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Macrophage antioxidant protection within atherosclerotic plaques

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1. ABSTRACT

Macrophage cells within inflammatory lesions are exposed to a wide range of degrading and cytotoxic molecules including reactive oxygen species. Unlike neutrophils, macrophages do not normally die in this environment but continue to generate oxidants, phagocytose cellular remains, and release a range of cyto-active agents which modulate the immune response. It is this potential of the macrophage cell to survive in an oxidative environment that allows the growth and complexity of advanced atherosclerotic plaques. This review will examine the oxidants encountered by macrophages within an atherosclerotic plaque and describe some of the potential antioxidant mechanisms which enable macrophages to function within inflammatory lesions. Ascorbate, α-tocopherol, and glutathione appear to be central to the protection of macrophages yet additional antioxidant mechanisms appear to be involved. γ-Interferon causes macrophages to generate 7,8-dihydroneopterin/neopterin and 3-hydroxyanthranilic acid both of which have antioxidant properties. Manganese superoxide dismutase is also upregulated in macrophages. The evidence that these antioxidants provide further protection, so allowing the macrophage cells to survive within sites of chronic inflammation such as atherosclerotic plaques, will be described.

2. INTRODUCTION

Macrophages function within the hostile environment of inflammatory lesions. As such they are exposed to a range of immune cell generated reactive oxidants which cause cellular damage and death. Atherosclerotic plaques are sites of chronic inflammation where macrophages play a key role in the pathological progression of the disease (1). The oxidants superoxide, hydrogen peroxide, hypochlorite, and hydroperoxides are all present within atherosclerotic plaques and are possibly generated by the macrophages. These reactive oxygen species (ROS) readily kill cells and will kill macrophage cells at elevated concentrations. Oxidation of proteins and lipids within the artery wall also creates secondary cytotoxic agents which, due to their stability and abundance, may eventually trigger macrophage cell death. The most significant of these secondary agents appears to be oxidized low density lipoprotein (oxLDL). OxLDL has been shown to be highly cytotoxic to a range of cells, including macrophages (2).

The oxidation of low density lipoprotein (LDL) to oxLDL and its subsequent uptake by macrophages to form relatively stable lipid loaded "foam cells" has been cited as a key process in plaque development (3). The macrophage foam cells appear to make up a significant

portion of an atherosclerotic plaque. As a result, the mechanism of LDL oxidation has been the focus of much research and argument. The failure of key dietary antioxidant supplementation studies to improve clinical outcomes in vascular disease has presented a major challenge to the oxidative model of vascular disease, especially the hypothesis that oxLDL formation is a major driver in initial development of the plaques (4-7). Yet oxLDL is found within atherosclerotic plaque and its cytotoxic properties strongly suggest to us that though oxLDL may possibly not be a significant factor in initial plaque formation, its presence will promote formation of a complex plaque by initiating macrophage cell death. A better knowledge of the process that prevents oxidant-induced death of macrophages is crucial to understanding complex plaque formation and stability. In this review we will explore the nature of the oxidative environment within an atherosclerotic plaque and the effect of these oxidants on macrophages. We then look at some possible antioxidant mechanisms operating within macrophages to ensure their survival.

3. PLAQUE OXIDANTS

Analysis of atherosclerotic plaques show that they are rich in products of oxidative damage. Hydroxylated fatty acid and oxysterols have been well characterized as well as a range of protein oxidation products. Much of the research emphasis has been on lipid oxidation products due to the high level of cholesteryl-esters found within plaques. The majority of the cholesterol esters appear to originate from cholesterol ester rich LDL particles which enter into the artery wall from the plasma. A high percentage of these cholesterol esters are esterified with polyunsaturated fatty acid (PUFA) which readily oxidizes to hydroperoxides in a lipid peroxyl radical mediated chain reaction (8). Lipid peroxyl radicals, when formed by a variety of oxidative and free radical based mechanisms, can abstract hydrogen atoms from surrounding PUFAs, generating more lipid peroxyl radicals and lipid hydroperoxides (9). In the presence of trace amounts of iron or copper ions, lipid hydroperoxides break down to an enormous range of products including cytotoxic oxysterols, pro-inflammatory isoprostanes and lipid aldehydes which rapidly react with proteins causing disruption to cell function. In addition to these lipid oxidation products, protein bound-DOPA (3,4-dihydroxyphenylalanine), 3-chlorotyrosine, dityrosine, protein carbonyls and protein hydroperoxides have also been identified within plaques (10-12). Of particular interest is protein bound DOPA (PB-DOPA) and protein hydroperoxides which, unlike other protein oxidation products, can initiate further oxidative damage (13). PB-DOPA is formed by hydroxyl radical and hypochlorous acid attack on tyrosine residues within protein (14, 15). Copper mediated LDL oxidation also generates PB-DOPA (16). Copper and iron ions are readily reduced by PB-DOPA enhancing metal catalyzed radical formation via the Fenton reaction. PB-DOPA can significantly amplify initial oxidant production as well as diffusing to other locations, transporting the oxidative stress with it (13, 17, 18). Protein hydroperoxides are the main primary products of hydroxyl radical and peroxyl radical reactions with proteins (19, 20). They have been observed to form on living cells, lipoproteins and a range of proteins (16, 21, 22). Though relatively stable, they readily react with ascorbate (Vitamin C) and glutathione (GSH), depleting cellular or extracellular antioxidant pools (13). The stability of the protein hydroperoxides suggests that the reported inhibition of caspase-3, a key effector caspase during apoptosis, by tryptophan and tyrosine hydroperoxides may also occur *in vivo* during oxidative stress (23). The ability to react with and cross-link DNA also suggests protein hydroperoxides as a major source of DNA damage and mutation (24).

Transition metal ions catalyze the breakdown of protein hydroperoxides as well as lipid hydroperoxides via protein peroxyl/alkoxyl free radical intermediates which may trigger further oxidative events (25) such as the oxidation of DNA (26). Unlike reactive lipid aldehydes, the protein carbonyls formed from protein hydroperoxide breakdown are relatively unreactive leaving the cell to remove them by proteolysis.

All these oxidative products appear to be the result of oxidant generating inflammatory cells within the plaque (Figure 1). Activated macrophages release significant amounts of superoxide which, though not very reactive in itself, dismutates to hydrogen peroxide. Hydrogen peroxide can react with reduced transition metal ions in a reaction known as the Fenton reaction, which generates the extremely reactive hydroxyl radical ($^{\bullet}OH$). Atherosclerotic plaques contain high levels of redox active copper and iron ions which can catalyze these reactions (27, 28). Reduced copper and iron ions also catalyze the oxidation of lipid PUFA so initiating lipid oxidation reactions on LDL found within plaques. These reactions generate significant amounts of lipid and protein hydroperoxides. Ferrous ions will also generate lipid hydroperoxides directly on the cell membrane of macrophage cells adding further oxidative stress (29, 30).

