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Neutrophil Research: The Future of Periodontal Diagnosis and Treatment

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Neutrophil Research: The Future of Periodontal Diagnosis and Treatment



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As a dentist why is it important to understand white blood cell/neutrophil biology?

While it is recognized that periodontal diseases and other oral infections are caused by bacteria in actuality it is the patients own innate immune system which is largely responsible for the destruction of the bone, ligament and gingival tissues that support the teeth. Neutrophils, the key white blood cells of the innate immune system, are responsible for detecting and eliminating the microbial invaders that make there way into the body (Cicchetti et al., 2002). These cells which are loaded with extremely toxic enzymes called proteases, are also responsible for the damage caused during the prolonged inflammatory phase which occurs when they become hyperactive or chronically activated by bacterial stimulus or immune regulators. Recent dental research has focused on translating our knowledge about these cells into diagnostic and treatment advances. In this brief review I will highlight what is known about how these key immune cells affect periodontal health and how the latest periodontal research is focusing on using this knowledge to develop novel diagnostic tools and novel treatment approaches for periodontal diseases.

What are neutrophils?

Neutrophils are the sentinels of the oral immune system (Bender et al., 2006). They are the most abundant white blood cell in our body, accounting for up to 70% of circulating leukocytes. Neutrophils are produced in the bone marrow at the rate of 60 billion cells per day (average male). This high rate of production is essential as these cells have a short circulation half life of about 10 hours and may only survive up to an additional 48 hours once they reach infected or damaged tissues. Monitoring neutrophil levels using complete blood counts is used to determine the presence of undiagnosed infections, to determine if a patient is able to mount a normal innate immune response, and to verify that a patient is able to produce a sufficient supply of these key microbe killing cells (Defraia and Marinelli, 2001). This is particularly important when planning any dental treatment in patients who have undergone any recent treatment or are taking any medications that may impact their bone marrow (e.g. chemotherapy or radiation therapy for cancer) or who may have genetic diseases that impact on the hematopoietic system (e.g. genetic disorders that result in neutropenia; Glauser, 2000).

How do neutrophils fight infections?

Neutrophils make essentially one way trips, from the bone marrow into the blood and finally into the infected tissue compartment (Cheretakis et al., 2006). See Figure 1. The neutrophil is the first line of defence against foreign microbes that make their way past primary physical barriers, such as the epithelium and bodily secretions that protect the external and lining surfaces of the body. Neutrophils appear only in infected or damaged tissues after being recruited by inflammatory mediators released from activated macrophages and endothelial cells or by chemical signals released by invading microorganisms themselves. At infected sites, the microbes are eliminated by the recruited neutrophils (chemotaxis) through the process of phagocytosis, which is defined as the engulfment, internalization, and degradation of extracellular material. The importance of these cells is seen in neutropenic patients (very low levels of circulating neutrophils) who are extremely susceptible to bacterial infections and often die as a result of these infections (Glauser, 2000).

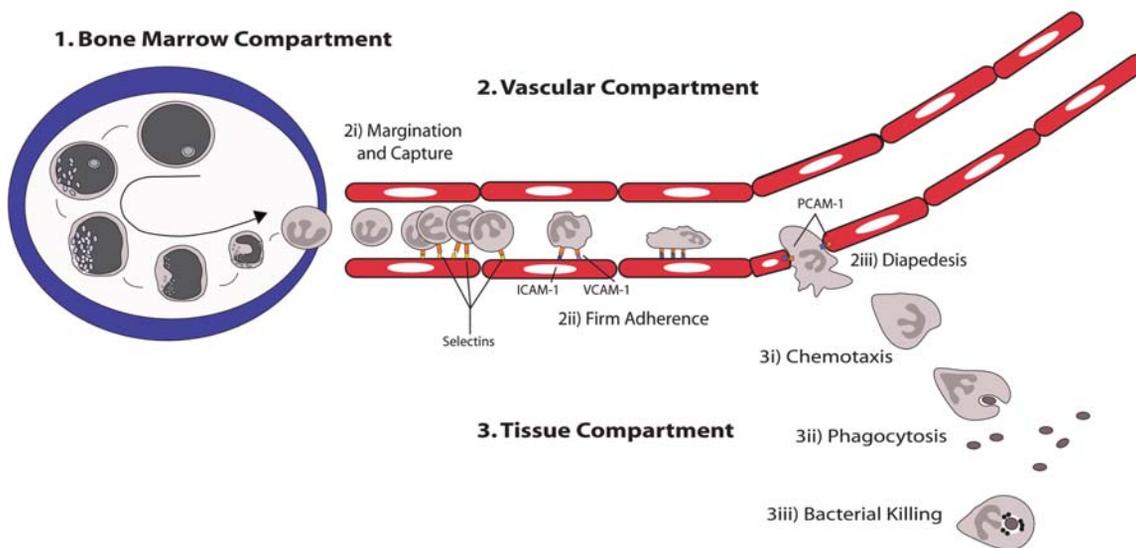


FIGURE 1: LIFESPAN AND FUNCTIONS OF THE NEUTROPHIL.

