that permits distribution of weight as evenly as possible across the surface of the foot.

What remains poorly understood is the underlying pathophysiology of the infections that occur in these wounds. The infections are poly-microbial with no specific pathogen identified as responsible for the progressive, invasive destructive process that descends from the initial superficial skin erosions through the dermis and muscle and then into bone (*Ge* et al., 2001). The wounds are often not particularly inflamed. Antibiotic treatment generally involves chronic systemic therapy and frequently fails to control the infection.

Recent discoveries on the mechanisms by which antimicrobial peptides are expressed and regulated on human skin suggest novel roles of the nervous system in skin immunity, and new insights in the pathophysiology of the diabetic foot ulcer that could have far reaching therapeutic consequences.

The initial neuropathy that develops in the diabetic is of a sensory type involving fine un-myelinated fibres ("C fibres") and can be demonstrated as a loss in pin prick sensation, and decrease in the sensation to heat and cold (*Ørstavik* et al., 2006). The cause of this neuropathy is unknown. Degeneration of sensory nerve fibres can be demonstrated within biopsies of the skin and soft tissues of the lower extremities (*Shun* et al., 2004). In addition, at about the same time as the onset of the neuropathy, a characteristic "diabetic dermopathy" is seen on the skin of many diabetic patients, characterized by melanotic papules (*Kiziltan* et al., 2006). The sensory innervation of the epidermis gradually becomes profoundly damaged as the course of diabetes continues.

The epidermis of man (and mouse) is designed both to restrict water loss and constrain or shape the microbiome that populates the skin. These functions are accomplished, in part, by the co-ordinated delivery of antimicrobial peptides and lipids that are packaged in structures called lamellar bodies (*Braff* et al., 2005). The lamellar bodies are produced by the keratinocytes as they begin to mature and move from their basal position in the epidermis. The

antimicrobial peptides and lipids penetrate the inter-keratinocyte spaces, as a "mortar", sealing the spaces between the flattening "brick-like" keratinocytes that comprise the superficial layers of the skin. What is remarkable, and somewhat counter-intuitive, is that upon injury to the skin, such as stripping the skin surface with tape, the recovery of the antimicrobial and permeability barrier requires the surface to dry. Within several hours lamellar bodies "ripen" and are delivered to the surface. Furthermore, signals are sent to the basal cells to initiate transcription and translation of antimicrobial peptides, insuring that adequate stores of these substances are available. However, if the surface is covered with a plastic film that maintains the moisture barrier, the barrier does not recover, and the induction of antimicrobial peptides is not observed (*Aberg* et al., 2008). Most likely, the osmotic pressure of the extracellular compartment in the injured skin is detected, and the receptors responsible for detection of the drift in osmolarity are one of the transient receptor potential vanilloid (TRPV) channels (*Liedtke*, 2006). These are also the receptors that sense temperature, and are expressed on the sensory nerve endings that lie at the junction between the epidermis and dermis. When activated these channels release calcium, and result in both transmission of an afferent stimulus to the brain as well as an efferent response from the nerve ending. The sensory nerve endings in human skin include peptidinergic nerves that release Substance P and Calcitonin Gene Related Peptide (CGRP) (*Schulze* et al., 1997). Each of these peptides has receptors on the keratinocyte. It is likely that activation of these receptors induces the antimicrobial/lipid barrier response and release of new, ripe lamellar bodies after surface injury.

Furthermore, each of the two neuropeptides has physiological properties that are important in tissue injury defence and recovery/healing. Substance P, for example, has direct antimicrobial activity, induces capillary leakage and vasodilatation, is a chemo-attractant of neutrophils and macrophages, degranulates mast cells, and induces expression of pro-inflammatory adhesion proteins on endothelial cells, epithelial, and phagocytic cells (*Brogden* et al., 2005). In addition, Langerhans cells, which taste and present antigens within the epidermis after superficial injury, are activated by Substance P (*Staniek* et al., 1997). CGRP is a potent vasodilator of the arterioles of human skin (*Brain* et al., 1986). Both Substance P and CGRP stimulate the proliferation of fibroblasts and keratinocytes and promote healing in *ex vivo* models of human cutaneous wounds (*Cheret* et al., 2014).