These reactions *in vitro* have been shown to be significantly enhanced by the release of reducing agents by macrophages. In tissue culture, macrophages release reduced thiols and directly reduce iron and copper by the action of a transplasma membrane electron transport system which transfers electrons from the NAD (P)H to external electron acceptors such as ferric and cupric ions (31, 32). So, in addition to superoxide, macrophages can further potentiate oxidative stress by enhancing the Fenton reaction through metal ion reduction.

Myeloperoxidase (MPO) and chlorotyrosine have also been detected within plaques (12, 33). Though made in low yields, chlorotyrosine is a very stable marker of hypochlorous acid reacting with protein tyrosine residues. The reaction also generates protein bound DOPA and dityrosine (15, 34). Hypochlorous acid (HOCl) is formed by the enzymatic action of MPO on hydrogen peroxide and chloride ions. Traditionally neutrophils have been considered the major source of this enzyme but macrophages within plaques have also been shown to

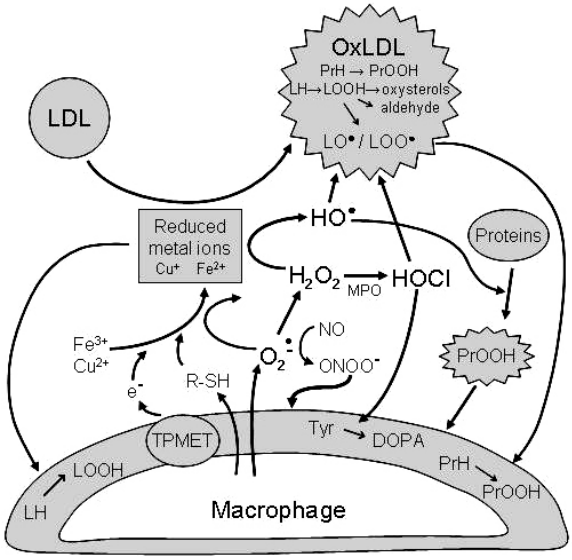

Figure 1. Summary of oxidants generated within atherosclerotic plaques. Oxidants are generated either through the release of superoxide ($O_2^{\bullet-}$) which can dismutate to hydrogen peroxide or be further converted to HOCl by myeloperoxidase (MPO). HOCl can oxidise both lipid and proteins in low density lipoproteins (LDL) to form oxLDL, or react with the cell plasma membrane. One of the products of HOCl damage to proteins is the oxidation of tyrosine (Tyr) to DOPA which occurs on protein amino acid residues. The Fenton reaction between hydrogen peroxide (H_2O_2) and reduced metal ions generates hydroxyl radicals ($HO^{\bullet}$) which can directly attack the cell, oxidise LDL or oxidise proteins to protein hydroperoxides (PrOOH). During oxidation of LDL to oxidised LDL (oxLDL) proteins (PrH) and lipids (LH) are oxidised to protein hydroperoxides (PrOOH) and lipid peroxides (LOOH). Lipid peroxyl ($LOO^{\bullet}$) and lipid alkoxyl radicals ($LO^{\bullet}$) can cause further lipid and protein oxidation. Macrophages potentially increase the pool of reduced copper and iron ions through the release of reduced thiols (R-SH) or direct electron transfer via the plasma membrane electron transport complex (TPMET).

release active MPO (35). HOCl also has the potential to diffuse through the plasma membrane and cause direct damage to cytosolic components (36).

Nitric oxide and peroxynitrite have also been implicated in the initiation of plaque growth as they are released from the endothelial cells of the artery wall (37). The ability of macrophages to generate nitric oxide has been highly controversial as human macrophages appear to be unable to synthesis significant levels of the iNOS cofactor 5,6,7,8-tetrahydrobiopterin, resulting in little or no nitric oxide generation (38, 39). However, there is a significant number of studies which have shown that macrophages taken from patients do release nitric oxide (40). We are therefore uncertain how important nitric oxide induced oxidative damage is to macrophages, though macrophages have been shown to be sensitive to these oxidants (41). In monocytes, the S-nitrosylation of key thiols within caspase-3 inhibits apoptosis promoting cell survival (42). This maybe a critical step promoting the collection and differentiation of monocyte/macrophages within the plaque.

Much of the research in this area has focused on identifying the primary oxidative events occurring within the plaque, yet the sum of the data strongly suggests that there is no single primary radical event. Metal catalyzed oxidation, cell-derived oxidants, MPO generated hypochlorite, lipid and protein peroxyl radicals are all present within the plaque and contribute to the overall oxidative stress as summarized in Figure 1. It is within this hostile environment that macrophages must function.

4. OXIDANT DAMAGE AND MACROPHAGE CELL DEATH

Free radicals and oxidants will kill any cell eventually if enough damage is inflicted on the cellular structure and machinery. In extreme oxidative stress, cellular death results from loss of the barrier function of plasma membranes and inactivation of key metabolic enzymes leading to the total failure of the cell (43-46).

Originally DNA was considered the major target of free radicals and oxidants, but it is now realized that with only 2% of the total cell dry mass comprising of DNA, and proteins making up 50% of the mass, there is very little direct DNA damage from external oxidants (20, 47). Cellular proteins, which make up the majority of a cells components, are therefore the primary target of free radicals (47).

Oxidation of the amino acid residues within enzymes causes inactivation through oxidative derivatization of active site amino acid groups, loss of enzyme shape due to peptide cleavage, or changes in polarity (23, 48-50). These reactions also occur on receptors with oxidative damage causing the loss of ligand binding or phosphorylation sites (51-54). The loss of plasma membrane ion channel function through protein oxidation causes the influx of calcium ions, osmotic cell swelling, and the activation of calcium dependent proteases, triggering both necrotic and apoptotic cell death mechanisms (55-57). If protein damage is extensive enough, the cell may no longer be able to function or even react fast enough to oxidative stress. Key apoptotic enzymes may be denatured and the cell's ability to generate ATP lost, causing the cell to decay into a necrotic, uncontrolled failure and death.