1. Neutrophils are produced at the rate of 60 billion cells per day from stem cells in the bone marrow. They migrate out of the bone marrow and into the circulation where they circulate until they are recruited to a site of infection or inflammation.

2. They are able to crawl through the blood vessel wall at sites expressing key signal proteins (e.g. VCAM) on the surface of the blood vessel walls. The expression of these “green light” proteins, which signal an infection in the area, is turned on by locally produced inflammatory mediators at the sites of infection or inflammation.

3. Once these cells have entered the tissue they carry out a discrete number of steps that ultimately leads to killing of the bacteria present. i) **Chemotaxis** involves the directed cell crawling towards the bacteria. This is analogous to a blood hound tracking a target. ii) **Phagocytosis** involves the neutrophil “eating” the bacteria to facilitate killing. iii) **Bacterial killing** is carried out within the neutrophils by a series of protein degrading enzymes and by oxygen radicals generated by systems within these white blood cells.

The neutrophil and periodontal diseases

Periodontal diseases are one of the most prevalent diseases occurring in man, with between 70 and 90% of the population experiencing this disease at some point during their lifetime. The human mouth has a constant bacterial presence that is kept under control, in large part, by a continual influx of neutrophils from the surrounding periodontal tissues (Bender et al., 2006). Neutrophils carry out a discrete set of processes that allow them to migrate from the blood into the infected tissues where they are able to phagocytose the invading bacteria and kill them utilizing enzymes and specialized killing machinery that generates oxygen radicals (Figure 1). Strong evidence demonstrates that a significant portion of the inflammatory mediated destruction of the tooth supporting tissues (periodontium) occurs as a result of collateral damage caused by the enzymes released by hyperactive neutrophils as they attempt to contain the bacterial infection (Gangbar et al., 1990; Lee et al., 1995). In fact neutrophil collagenase (matrix metalloproteinase 8 [MMP-8]), released during periodontal infections is one of the major enzymes responsible for degradation of tooth supporting bone and ligament. Long-term use of low dose doxycycline has been shown to inhibit neutrophil collagenase and periodontal destruction in patients with periodontal disease which is resistant to conventional periodontal therapy (McCulloch et al., 1990).

Diagnostic tools and the innate immune system

Due to the critical role played by neutrophils in the oral immune response to bacteria, work over the past decade has focused on developing novel diagnostic tools that would enable the dentist to identify those patients experiencing active periodontal breakdown. Previous attempts at diagnostic tests have utilized biochemical detection methods for measuring the presence of inflammatory markers or proteases that are released during periodontal inflammation and tissue breakdown (Eley and Cox, 1998). While these approaches have resulted in some commercially available tests their success has been limited by practical limitations and the need for expensive specialized equipment. Recent work from my lab at the University of Toronto has returned to a previous approach from the 1960s that focused on the monitoring of salivary neutrophil levels. Previous work demonstrated that the saliva has a constant supply of incoming neutrophils that arrive through the crevicular fluid that flows in to the oral cavity from the periodontal sulcus. The rate at which neutrophils migrate through the gingival sulcus into the oral cavity is increased in the

presence of periodontal inflammation and also correlates with increased pocket depth and the gingival inflammatory index. The idea of using neutrophil quantification to assess periodontal disease status and the effectiveness of therapy was first proposed by Raeste in 1978 (Raeste and Aura, 1978). That study described an oral rinse assay for neutrophil counts that used a series of 12 sequential rinses to assess oral neutrophil levels. Since that time this approach was largely abandoned due to the impracticality of using large numbers of successive rinses and labor-intensive counting methods to measure oral neutrophil levels.

Translating laboratory research into chair side clinical tests

Since saliva collection is non-invasive and with new biochemical techniques available we have developed a simple and rapid approach for monitoring oral neutrophil levels, periodontal breakdown and the effectiveness of periodontal therapy. This simple and rapid method for the quantification of neutrophil levels in the oral environment utilizes a 30 second oral rinse that is collected from the patient. A biochemical agent is added that results in a colour change that reports the number of oral neutrophil present. The colour change is then matched to a colour chart that identifies the severity of the periodontal inflammation. See Figure 2. In clinical studies we have carried out the oral neutrophil



FIGURE 2: ORAL RINSE TEST TO DIAGNOSE PERIODONTAL DISEASES.

