Periodontitis – understanding the relationship between pathogens, neutrophils, macrophages and chronic inflammation.

Dr Andrew Devitt; Dr Mike Milward; Dr Paul Cooper

Acute inflammation results in the accumulation of immune cells within tissues and is subsequently resolved by their removal via a programme of ordered cell deletion known as apoptosis. A defining characteristic *in vivo* of apoptosis is rapid, controlled removal of dying cells by phagocytes (e.g. macrophages). This ultimate step in the programme, sometimes known as efferocytosis, is now defined as one that not only removes unwanted cells and debris but tailors immune responses and appears actively anti-inflammatory.

We have shown that components of the innate immune system (e.g. CD14) that normally mediate protective, pro-inflammatory responses to pathogens are intimately involved in apoptotic cell clearance (Devitt *et al*. (1998). *Nature.* 392: 505-9). Whilst ligation of innate immune receptors (e.g. CD14) with ligands of microbial origin (e.g. Lipopolysaccharides/LPS) results in inflammation, ligation with apoptotic cells results in an anti-inflammatory phenotype (Devitt *et al*. (2004) *J Cell Biol.* 167: 1161-70). As such apoptotic cell removal is an important homeostatic mechanism for both the control of cell numbers and immune function. This is clearly demonstrated by observations that failure to clear apoptotic cells efficiently can lead to a range of persistent inflammatory and proliferative conditions e.g. deficiency of the complement component C1q inhibits apoptotic cell clearance *in vivo* resulting in autoimmunity. Removal of apoptotic cells by phagocytes is central to controlling immune responses and maintaining homeostasis within the body and failure of this process leads to accumulation of dead/dying cells within the tissues contributing to pathogenesis of several chronic inflammatory and autoimmune diseases (Khanna *et al*. 2010 *PLoS One* **5**:e9539).

Periodontitis is an oral disease that results in loss of hard and soft tissue tooth support and ultimately results in tooth loss. The disease associates with other systemic, chronic auto-immune/-inflammatory diseases including rheumatoid arthritis and cardiovascular disease. Key to the disease process is an aberrant inflammatory response which occurs in susceptible individuals in response to oral bacterial plaque. Several risk factors associate with pathogenesis, including genetics, ageing, smoking and Type-2-diabetes. Neutrophils are inflammatory cells key to periodontitis pathogenesis and they accumulate within the gingival tissues in response to chemotactic signals derived from oral epithelial cells and sub-mucosal mesenchymal/stromal cells which become stimulated by oral plaque bacteria. Once present in the tissues neutrophil dys-regulated activity, including release of reactive oxygen species (ROS), metalloproteinases (MMPs) and neutrophil extracellular traps (NETs), cause the tissue damage which underpins disease phenotype.

To date efferocytosis has not been studied in periodontitis although data indicate that key periodontitis risk factors may impede this process and thereby inhibit disease resolution. Indeed it has been hypothesized that gingipain proteases from the key periodontitis pathogen, *Porphyromonas gingivalis*, have potential to cleave phosphatidylserine structures (cell surface death signals) on dying neutrophils thereby making them unrecognisable to phagocytes. The aging process may also limit the

externalization of phosphatidylserine and cigarette smoke can impair macrophage apoptotic cell clearance (Richens *et al.* 2009 *Am J Respir Crit Care Med* 179:1011).

Programme. This project will establish an exciting and novel collaboration between Aston University, School of Life & Health Sciences and the University of Birmingham, School of Dentistry. The hypothesis underlying the current programme is that **during periodontal disease phagocytes lose their ability to mediate a dominant anti-inflammatory response to apoptotic cells**. This may be the result of reduced apoptotic cell clearance or responsiveness by macrophages; increased inflammatory signalling following ligation of immune receptors and/or reduced production of anti-inflammatory mediators (e.g. TGFβ1, IL10). Specific aims of this study include:

1) Characterisation of the competence of macrophages to migrate to (chemotaxis assays), bind and remove apoptotic cells (e.g. neutrophils & oral epithelial cells). This will include quantitative analysis of the ability of macrophages to bind and phagocytose apoptotic cells *in vitro* in the presence or absence of key components of the periodontal inflammatory lesion combined with disease risk factors including neutrophils undergoing cell death, neutrophil extracellular traps, periodontal pathogenic bacteria and their products (e.g. *Porphyromonas gingivalis* gingipains) and cigarette smoke extract.

2) Characterisation of the responses of macrophages following removal of apoptotic cells in the presence or absence of key components of the periodontal inflammatory lesion as described above.

3) Following establishment of the *in vitro* model assays crossover studies may be performed using patient and healthy immune cells, i.e. apoptotic neutrophils and macrophages, to determine whether inherent defects in efferocytosis likely underpin disease.

These studies will use a range of cell models, primary cells from humans and a broad range of established cell and molecular biology techniques including immuno-fluorescence flow cytometry and microscopy (including real time analyses of cellular interactions and chemotaxis); ELISA, isolation and culture of primary cells, induction and analysis of cell death and macrophage function (both *in vivo* and *in vitro*) (e.g. see Devitt & Gregory, 2004 *Methods Mol Biol* 282:207).

Results from this study will generate novel data that may implicate defective efferocytosis in periodontitis pathogenesis thereby advancing current knowledge of the pathophysiology of this disease.