At lower oxidant levels, the mechanisms triggering cell death are far more subtle, with many cells appearing to be programmed to die through apoptosis when only minor oxidative damage occurs within the cell. There is considerable discussion about whether at these lower levels of oxidative damage, the oxidants are acting as a type of intracellular signal to trigger apoptosis or whether the oxidant damage is itself the trigger of cell death (58-60). Though the hypothesis of reactive oxygen species acting as intracellular messenger molecules may be

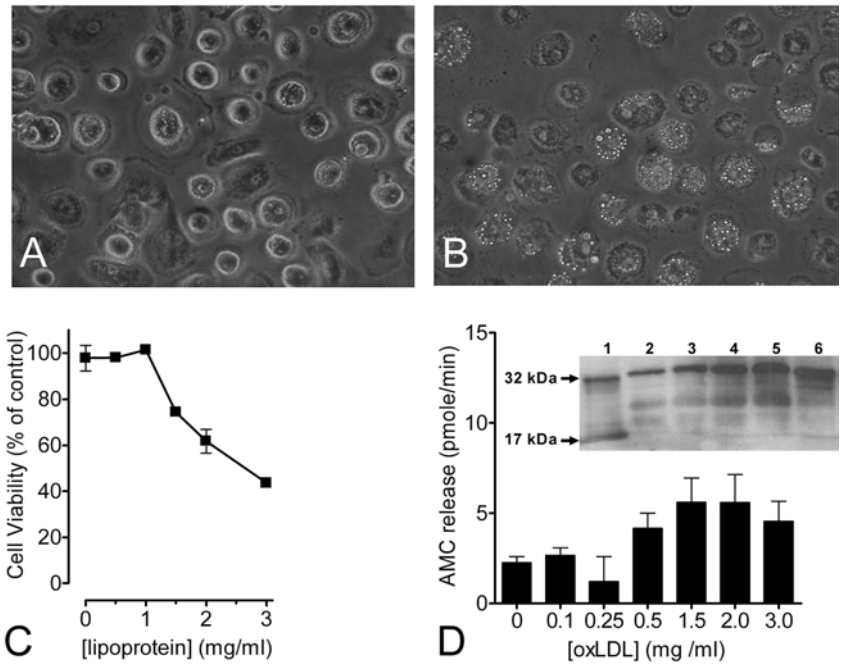

Figure 2. OxLDL induced Human monocyte-derived macrophage cell death. HMDM cells prepared from whole human blood (61) were incubated in varying concentrations of either LDL or oxLDL in RPMI-1640 media supplemented with 10% heat inactivated human serum. OxLDL was prepared by copper oxidation (71) and concentration is given in total mass (154). After 24 hours the untreated cells (A) look normal, while the cells exposed to 1.5 mg/ml oxLDL (B) show loss of membrane integrity, cell lysis, and blebbing with the cytoplasm full of lipid filled endosomes. After 24 hours incubated with 1.5 mg/ml oxLDL or greater, there is a metabolic loss in cell viability (C) measured by the cells' ability to reduce MTT with NAD (P)H dependent reductases (155). No significant increase in caspase-3 enzyme activity (D) was detected by measuring the cleavage of Ac-DEVD-AMC (107), nor was there any conversion of the pro-caspase-3 to its active 17 kDa forms visualized by immune blotting with a mouse monoclonal caspase-3 antibody (E-8) (Santa Cruz Biotechnology Inc., USA) of an SDS-PAGE electrophoresis of cell homogenates consisting of; lane 1, Jurkat cells treated with anti-Fas antibody at 200 ng/ml for 2hours, Lines 2-6 human macrophages treated with 0 (lane 2), 0.5 mg/ml (lane 3), 1.5 mg/ml (lane 4), or 3.0 mg/ml oxLDL (lane 5) for 24hours, or anti-Fas antibody at 200 ng/ml for 22 hours (lane 6). The 32 kDa band for pro-caspase-3 is overexposed to enable visualization of the 17 KDal active form.

attractive, until the molecular switches or receptors involved are identified, it is difficult to distinguish between these two mechanisms.

What happens with macrophages during oxidative stress is not completely clear as the cells' sensitivity and response to oxidants is dependent on the type or source of macrophages under study as well as the oxidants used. Reported research has generally used the monocyte-like human leukemia derived cells lines THP-1 or U937; mouse macrophage-like Raw 264.7 cells, J774 cells, or mouse peritoneal macrophages. The number of oxidant studies on actual human-blood derived monocytes and human monocyte-derived macrophages (HMDM) is surprising low in comparison. The difference between these different cell lines can be striking.

Protein oxidation predominates over lipid oxidation in U937 and THP-1 exposed to peroxyl or hydroxyl radicals (21, 22, 47), but in cholesterol ester rich HMDM cells, peroxyl radicals, generate predominately lipid hydroperoxides with very little protein oxidation occurring (61). There appears to be enough PUFA within the HMDM cells to sustain a lipid oxidation chain reaction, while in THP-1 and U937 cells, the PUFA content is too low to sustain a significant lipid peroxidation reaction and the peroxyl radicals are rapidly scavenged by the cellular proteins.

This variation in cellular response is even more striking with oxLDL which causes a caspase-3-dependent apoptosis in THP-1 cells, while with U937, a necrotic death occurs with the loss of intracellular GSH, caspase inhibition, and cell swelling (62). With HMDM cells, oxLDL has been reported to induce caspase-3 activation (63) though cell death was not dependent on this activation (64). However, in our laboratory we have repeatedly failed to see any oxLDL induced caspase activation in HMDM cells either by immunoblotting for active caspase-3 or measurement of caspase enzyme activity (Figure 2). These differences may be due to variation in oxLDL preparations giving variable amounts of cytotoxic lipids (65), or changes to the oxidant generated oxLDL ligands to which the different scavenger receptors may bind, changing the

