Oxidative stress and macrophage function: a failure to resolve the inflammatory response

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Abstract
The suppression of pro-inflammatory gene expression along with the clearance of apoptotic cells by phagocytosis can play an important role in resolving the inflammatory response. Any impairment of these processes can therefore lead to a chronic inflammatory state. Oxidative stress can have both direct and indirect effects on macrophage function. This mini-review highlights a mechanism through which oxidative stress via the production of reactive carbonyls alters the ECM (extracellular matrix) environment of macrophages, thereby altering their behaviour. Carbonyl modification of ECM proteins causes increased macrophage adhesion and activation through receptors that are also involved in phagocytosis. Moreover, interaction of macrophages with these carbonyl-modified ECM proteins leads to decreased phagocytic activity towards apoptotic cells. At a more direct level, both oxidative and carbonyl stress inhibits activity of the transcriptional co-repressor HDAC-2 (histone deacetylase 2), which under normoxic conditions helps to suppress pro-inflammatory gene expression. Consequently, macrophages activated under conditions of oxidative or carbonyl stress can lead to a more enhanced inflammatory response. Coupled with an impairment of the phagocytic response, this can lead to ineffective clearance of apoptotic cells and secondary necrosis, with the result being failure to resolve the inflammatory response and the establishment of a chronic inflammatory state.

Introduction
In the last 20 years, free radicals in the form of ROS (reactive oxygen species) have become increasingly recognized as playing a major role in many disease processes. Indeed, biological systems are continually exposed to ROS, none more so than the lung. With its large surface area and blood supply, the lung is highly susceptible to injury mediated by oxidative stress. Indeed the main causal factor of COPD (chronic obstructive pulmonary disease), a disease characterized by chronic steroid-insensitive inflammation leading to progressive airway obstruction and emphysema [1], is oxidative stress as a result of long-term cigarette smoking [2,3]. It is the imbalance in the oxidant/antioxidant mechanism triggered by cigarette smoking that can induce tissue damage, either directly or indirectly, that has led to the proposal that it plays a central role in the pathogenesis of COPD [4]. At a cellular level, however, the macrophage is believed to be the principal cell type responsible for disease pathogenesis [5]. Indeed, there are increased numbers of activated alveolar macrophages in the lungs of smokers [6]. This mini-review discusses how oxidative stress, both intracellularly through the activation of redox-sensitive transcription factors [7,8] and impairment of chromatin regulation, and extracellularly through its impact on modification of the extracellular environment, can influence macrophage function [9,10]. Moreover, the receptors used to sense these modifications in the extracellular environment are also utilized in phagocytosis, with important consequences for the removal of apoptotic cells and the resolution of any inflammatory response. Indeed, macrophage function such as phagocytosis has been shown to be impaired in COPD [11]. Therefore attempts to highlight at a mechanistic level how ROS can impact on macrophage function may help to explain, in part, how this might help drive the chronic inflammatory state observed in diseases such as COPD.

Sources of oxidative stress
There are essentially two sources of ROS, environmental and cellular. Environmental-derived ROS consists of both gaseous and particulate air pollution and will principally affect the lung. This ranges from cigarette smoke and oxidant gases, such as ozone, nitrogen dioxide and sulfur dioxide, to airborne PM10 (particulate matter <10 µm) from diesel car exhaust fumes, which can promote ROS production in situ [12]. Moreover, cigarette smoke contains over 4700 chemical compounds and high concentrations of oxidants (10^14 molecules/puff) and 3000 p.p.m. NO (nitric oxide)/puff [13]. The nature of ROS found within cigarette smoke varies from short-lived oxidants, such as the superoxide radical (O_2•-) and the NO molecule, to long-lived organic radicals, such as semiquinones which can undergo redox cycling within the epithelial lining fluid of smokers for some considerable period of time [14,15].

Cellular-derived ROS is enzymatically produced by inflammatory and epithelial cells [16] within the lung as part of an inflammatory-immune response towards a pathogen or

Key words: chronic obstructive pulmonary disease (COPD), histone deacetylase (HDAC), inflammatory response, macrophage, oxidative stress, reactive oxygen species.

Abbreviations used: AP-1, activator protein 1; 4-HNE, 4-hydroxy-2-nonenal; AGE, advanced glycation end-product; ALE, advanced lipoxidation end-products; COPD, chronic obstructive pulmonary disease; ECM, extracellular matrix; HDAC, histone deacetylase; MSR-A, macrophage scavenger receptor type A; NF-κB, nuclear factor κB; ROS, reactive oxygen species.