The sensory nerves of the skin that express TRV1 can also communicate directly with dermal dendritic cells (*Riol-Blanco* et al., 2014). When the skin of a mouse is exposed repeatedly to imiquimod, TRV1+ sensory nerves in the skin stimulate dermal dendritic cells to secrete IL23, which in turn appears to induce the expression of IL22 and IL17 in nearby γδ intra-epithelial lymphocytes. These cytokines, in turn, should induce robust keratinocyte antimicrobial peptide expression, as well as keratinocyte proliferation. This recently reported discovery adds another dimension to the nervous system in the immune defence of the skin.

Sensory nerves within the epidermis are also capable of recognizing the presence of bacteria, by a direct sensing mechanism. A recent study in mice has demonstrated that *S. aureus* expressing lytic toxin reduces the threshold of heat and pain stimuli in afferents draining the sites in which microbes had been introduced (*Chiu* et al., 2013).
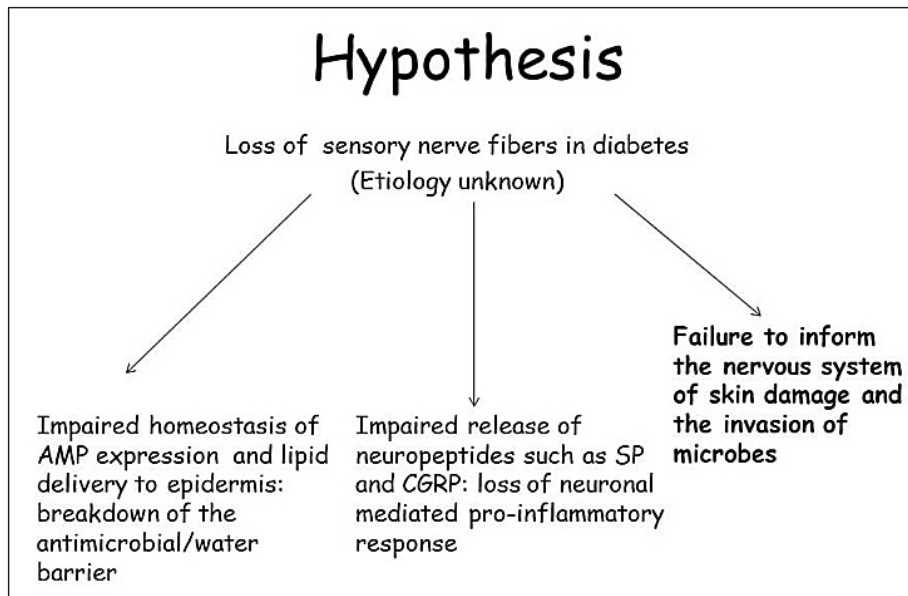
**Figure 1**: Hypothesis on the events leading to the non-healing skin ulceration and soft tissue destruction in the diabetic patiënt.

The receptors involved have not been identified, but they appear not be related to any of the known Toll-like receptors (TLRs). Furthermore, stimulation of the sensory nerves by this toxin leads to the release of CGRP, as measured in the dorsal root ganglion. This study demonstrates, however, that the nervous system can detect the presence of bacteria and therefore can "know" the anatomical location of an infection and could in principle utilize this information in ways that optimize the immune response.

In the diabetic who has developed a sensory neuropathy, these defensive and protective functions of the nervous system are lost. We hypothesize that the breakdown of the immune functions supported by the sensory nerves of the skin leads to the non-healing skin ulceration and soft tissue destruction in the diabetic (Figure 1).