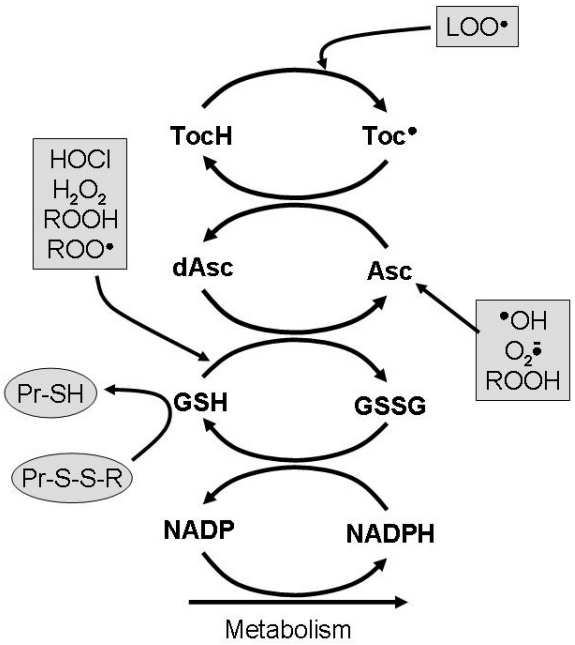

Figure 3. Cellular Antioxidant Scavenging. Lipid peroxyl radicals ($LOO^{\bullet}$) are scavenged by tocopherol to generate the tocopheroxyl radical which is regenerated to tocopherol by ascorbate (Asc). Ascorbate can neutralize hydroxyl and water-soluble peroxyl radicals and superoxide. The resulting ascorbate radical is "recycled" back to ascorbate by the reaction with glutathione (GSH). GSH reacts with hypochlorite (HOCl), peroxyl radicals and hydroperoxides by both enzymatic and non-enzymatic mechanisms. GSH also maintains various proteins in a reduced state. The oxidised GSH (GSSG) is converted back to GSH by the action of GSH reductase using NADPH.

response to oxLDL (66). Minor variations in the purification protocol for HMDM cell preparation will alter cellular antioxidant mechanisms or change the balance of scavenger receptors (67). For example CD36 uptake of oxLDL appears to favor caspase initiated apoptosis (63) while the class A macrophage scavenger receptor may promote foam cell formation (68, 69). Which receptors are present, and a difference in the receptors which the oxLDL binds to will alter the cellular response observed.

How oxLDL actually causes macrophage death *in vitro* is unclear, especially as heavily oxidized LDL is relatively unreactive. OxLDL is traditionally prepared by 24 hours incubation with copper chloride or sulphate (2, 70, 71). Cell mediated LDL oxidation is metal ion dependent and redox active metal ions are present with atherosclerotic plaques (27, 28, 72). After 24 hours with copper ions all the LDL PUFAs have oxidized to hydroperoxides before breaking down to various aldehydes, ketones, and secondary alcohols. The reactive aldehydes (HNE, MDA) have reacted with and derivatised the apoB100, whose protein hydroperoxides have also broken down to various unreactive amino acid derivatives. OxLDL is readily taken up by macrophages but at higher concentrations it triggers a cell death process that centers on the mitochondria with the release of cytochrome c (73, 74) and the loss of mitochondrial membrane potential (64). One of the early events observed during oxLDL induced macrophage death is intracellular oxidant production (69, 75) and GSH loss (76). Whether GSH loss is the cause or the consequence of oxidant production is unclear but the fall in cellular redox potential should reduce the stability of redox sensitive thiol dependent enzymes, a number of which are found in the key glycolytic and Krebs cycle pathways. Cytochrome c release and mitochondrial dysfunction through membrane pore formation may also be a source of oxidative stress.

Oxysterols have been cited as the main cytotoxic agent within oxLDL (77). Though not all oxysterols are cytotoxic, 7-ketocholesterol, a significant product of advanced LDL of oxidation (65), has been shown to modify lipid raft domains in THP-1 cells causing an increase in cytosolic calcium and activation of calcium dependent proteases (57). 7-Ketocholesterol, also initiates oxidative stress in mouse macrophage J774A.1 cells through the activation of NADPH oxidase; a possible source of oxLDL induced oxidative stress (78).

Though oxLDL appears to be the main cytotoxic agent present in the plaques, the extreme reactivity of HOCl must also be considered. HOCl rapidly causes loss of plasma membrane function followed by inactivation of cellular enzymes within the cytosol of murine macrophage-like P388D1 cells (79). The reaction with proteins is very rapid with the amino residues cysteine, methionine, disulfide bonds, tryptophan, α-amino, lysine and tyrosine being preferential targets in that order (80). Like hydroxyl and peroxyl radicals, HOCl causes loss of cell viability through the destruction of the cellular machinery (81, 82). As it is generated in significant quantities during both chronic and acute inflammation, it is a major source of oxidative stress to cells and needs to be mitigated by macrophages within the inflammatory site.

5. ANTIOXIDANTS VITAMIN C, E, AND GSH

Ascorbate (vitamin C) is one of the main water-soluble antioxidants in extracellular and intracellular fluids. It is present in plasma at concentrations of about 43 μM and can be increased to about 59 μM by supplementation (6). The concentration of ascorbate inside cells is usually much higher and can be several millimolar (83). Ascorbate scavenges hydroxyl, superoxide, glutathionyl and urate radicals, aqueous peroxyl and alkoxyl radicals, and also singlet oxygen (84). It can also recycle α-tocopheroxyl radicals back to α-tocopherol, including those in LDL (Figure 3) (85). Ascorbate also breaks down protein hydroperoxides to the unreactive hydroxylated amino acid residues (13). Ascorbate therefore has an important role inhibiting oxidative damage *in vivo*.

Ascorbate also inhibits copper or cellular-mediated LDL oxidation, potentially lowering the concentration of oxLDL within plaque (86-89). It has been reported that one mechanism where ascorbate protects LDL from oxidation involves the conversion of ascorbate to dehydroascorbate which can modify histidine residues on

apoB-100. The resulting 2-oxo-histidine residues do not bind redox active copper ions so preventing LDL oxidation (87, 90). Ascorbate can also be a pro-oxidant, because it reduces iron and copper ions to their more active lower valency states, which can rapidly decompose lipid hydroperoxides into lipid radicals. Ascorbate can therefore increase the rate of oxidation of partially oxidised LDL containing pre-existing lipid hydroperoxides (89, 91). Consequently, ascorbate can have a 'Jekyll and Hyde' effect on oxidation of native or partially oxidised LDL. As well as affecting the oxidation of LDL, ascorbate can also affect the way cells respond to oxidised LDL. It should be noted that cells in culture are often deficient in ascorbate and adding ascorbate at physiological concentrations can increase the intracellular concentrations of ascorbate many fold, up to about 10 mM (92, 93). Apoptosis of human umbilical artery smooth muscle cells induced by oxidised LDL with a high content of hydroperoxides or by a fatty acid hydroperoxide is decreased greatly by pre-incubation of the cells with ascorbate at physiological concentrations (94). Ascorbate may protect smooth muscle cells from death in atherosclerotic lesions and help maintain the stability of these lesions. Remarkably, ascorbate did not protect against apoptosis induced by oxidised LDL in macrophages, but produced a modest increase in apoptosis (92). This was not due to a pro-oxidant effect of ascorbate in the cells because the oxidised LDL-induced increase in the levels of heme oxygenase-1 were prevented by pre-treatment with ascorbate. It appeared that ascorbate was protecting the cells effectively against the oxidative stress induced by oxidised LDL. The modest increase in apoptosis may possibly have been due to a protection of the apoptotic machinery by ascorbate, as proposed by Vissers *et al.* (95).