Oral rinse samples with diagnostic agent added from patients with varying degrees of periodontal disease are placed against the standard colour swatch corresponding to disease levels. **The degree of blue corresponds to the level of inflammation and periodontal disease present in the patient's mouth.**

quantification approach has been demonstrated to accurately identify the presence and severity of periodontal disease in adult patients and has also been shown to identify those patients responding to phase 1 therapy (Bender et al., 2006).

How would this diagnostic test benefit dental care?

A significant problem in society is being able to rapidly and inexpensively identify geriatric and other institutionalized patients in need of dental care. This is a significant healthcare issue as recent studies have identified significant links between periodontal diseases and common systemic diseases such as cardiovascular disease including heart attacks and strokes, diabetes and aspiration pneumonia. Being able to identify periodontal diseases in institutionalized elderly patients using a rapid non-invasive test that can be administered by non-dental staff has the potential to have a significant impact on this population and the healthcare system.

While this test is currently in clinical trials it is our expectation that this type of screening tool may be useful in multiple clinical settings:

- 1) In long-term care facilities this test will be able to inexpensively identify patients in need of dental treatment.
- 2) In the primary care medical setting physicians treating diabetics will be able to rapidly identify those patients who need a dental referral for periodontal disease that may be impacting their diabetic management.
- 3) In the dental office this tool will be useful during dental recalls and exams to help screen for and identify patients who need periodontal treatment and perhaps more importantly it can serve as a powerful visual educational tool to help patients understand the severity of their periodontal disease.

Why are some patients more susceptible to periodontal diseases in spite of healthy lifestyles and good oral hygiene?

Research over the past 10 years has implicated specific genes that regulate key aspects of the immune response as being responsible for a genetic predisposition to inflammatory disease including periodontal diseases (Jansson, 2006; Kornman and Duff, 2001). The best example of this is an Interleukin-1 gene polymorphism which has been shown to lead to the overproduction of this potent immune system activating cytokine in patients carrying a version of the gene (Kornman et al., 1999). This hyperactive version of the gene leads to not

only periodontal disease but also increases a patient's risk of cardiovascular diseases and heart attacks. As a result recent work has focused on identifying drug targets or key inflammatory regulators that could dampen the hyperactive immune responses that are responsible for periodontal destruction.

Pharmacologic manipulation: Resolution of the inflammatory response

A leading biochemist, Dr. Charles Serhan at Harvard, has identified two new groups of inflammatory mediators he calls lipoxins and resolvins, which are generated at the tail end of the inflammatory response in order to halt and resolve the immune response (Serhan, 2006).

Lipoxins are anti-inflammatory compounds derived from arachadonic acid found in cell membranes at sites of inflammation while resolvins are derived from omega-3 fatty acids (Serhan, 2005). These compounds actually block further neutrophil migration into the inflamed tissue and could also partly explain why a diet rich in omega-3 fatty acids helps to reduce inflammatory mediated diseases such as arthritis and cardiovascular disease.

He has demonstrated that resolvins applied topically in an animal periodontitis model protected against the immune mediated tissue destruction. This remarkable finding identifies compounds which may serve as excellent targets for preventing the hyperactive neutrophil mediated inflammatory response that is directly responsible for periodontal tissue destruction (Van Dyke and Serhan, 2003).

What does this mean for my patients?

This finding will revolutionize the pharmacological approach to anti-inflammatory therapy. The key point is that these newly identified mediators are involved in resolving the normal immune response that has already been initiated as part of the innate immune response required for host protection. Commonly used non-steroidal inflammatory drugs (NSAID) such as ibuprofen and diflusal block the acute inflammatory phase by arresting the conversion of arachadonic acid into potent inflammatory mediators such as prostaglandins and leukotrienes. This "old" approach prevents normal healing as these drugs also block the resolution phase of the inflammatory response required for normal tissue healing. This may also explain why NSAID do not work long-term in preventing periodontal destruction. There is little doubt that new drugs that will revolutionize how we treat inflammatory diseases are just around the corner (Serhan and Chiang, 2004).

The future will be bright ...only with research

These are only two examples of how basic science research is being translated in to improving patient care. We are truly living in a remarkable age of scientific discovery where research will rapidly have dramatic and tangible effects on how we treat and manage our patients. It is up to the dental profession to understand the latest research findings and support ongoing scientific discovery as only through ongoing well funded research will we be able to improve how we treat and care for our patients.

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Disclaimer: The author has declared a potential financial interest in the diagnostic test described in this article.



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