## ERYSIPELAS AND THE REGRESSION OF SARCOMA

Erysipelas is a superficial self-limiting infection of the epidermis and dermis by Group A *Streptococcus*. The infection is associated with a rapidly rising fever and a painful red indurated thickening of the skin. It begins as a small lesion, which grows in area over 4-6 days. The margins are raised and have a firm border. The fever and systemic symptoms of illness subside when the growth of the rash stops. Of particular interest is the curious finding that streptococcal organisms can rarely be identified in the bloodstream in this infection, unlike what is observed in a deeper streptococcal soft tissue infection (*Linder* et al., 2010). It is this observation associated with erysipelas that I believe suggests an underlying immune role being played by the nervous system in defence of the skin.

As I have noted above, the nervous
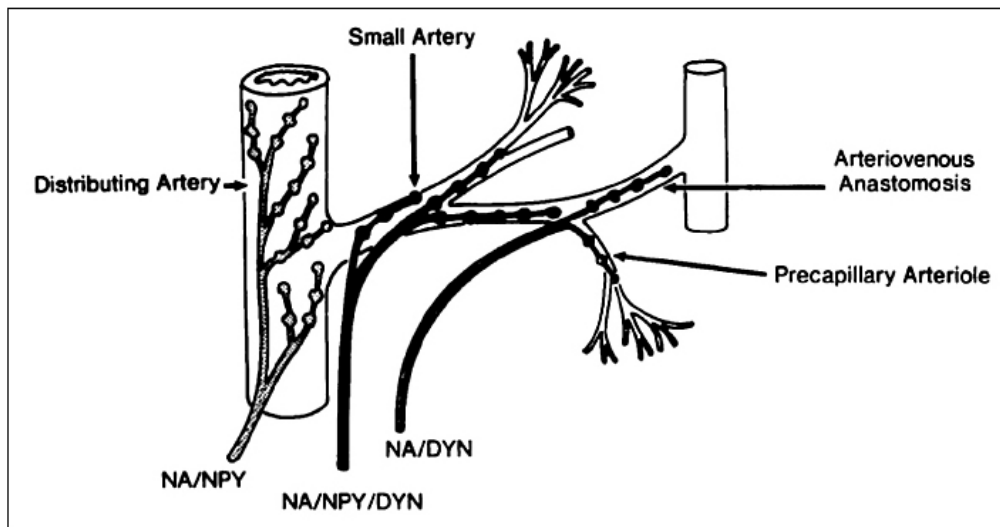
**Figure 2**: Diagram summarizing the microvasculature in the guinea pig ear (from: *Gibbins* and *Morris*, 1990).

system has the ability to detect the presence of microbes in the skin and underlying tissues through activation of sensory nerves. A question that we can ask is how this information is utilized by the central nervous system. In the case of erysipelas I would suggest that the response to the superficial streptococcal infection is a highly selective vasoconstriction in which the capillaries that perfuse the site of infection are made less accessible to the bacteria in their midst, in order to prevent their entry into the intra-vascular space. In addition, I suggest that the nervous system also responds by reducing the flood of blood through distant capillary beds to reduce the likelihood that a microbe that has entered the blood stream can escape and infect a distant tissue compartment. In other words, the nervous system shuts down access of streptococci both into and out from the vascular space. Local antimicrobial defences then deal with the primary skin infection. The spleen and other organs with phagocytic capacity eliminate bacteria that have gained intravascular access.

The nervous system is anatomically designed to regulate the flow of blood through capillary beds through control of pre-capillary sphincters. What is surprising is that the pre-capillary sphincter appears to be innervated by a specific nerve, different from that which controls blood flow through proximal arterioles (*Gibbins* and *Morris*, 1990) (Figure 2). As demonstrated in the vascular bed of the ear of the guinea pig the nerves that innervate the most distal portions of the arterial bed, the precapillary sphincter and the arteriovenous anastomosis, are distinctly different (noradrenaline/dynorphin containing) than those that innervate the more proximal arteriole (noradrenaline/Neuropeptide Y (NPY)/dynorphin) or the distributing artery (noradrenaline/NPY) (*Gibbins* and *Morris*, 1990). Thus, we can imagine that the central nervous system has the capacity to direct the vasoconstriction of specific pre-capillary beds by directing efferent signals through neurons that communicate with these vascular beds. Since gas exchange can occur across small diameter arterioles and venules as