OxLDL increases the level of the major intracellular antioxidant GSH in macrophages (96) and human umbilical artery smooth muscle cells (97) and increases the uptake of the GSH precursor L-cystine by the transporter x_c^- (97). The increase in GSH was prevented by pre-incubation with physiological concentrations of ascorbate, presumably because the ascorbate protected the cells from the oxidative stress induced by the oxidised LDL (97). Ascorbate also decreases HOCl-induced DNA base and protein damage in smooth muscle cells (98).

α-Tocopherol (vitamin E) is probably the most important lipophylic antioxidant in biological systems and has been the subject of many reviews with much debate. α-Tocopherol rapidly reacts with and neutralizes lipid peroxyl radicals, inhibiting the propagation of lipid peroxidation reactions (9). This reaction has been well characterized in LDL, where the high concentration of PUFAs provide a highly oxidative environment as described in section 3 of this review. At the low radical/oxidant concentrations that may be present within plaques, α-tocopherol can also act as a pro-oxidant. The antioxidant activity of α-tocopherol is dependent on the tocopheroxyl radical formed in the neutralising of a lipid peroxyl radical, going on to react with a second lipid peroxyl radical to form an unreactive tocopherylquinone. At low radical flux (low oxidant concentrations), the tocopheryl radical has time to react with PUFAs to generate a new lipid peroxyl radical before reacting with another radical in the LDL particle, while regenerating the tocopherol (99). This tocopherol-mediated peroxidation (TMP) is self limiting as an increase in lipid peroxyl radicals causes the reaction between the tocopheryl-radical and lipid peroxyl radical to dominate so preventing the reaction between the tocopheryl-radical and the PUFAs (100). A further limiter to this TMP mechanism is the very rapid reaction between ascorbate and the α-tocopheryl radicals discussed above with the resultant regenerated tocopherol. This TMP mechanism has been used by some to argue that tocopherol has little effect in inhibiting lipid oxidation, but this argument ignores the fact that in the absence of tocopherol, a rapid, lipid peroxyl-mediated chain reaction will occur which is faster than the TMP mechanism.

These reactions all appear to occur to a varying extent within the plasma membranes of cells and depend on the amount of phospholipid PUFA present and the concentration of ascorbate. Resistance of red blood cell membranes to peroxyl radicals is partially dependent on tocopherol (101) and the recycling of tocopherol by ascorbate (102). As discussed in section 4, oxidation of plasma membrane lipids in U937 and THP-1 cells appears to be a minor event but is the predominant oxidative damage to HMDM cells grown in human serum (21). With HMDM cells, tocopherol may be an important part of the cell protection mechanism, though this is as yet unconfirmed (61, 103). α-Tocopherol supplementation does not alter the HMDM cells' susceptibility to oxLDL (104), suggesting that plasma membrane oxidation is not a significant factor in oxLDL toxicity. Though not strictly a defense mechanism, α-tocopherol supplementation of human monocytes does inhibit respiratory burst activity decreasing the level of superoxide and superoxide-derived oxidants within the plaque (105). The mechanism appears to be an impairment to the NADPH-oxidase assembly through inhibition of the action of protein kinase C which phosphorylates the cytosolic factor $p47^{phox}$ during translocation to the plasma membrane. How α-tocopherol causes these intracellular effects and whether they have anything to do with its antioxidant activity has yet to be determined. How this observation relates to the superoxide release observed with 7-ketocholesterol treated J774A.1 cells is uncertain (78).

Though tocopherol and ascorbate are important cellular defense molecules for the macrophage, their concentration is very much influenced by dietary intake. The pro-oxidant activities of these vitamins may also set an upper limit to the concentration the body will allow and therefore the maximum radical/oxidant flux or concentration they will be able to neutralise.

Glutathione (GSH) however is synthesized and regenerated intracellularly at the expense of NADPH (Figure 3). Intracellular protein thiols are in equilibrium with GSH, which is kept at millimolar concentrations in the cell. GSH is the key thiol controlling the redox potential and state of reduction within the cell. A number of the key enzymes in metabolism such as glyceraldehyde-3-

phosphate dehydrogenase (GAPDH), as well as regulatory enzymes like caspases, require key cysteine residues to be in a reduced free thiol state for activity (106, 107). GSH levels are partially dependent on the cell's ability to maintain a metabolism that generates enough NADPH to maintain a large pool of reduced GSH.

GSH reacts rapidly with a range of oxidants including hydroxyl and peroxyl radicals and of course HOCl, which is also highly reactive with any free thiols. GSH is also the cofactor for the enzyme GSH peroxidase responsible for breaking down intracellular hydrogen peroxide and lipid hydroperoxides. It is estimated that approximately 70% of the cells total antioxidant capacity is due to the presence of free thiols, whose reduction is partially dependent on the GSH concentration (108). Though this does sound impressive it must be remembered that the majority of researchers (including ourselves) do not control for ascorbate levels within cells grown in tissue culture so this figure may reflect protection in cell culture rather than *in vivo*. For example, ascorbate reduces the level of GSH loss and subsequent DNA base and protein damage in human arterial smooth muscle cells (98). However, considering the level of GSH found in a cell, and the interaction with ascorbate, it is still an essential part of the cellular defense mechanism.

GSH loss appears from many studies to be one of the first indicators of oxidative stress within the cell. Whether this means it is also the first line of defense or whether it is the most reactive and susceptible to oxidants is a matter of semantics. GSH regeneration by NADPH reduction from GSSG by the enzyme GSH reductase means the cell can withstand a certain level of oxidative stress. The overwhelming of this protection through complete oxidation of the GSH, or inhibition of GSH regeneration through oxidative damage to metabolic enzymes, results in further loss of cell function and death. GSH has been suggested as the main determinant of cell death (109). Protein oxidation and cell death in THP-1 cells treated with peroxyl radicals only becomes significant once GSH levels have almost been depleted (103). One of the earliest cellular events in oxLDL treated U937 and HMDM cells is a rapid loss in cellular GSH though only a small 20% loss is observed in THP-1 cells (62, 76). 7,8-Dihydroneopterin protection of the cells' GSH store seems to prevent cellular death in U937 cells exposed to peroxyl radicals or oxLDL (71, 110). Depletion of GSH from macrophages, does not appear to trigger apoptosis but does make the cells far more susceptible to oxidative damage and cell death through peroxyl radicals, nitric oxide, and oxLDL (76, 103, 109). Inhibition of the GSH synthesis enzyme increases the toxicity of oxLDL to THP-1 cells (111).