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irritant. Several sources for ROS production exist within a cell and include mitochondrial respiration, NADPH oxidase and the xanthine/xanthine oxidase system, of which the principal ROS generator is NADPH oxidase. This results in the release of $\text{O}_2^\cdot+$, which can either react with NO to form highly reactive peroxynitrite ($\text{ONOO}^-$) or, alternatively, be rapidly converted into $\text{H}_2\text{O}_2$ under the influence of SOD (superoxide dismutase). However, $\text{H}_2\text{O}_2$ in the presence of $\text{Fe}^{2+}$ can also produce the more damaging hydroxide radical ($\cdot\text{OH}$) through Fenton chemistry. Moreover, redox cycling of $\text{Fe}^{2+}$ and $\text{Fe}^{3+}$ through a combination of Haber–Weiss and Fenton chemistry can also result in the formation of the more damaging hydroxide radical ($\cdot\text{OH}$) from superoxide [17]. This can be particularly relevant to COPD where smokers have been found to have significantly higher levels of iron in their lungs thereby increasing the potential ROS burden [18]. The haem peroxidases, myeloperoxidase or eosinophil peroxidase, secreted by inflammatory cells, can also catalyse the formation of the very potent and damaging oxidants hypochlorous acid ($\text{HOCl}$) and hypobromous acid ($\text{HOBr}$) from $\text{H}_2\text{O}_2$ in the presence of chloride ($\text{Cl}^-$) and bromide ($\text{Br}^-$) ions respectively. Once produced, ROS can interact with a wide variety of molecules through electron donation in biological systems.

**Molecular impact of ROS**

ROS when generated close to cell membranes oxidize membrane phospholipids (lipid peroxidation), a process that may continue as a chain reaction. This can impair membrane function, inactivate membrane-bound receptors and enzymes and increase tissue permeability, processes that have been implicated in the pathogenesis of many forms of tissue injury [19]. The products of lipid peroxidation which results in the formation of reactive aldehydes such as acrolein, 4-HNE (4-hydroxy-2-nonenal), malonaldehyde and glyoxal [20], along with other bioactive molecules, such as the isoprostanes and platelet-activating factor mimetics [21], have also been referred to as carbonyl stress by some groups. Reactive carbonyls are also found in cigarette smoke in high concentrations [22, 23]. They are highly diffusible end-products of lipid peroxidation and are able to induce various cellular events, such as proliferation, apoptosis and activation of signalling pathways [24, 25]. Reactive carbonyls such as acrolein and 4-HNE attack residues such as lysine, arginine, histidine and cysteine through Michael addition reactions. Alternatively the carbonyl group can react with free amino groups to form Schiff bases. The result is covalent protein modifications, commonly referred to as AGEs (advanced glycation end-products) or ALEs (advanced lipoxidation end-products) [26, 27]. The immediate impact is that these carbonyl-derived protein modifications have been shown to be markers for oxidative stress-derived tissue damage in various chronic diseases [28].

Intracellularly, oxidative stress can also have a major impact on redox-sensitive signalling pathways as well as act as a second messenger itself [21, 29]. Redox-sensitive molecular targets usually contain highly conserved cysteine residues, and their oxidation, nitrosylation or the formation of disulfide links are crucial events in oxidant/redox signalling. Such molecular targets include transcription factors [NF-κB (nuclear factor κB) and AP-1 (activator protein 1)], signalling molecules such as Ras/Rac or JNK (c-Jun N-terminal kinase), protein tyrosine phosphatases, p21 [30] and redox sensors such as thioredoxin which can sequestrate and inactivate kinases such as Ask-1 (apoptosis signal-regulating kinase 1) [31, 32]. The activation of redox-sensitive transcription factors, such as NF-κB and AP-1, is a necessary prerequisite for induction of pro-inflammatory gene expression. However, at another level of gene regulation, chromatin topology also plays an important part, through which ROS can have a dramatic impact. Chromatin consists of DNA tightly wrapped around a tetrameric set of core histone proteins. Acetylation of the histones by transcriptional co-activators such as CBP [CREB (cAMP-response-element-binding protein)-binding protein]/p300 results in uncoiling of the DNA around the core histones, facilitating access for DNA polymerases so as to initiate gene transcription. In contrast, HDACs (histone deacetylases) remove the acetyl groups allowing condensation of the DNA around the histone core, expelling the DNA polymerases, thereby shutting off gene expression. Within this complex regulatory mechanism, ROS has been shown by us and others to inactivate HDAC-2. This is achieved through increased nitrification or carbonylation [33]. Coupled with ROS-induced histone acetylation through activation of NF-κB and AP-1 transcription factors, this has the net effect of promoting pro-inflammatory gene expression [34]. The impact of ROS on HDAC-2 is particularly important as it has also been shown to be required for corticosteroid-mediated inhibition of the inflammatory response [35].