## Hypothesis

The presence of certain species of microbe (e.g., Group A Streptococci) in skin stimulates a central autonomic vascular response that involves vasoconstriction of pre-capillary arterioles locally and at distant sites

Vasoconstriction prevents entry of microbes into the blood stream through capillary beds and their escape through distant capillary beds thereby reducing systemic spread

This mechanism exploits a closed vascular system and a CNS that can control the flow of blood through pre-capillary arterioles

**Figure 3**: Hypothesis on how the central nervous system can restrict the spread of microbes throughout the body from an infected site by control of the flow of blood through capillary beds.

effectively as across capillary beds (*Vovenko*, 1999), the closure of the capillary beds would restrict nutrient passage, lymphatic flow, and cellular (and microbial) traffic, but would not necessarily reduce gas exchange.

These observations lead us to the hypothesis that the central nervous system can restrict the spread of microbes throughout the body from an infected site by control of the flow of blood through capillary beds. The hypothesis, which is presented in Figure 3, can explain one of the more extraordinary and mysterious stories in the history of medicine. In the late 1800's, William Coley, an orthopaedic surgeon, reported that patients with aggressive soft tissue sarcomas would experience complete regression should they also develop erysipelas (*Nauts* et al., 1946). Coley's observation was based on the co-incidental infections that occurred in his cancer patients, and then on the many positive responses of patients "therapeutically" infected with streptococcus to cause erysipelas. Unfortunately, streptococcal infection was itself rather dangerous with a significant

independent mortality. Coley attempted to refine the method by extracting non-infectious microbial components. As the preparations became better characterized, they lost efficacy. Coley believed that sarcoma was caused by a bacterial infection, and his use of the bacterial preparations somehow stimulated an immune response.

It is interesting to review Coley's description of the response of the sarcoma in the setting of an induced erysipelas in a patient with a soft tissue sarcoma (*Coley*, 1910):

*"...Finally in October 1891, with 5 decigrams of a bouillon culture of Streptococcus of erysipelas just brought me from Koch's laboratory in Germany by Dr. Frank Ferguson, the pathologist of the New York Hospital, a most severe attack of erysipelas developed, nearly causing the death of the patient. Within an hour after the injection a severe chill occurred followed by a temperature of 105°F. After an interval of 12 hours a typical attack of erysipelas developed starting at the point of injection and extending over the neck and face. It ran its usual course.*

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*The tumour of the neck began to break down on the second day, and a discharge of broken down tumour tissue continued until the end of the attack. At the end of two weeks the neck tumour had disappeared and the tonsil tumour had decreased in size. The patient remained well for 8 years."*

Coley carefully documented the usual changes that he observed in the tumour following the induction of erysipelas:

*"...First, the tumour becomes much paler owing to decreased vascularity; second, it becomes much more movable and less fixed to the surrounding tissues; third it soon begins to show areas of softening, due to caseous degeneration or necrobiosis of the tumour elements; fourth, gradual disappearance, either by absorption..or in other cases by breaking down and liquefaction."*

I would suggest that what Coley was observing was a specific neurally mediated immune vasocontrictive response to presence of *Streptococcus* that interfered with blood flow to the sarcoma (Figure 3).

The role of the nervous system in the immunity of the skin as highlighted here involves the capacity of the nervous system to spatially identify the anatomical site of injury or infection, its ability to direct neuropetides that promote wound healing and provide antimicrobial defense to that site, and its capacity to prevent metastatic spread of infection through the vascular space. I propose that this system of immunity represents the basic outline of the immune function of the nervous system of all vertebrates.

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