Considering the importance of GSH, it is not surprising to find that exposing THP-1 cells to oxLDL or HNE (a reactive aldehyde formed during LDL oxidation (112)) or human arterial smooth muscle cells to oxLDL (97) increases cellular GSH levels, so increasing potential antioxidant protection (96). Similarly increasing the GSH level by treatment with GSH-ethyl ester decreased mouse derived IC21 macrophage death from oxLDL induced apoptosis (113). We have not observed this GSH increase in HMDM cells with oxLDL treatment (unpublished results).

The regeneration of the GSH does appear to become rate limiting as increasing the expression of GSH reductase using a viral vector decreases the susceptibility to the effects of oxLDL. When applied to a whole mouse, the treatment showed a significant decrease in atherosclerotic plaque size (114). So in the case of the mouse model, oxidant induced cell death appears to be key driver in the growth of atherosclerotic plaques.

Like most thiols, GSH reacts rapidly with HOCl (79, 115). The loss of GSH in red blood cells occurs prior to cell lysis but surprisingly GSH loss and cell lysis do not appear to be linked. HOBr causes ten times more cell lysis than HOCl, but with the same level of GSH loss (116). However, in THP-1 and J774 cells, HOBr and HOCl are equally cytotoxic suggesting that the mechanism of action is very different between the cell types (117). GSH is rapidly lost from endothelium cells with HOCl exposure (118). Increasing endothelial cell GSH levels with GSH monoethyl ester decreased the damage caused by HOCl (119). Whether of GSH levels is the central determinate of macrophage cell survival has yet to be full determined.

The balance of these protection mechanisms within macrophages in a plaque, compared to those observed in the tissue culture dish, is difficult to determine. The regeneration of tocopherol through ascorbate, which is regenerated through reduction by GSH, which in turn is regenerated by NADPH dependent GSH-reductase activity, should be a very responsive mechanism to oxidative stress *in vivo* (Figure 3). The importance of cell metabolism in generating NADPH and cellular ascorbate levels requires further study to understand how and when this defense mechanism fails leading to macrophage cell death.

6. NEOPTERIN, 7,8-DIHYDRONEOPTERIN, AND 3HAA

Though α-tocopherol, ascorbate, and glutathione provide a considerable oxidant scavenging capacity to cells, macrophages appear to respond to inflammatory mediators by increasing their antioxidant capacity. γ-Interferon acts to prepare immune cells for inflammation. Macrophages respond to γ-interferon by various cellular changes such as increasing the NADPH-oxidase superoxide generating capacity (120, 121). Interferon also up regulates the synthesis of two different compounds; 7,8-dihydroneopterin and 3-hydroxyanthranilic acid (3HAA). Both these compounds have antioxidant activity *in vitro* suggesting a role in the protection of macrophages from oxidative stress generated during inflammation (122). 7,8-Dihydroneopterin is formed by the breakdown of GTP by GTP-cyclohydrolase (123, 124) while indoleamine 2,3-dioxygenase (IDO) catalyses the degradation of the amino acid tryptophan to a range of products including kynurenine and 3HAA (125, 126).

Both 7,8-dihydroneopterin and 3HAA have strong antioxidant properties suggesting they may function

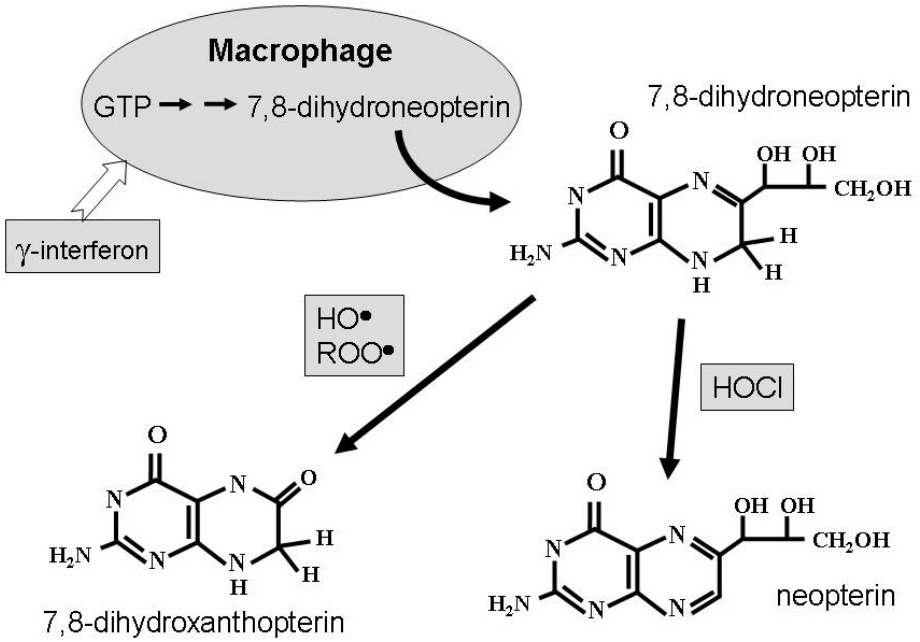

Figure 4. Generation and oxidation of 7,8-dihydroneopterin. γ-Interferon stimulation of macrophages causes the enzymatic breakdown of intracellular GTP to 7,8-dihydroneopterin which can either be oxidized to the high fluorescent neopterin by HOCl or to 7,8-dihydroxanthopterin by peroxyl and hydroxyl radicals.

within inflammatory sites to protect macrophages from the oxidants present. Surprisingly this response is only seen in primate (including human) macrophages as they lack sufficient expression of the enzyme 6-pyruvoyltetrahydropterin synthase. This enzyme is part of the pathway converting 7,8-dihydroneopterin-triphosphate to 5,6,7,8-tetrahydrobiopterin, the cofactor for iNOS. Thus primate macrophages do not release significant amounts of nitric oxide when stimulated with γ-interferon, but make 7,8-dihydroneopterin instead (38).

One of the oxidation products of 7,8-dihydroneopterin is the highly fluorescent compound neopterin. Plasma and urine neopterin levels have been used for a number of years as a marker of immune cell activation in a variety of diseases and conditions including allograft rejection (127), pathogen infection (128) and autoimmune disease (129). The rise in plasma neopterin with increasing severity of vascular disease clearly demonstrates the inflammatory nature of atherosclerosis (130).

