Chapter 5

Conclusion

Conclusions

Adverse respiratory health effects are associated with occupational and/or environmental exposure to heavy metals (Hughes, 1980; Goering 1992; Vanadium, 2001). Neutrophils are mobilized to the airways following inhalation of toxic gases and particles, and are the probable perpetrators of inflammation-related airway damage (Bassett *et al,* 2000; Douwes *et al*; 2002; Saldiva *et al*, 2002). Cobalt, palladium, platinum and vanadium are all metals of environmental and occupational significance, particularly in South Africa, and the laboratory research presented in this thesis was undertaken with the primary objective of identifying possible pro-oxidative and pro-inflammatory interactions of these metals with human neutrophils *in vitro,* using sophisticated procedures, such as electron spin resonance spectroscopy, electrophoretic mobility shift assay combined with phosphor-scanning, and the Bio-Plex suspension array system. The major conclusions of the study are as follows:

- Inclusion of vanadium in the +2, +3, and +4, but not in the +5 valence states to activated human neutrophils promotes the formation of hydroxyl radical, one of the most reactive and damaging free radicals in biological systems (Cheng *et al*, 2002). In the physiological setting, exposure to Fe^{2+} presents the highest risk of hydroxyl radical toxicity via the Fenton reaction; however, this is stringently controlled *in vivo* by iron-binding proteins, which limit the availability of free iron. Exposure to V^{2+}, V^{3+} and V^{4+}, as is likely to occur in the environmental setting and occupational setting in particular, is likely to pose the potential threat of hydroxyl radical toxicity.

- Exposure of neutrophils to Co^{2+}, Pd^{2+}, Pt^{2+} or V^{2+-5+} was not accompanied by activation of NF-κB or synthesis of IL-8, which was underscored by the failure of the metals to activate cytosolic signalling mechanisms involved in activation of NF-κB, as well as lack of effects on other transcription factors which cooperate with NF-κB in the activation of target genes.

- Palladium, but not cobalt, platinum, or vanadium, attenuates the neutrophil-activating and -mobilizing properties of the chemoattractants, C5a and IL-8, both

of which are critical components of the host innate immune response. C5a is generated during complement activation and is a potent chemoattractant for neutrophils, monocytes and macrophages (Hopken *et al*, 1996). IL-8 is produced by epithelial cells, monocytes, macrophages and neutrophils and possesses selective chemotactic activity for neutrophils. This represents a previously undocumented mechanism by which exposure to a heavy metal may compromise innate host defences.

- Palladium also neutralizes the pore-forming action of the pneumococcal toxin, pneumolysin, resulting in attenuation of toxin-mediated Ca^{2+} influx, with consequent attenuation of nuclear translocation of NF-κB proteins and production of IL-8. Because many different bacterial pathogens produce cholesterol-binding, pore-forming toxins (Andrew *et al*, 2000), exposure to palladium may favour microbial persistence in the airways, possibly predisposing to pneumococcal infection.

During occupational, as well as environmental, exposure to the metals, inhalation occurs over an extended period and the first cells to come into contact with the inhaled matter are alveolar macrophages and respiratory epithelium. It has been demonstrated that production of GM-CSF, IL-6, IL-1β, TNF-α, IL-8, and MCP-1 by alveolar macrophages is increased after exposure to particulate air pollution matter (Goto *et al,* 2004). Furthermore, exposure to air pollution causes a systemic inflammatory response, subsequently leading to bone marrow stimulation and the release of granulocytes into the circulation (Terashima *et al,*1997, Tan *et al,* 2000) . Functional studies showed that these immature granulocytes are less deformable and less chemotactic, and migrate less efficiently to the sites of inflammation (Van Eeden *et al*, 1997; Van Eeden *et al*, 1999). However, neutrophils will eventually reach the airways at sites of inflammation and come into contact with the inhaled particulate matter containing the metals. Apart from contact between phagocytes and inhaled pollutants within the respiratory tract, neutrophils might also encounter metals in the circulation from which they are transported by blood proteins to various tissues (Vanadium, 2001).

Taken together with previous published findings (Theron *et al,* 2004; Ramafi *et al,* 2004), the results of the current studies appear to demonstrate that the primary consequence of the interaction of Co^{2+}, Pd^{2+}, Pt^{2+} and V^{2+-4+} with human neutrophils is to increase the reactivity, as opposed to the generation of reactive oxidant species generated by these cells, presumably by functioning as catalysts of oxidation/reduction reactions. This clearly presents the potential threat of oxidant-mediated toxicity and carcinogenesis. Unlike the other test metals, Pd^{2+} may compromise innate host defences by inactivating host- and bacterial-derived proteins with neutrophil activating/ mobilizing properties.