**Extracellular environment and macrophage function**

Macrophages are multifunctional cells. Their roles extend from clearance of micro-organisms, xenobiotic material and apoptotic cells to regulating both innate and acquired immune responses through antigen presentation to secretion of various cytokines and chemokines. In order to undertake these tasks macrophages have evolved several strategies; these include the recognition and adherence to particular substrates, migration in response to chemoattractants, activation to elicit inflammatory responses towards a pathogen, and phagocytosis of foreign bodies or apoptotic cells. Phagocytosis of apoptotic cells is an immunologically silent process [36] that plays a major role in the resolution of an inflammatory response [37]. In a disease such as COPD, where cigarette smoking causes a rise in both neutrophil and macrophage numbers in lung tissues, it has been shown that COPD alveolar macrophages have a decreased propensity for phagocytosis [11]. The ultimate fate of many recruited inflammatory cells is death, either through apoptosis or necrosis. It is the balance between inflammatory cell recruitment and the removal of apoptotic cells by phagocytosis that ultimately
determines whether the inflammatory response will resolve. Consequently, failure to remove inflammatory cells can result in secondary necrosis causing further tissue damage and exacerbating the inflammatory response [37,38]. This can ultimately contribute towards a chronic inflammatory state [39].

Regulating the clearance of apoptotic cells can be viewed from two perspectives: first, influencing the rate at which cells enter apoptosis and, secondly, controlling the phagocytic removal of apoptotic cells [38,40,41]. The former is known to be affected by inflammatory mediators and growth factors [42]. Likewise, phagocytosis can also be controlled by pro-inflammatory cytokines [43], as well as other factors, such as the ECM (extracellular matrix) proteins fibronectin [41] and collagen [44]. More recently, we have shown that ECM proteins modified by AGE and/or ALE adducts have a negative impact on the phagocytosis of apoptotic cells [10]. The mechanism of this impaired phagocytic response is proposed to be one of sequestration of the receptors involved in recognition of apoptotic cells towards that of adhesion to the modified ECM proteins. This is similar to an earlier finding of El-Khoury et al. [45].

Numerous receptors, including MSR-A (macrophage scavenger receptor type A) and CD36, have been shown to be involved in the uptake and recognition of apoptotic cells [46–48]. Both MSR-A and CD36 belong to the scavenger receptor superfamily of which there are five classes (A–E) that are structurally unrelated. The ligand-binding properties for these receptors are very broad. Intriguingly, one group has reported [49] that there may be a genetic association between COPD and a binding-site mutation in MSR-A with COPD. Whether this mutation alters the ligand-binding profile for this receptor remains unclear at present, but it does raise some interesting questions. Both MSR-A and CD36 are involved in macrophage adhesion to ECM proteins post-translationally modified by lipid peroxidation products or cigarette smoke [9,45,50]. We have shown that both 4-HNE and acrolein adducts on collagen IV can facilitate macrophage adhesion in a non-integrin-related manner through MSR-A. Moreover, this adhesion can also result in macrophage retention and inhibition to migrate in response to chemotactic stimuli. Another property of modified ECM proteins was the ability to activate macrophages as demonstrated by us and others [9,50,51]. Interestingly, adhesion to 4-HNE adducts of ECM were not only greater but could be achieved with much lower exposure concentrations of 4-HNE than that seen with acrolein. It is possible therefore that 4-HNE modifications may be pathophysiology more important than acrolein. Indeed, Rahman et al. [52] have previously shown that there are increasing levels of 4-HNE carbonyl adducts within lung tissue with increasing severity of COPD status as defined by a worsening FEV1: (forced expiratory volume in 1.0 s). However, it should be remembered that in vivo the antioxidant capacity as well as the frequency of exposure to carbonyls or oxidative stress will have a big impact on the level of protein modifications attained.

Concluding remarks
The ability of macrophages to adhere to, and be activated by, modified proteins resulting in localized tissue retention and pathology has been implicated in several chronic diseases, such as atherosclerosis and Alzheimer’s disease. Furthermore, similar mechanisms may operate in other chronic diseases that include COPD, chronic renal failure, diabetic complications and asbestosis. Coupled with the observation that changes in cellular environment through the production of modified ECM proteins can also prevent apoptosis, the implications in vivo would be a failure to resolve the inflammatory response and the potentiation of a chronic inflammatory state.

References

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