Neopterin and 7,8-dihydroneopterin have both been shown to have some pro-apoptotic activity in some cells (131-133), but in macrophages, we and others found 7,8-dihydroneopterin to be a relatively good antioxidant and inhibitor of oxidant induced cell death when added at low micro-molar concentrations to the media. In solution, 7,8-dihydroneopterin rapidly scavenges peroxyl radicals (134) which is how it inhibits both copper and AAPH-peroxyl radical (2,2'-azobis (2-amidinopropane) dihydrochloride) mediated LDL oxidation. *In vivo* this reaction may be important in slowing the rate of LDL oxidation and therefore lowering the concentration of oxLDL within plaques. In protein solutions, 7,8-dihydroneopterin inhibits protein hydroperoxide formation by scavenging hydroxyl and aqueous peroxyl radicals (110). With cellular proteins the picture is somewhat more complicated. As described earlier, treatment of U937 and THP-1 cells with AAPH-derived peroxyl radicals causes the formation of protein hydroperoxides, GSH loss, and cellular necrosis (21, 103). Micro-molar concentrations of 7,8-dihydroneopterin protect U937 and THP-1 cells from this damage (71, 135), but not HMDM cells (61). The HMDM cells, take up a considerable amount of lipid when cultured in human serum, which appears to cause lipid oxidation to predominate over protein oxidation when driven by AAPH peroxyl radicals. The failure of 7,8-dihydroneopterin to inhibit this oxidative damage is somewhat perplexing. During LDL oxidation 7,8-dihydroneopterin effectively scavenges lipid peroxyl radicals. Iron readily catalyses the formation of lipid peroxides on U937 cells and this is also effectively inhibited by 7,8-dihydroneopterin (30). There is a specific cellular interaction occurring beyond simple radical scavenging. This difference is clearly highlighted by the fact that 7,8-dihydroneopterin inhibits oxLDL-induced loss of GSH and cell death in U937 (71) and HMDM cells (data not shown) but not THP-1 cells (71).

7,8-Dihydroneopterin also rapidly reacts with HOCl, and neutralizes this potent oxidant thus preventing cellular damage (30). Interestingly, the product of this reaction is neopterin while the product of peroxyl and hydroxyl radical scavenging by 7,8-dihydroneopterin is 7,8-dihydroxanthopterin (Figure 4) (30, 136, 137). The reaction with HOCl is at present the only known reaction occurring *in vivo* that could generate neopterin from 7,8-dihydroneopterin. This suggests that the majority of

plasma neopterin measured during inflammation (both acute and chronic), originates from the scavenging of HOCl.

Though the reactivity of 7,8-dihydroneopterin in tissue culture strongly supports an antioxidant/protective function during inflammation, the inability to get macrophages to generate 7,8-dihydroneopterin above nanomolar concentration is a concern. Studies of atherosclerotic plaque have shown total neopterin levels (7,8-dihydroneopterin + neopterin) to reach low micro-molar concentrations in advanced plaques suggesting there are additional factors other than γ-interferon controlling 7,8-dihydroneopterin synthesis and release (122).

Considerably less is known about the antioxidant activity of 3HAA. It is a potent inhibitor of macrophage mediated LDL oxidation so may also reduce oxLDL concentrations within plaques (138-140). 3-HAA has been reported to react with AAPH-peroxyl radicals inhibiting lipid oxidation in rat brain homogenates, as well as scavenging hydrogen peroxide and chloramines (141, 142). Whether 3HAA can protect macrophages from oxidants, including oxLDL, does not appear to have been investigated. Interestingly, nitric oxide can effectively inhibit IDO so reducing or preventing significant 3HAA synthesis (143). The switch from nitric oxide to 7,8-dihydroneopterin synthesis in primate macrophages may also be linked to 3HAA synthesis.

7. SUPEROXIDE SCAVENGING BY MnSOD

A glance at Figure 1 will show that superoxide generation is central to much of the oxidant generation within an inflammatory site. Though generated mainly in phagosomes, any superoxide that enters the cells cytosol should be removed by the large capacity of CuZn-superoxide dismutase (CuZnSOD). The resulting hydrogen peroxide is then either broken down by catalase or GSH peroxidase. It is therefore surprising that it is not CuZnSOD that is up regulated during inflammation, but the mitochondrial MnSOD. Tumor necrosis factor-α, interleukin-1 and in some cells lipopolysaccharide, trigger the increase in mRNA levels of MnSOD (144-146). OxLDL treatment of mouse J-774, human Mono-Mac-6, rabbit and human monocyte derived macrophages all cause an increase in the amount of MnSOD protein detected by immunoblotting (147, 148) while gene array analysis suggests that in atherosclerotic plaques and oxLDL treated THP-1 cells, CuZnSOD is down regulated (149-151). Hydrogen peroxide treatment also increases HMDM cell MnSOD expression (152). Within plaques, regions of elevated cell death markers also show increased MnSOD protein (147, 149). From this observation it was suggested that the oxidative stress which eventually caused the cell death, was the initial signal causing the cells to increase MnSOD expression. As discussed earlier, oxLDL causes an intracellular oxidant flux, either from NADPH-oxidase (150) or lipoxygenase activity (153) or the mitochondria (75). The observed MnSOD increase could be a protective effect from this radical flux or part of the apoptosis' mechanism acting to localize the hydrogen peroxide formation to the mitochondria, without peroxynitrite formation. To further confuse this picture, oxLDL induced apoptosis in HMDM cells has also been reported to be dependent on MnSOD expression, as oxLDL induced apoptosis was inhibited by antisense oligonucleotides to MnSOD (152). In this model the conversion of superoxide to hydrogen peroxide appears to be a necessary part of the apoptosis mechanism. Whether this hydrogen peroxide is part of a intracellular signal as the researchers argue or whether it is to remove potential reactions of superoxide is uncertain.

In complete conflict with this result the increase in MnSOD caused by sublethal exposure to oxLDL is associated with inhibition of cell death at higher oxLDL concentrations in human Mono-Mac cells (150). Whether this actually occurs within plaques is uncertain as we have not observed this protective response with HMDM cells (unpublished results).

8. CONCLUSION

In this review we have summarized some of the complex and interlinked antioxidant defense mechanisms potentially used by macrophages within the chronic inflammatory site which is the atherosclerotic plaque. A number of these antioxidants are up regulated by cytokines and appear to allow macrophages to function in the presence of inflammation-generated oxidants. Atherosclerotic plaques contain a large range of reactive oxidants, many of which are generated by the macrophages themselves, either directly (i.e. superoxide) or indirectly (i.e. oxLDL). The macrophage's antioxidant system appears to be capable of neutralising this oxidative stress, yet in advanced plaques macrophage cell death is a prominent feature. Why this defense mechanism fails is uncertain. It may simply be overwhelmed by the increasing concentration of reactive material such as oxLDL, or the lack of nutrients (oxygen, glucose) to sustain cells deep within the plaque. There is a large gulf between what is seen in tissue culture and observed within atherosclerotic plaques. Though oxLDL is found within plaques it is uncertain whether it is the cause of cell death within the plaque. Markers of HOCl are also found within the plaque along with a range of reactive protein and lipid oxidation products. It is most likely that all these agents contribute to the oxidative stress within the cells. The question is: when are they produced and are they generated at sufficient quantity to cause the hypothesized reactions?

How macrophages mitigate this oxidative stress does appear to depend on their ability to synthesize and up regulate antioxidants. γ-Interferon dependent synthesis of 7,8-dihydroneopterin appears to be a potent protective system, yet in tissue culture only nanomolar concentrations can be generated while micromolar concentrations are observed within plaques. This suggests that the 7,8-dihydroneopterin produced by cells in culture is diluted by the large volume of culture medium relative to the cells or that there are other factors controlling 7,8-dihydroneopterin production within the macrophage. Whether the large levels of neopterin within plaques are due to HOCl scavenging also needs to be examined. The role of 3HAA

in this system is even more uncertain as it has only been shown to inhibit cell-and metal ion-mediated oxLDL formation. Why the neopterin/7,8-dihydroneopterin system even exists is a mystery. The possible advantage of primate macrophages changing from nitric oxide production to 7,8-dihydroneopterin release and prevention of nitric oxide IDO inhibition requires further study, especially on how these two γ-interferon controlled pathways function. The role of ascorbate also needs to be addressed. Though it appears not to protect macrophages from oxLDL it may be important to the control of a range of repair and inhibitory mechanisms, many of which are outside the scope of this review.

The continued functioning of macrophage cells within the chronic inflammatory site of the atherosclerotic plaque depends on the balance between oxidants and antioxidants. Understanding this balance and how it fails within atherosclerotic plaques may provide valuable insights for future therapeutic measures.

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10. REFERENCES

1. P. Libby, P. M. Ridker and A. Maseri: Inflammation and atherosclerosis. *Circulation* 105, 1135-1143 (2002)

2. V. C. Reid and M. J. Mitchinson: Toxicity of oxidised low density lipoprotein towards mouse peritoneal macrophages *in vitro*. *Atherosclerosis* 98, 17-24 (1993)

3. D. Steinberg, S. Parthasarathy, T. E. Carew, J. C. Khoo and J. L. Witztum: Beyond Cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 320, 915-924 (1989)

4. The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group: The effect of Vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 330, 1029-1035 (1994)

5. Collaborative GRP Primary Prevention: Low-dose aspirin and Vitamin E in people at cardiovascular risk: A randomised trial in general practice. *Lancet* 357, 89-95 (2001)

6. R. Collins, J. Armitage, S. Parish, P. Sleight and R. Peto: MRC/BHF Heart protection study of antioxidant vitamin supplementation in 20536 high-risk individuals: A randomised placebo-controlled trial. *Lancet* 360, 23-33 (2002)

7. D. Magliano, J. Mcneil, P. Branley, L. Shiel, L. Demos, R. Wolfe, D. Kotsopoulos and B. Mcgrath: The Melbourne atherosclerosis vitamin E trial (Mavet): A study of high dose vitamin E in smokers. *Eur J Cardio Prev Rehab* 13, 341-347 (2006)

8. H. Esterbauer, G. Jurgens, O. Quehenberger and E. Koller: Autoxidation of human low density lipoprotein:Loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. *J Lipid Res* 28, 495-509 (1987)

9. H. Esterbauer, J. Gebicki, H. Puhl and Jurgens G.: The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Rad. Biol. & Med.* 13, 341-389 (1992)

10. S. Fu, M. Davies, R. Stocker and R. T. Dean: Evidence for role of radicals in protein oxidation in advanced human atherosclerotic plaque. *Biochem J* 333, 519-525 (1998)

11. J. M. Upston, X. Niu, A. J. Brown, R. Mashima, H. Wang, R. Senthilmohan, A. J. Kettle, Dean R.T. and R. Stocker: Disease stage-dependent accumulation of lipid and protein oxidation products in human atherosclerosis. *Am J Pathol* 160, 710-720 (2002)

12. A. A. Woods, S. M. Linton and M. J. Davies: Detection of HOCl-mediated protein oxidation products in the extracellular matrix of human atherosclerotic plaques. *Biochem J* 370, 729-735 (2003)

13. J. A. Simpson, S. Narita, S. P. Gieseg, S. Gebicki, J. M. Gebicki and R. T. Dean: Long-lived reactive species on free-radical-damaged proteins. *Biochem J* 282, 621-624 (1992)

14. S. P. Gieseg, J. A. Simpson, T. S. Charlton, M. W. Duncan and R. T. Dean: Protein bound 3,4-dihydroxyphenylalanine is a major reductant formed during hydroxyl radical damage to proteins. *Biochemistry* 32, 4780-4786 (1993)

15. W. H. F. Sutherland, S. P. Gieseg, R. J. Walker, S. A. De Jong, C. A. Firth and N. Scott: Serum protein-bound 3,4-dihydroxyphenylalanine and related products of protein oxidation and chronic hemodialysis. *Ren Fail* 25, 997-1009 (2003)

16. S. P. Gieseg , J. Pearson and C. A. Firth: Protein hydroperoxides are a major product of low density lipoprotein oxidation during copper, peroxyl radical and macrophage-mediated oxidation. *Free Radic Res* 37, 983-991 (2003)

17. B. Morin, M. J. Davies and R. T. Dean: The protein oxidation product 3,4-dihydroxyphenylalanine (DOPA) mediates oxidative DNA damage. *Biochem J* 330, 1059-1067 (1998)

18. D. I. Pattison, R. T. Dean and M. J. Davies: Oxidation of DNA, proteins and lipids by DOPA, protein-bound DOPA, and related catechol (smine)s. *Toxicology* 177, 23-37 (2002)

19. S. Gebicki and J. M. Gebicki: Formation of peroxides in amino acids and proteins exposed to oxygen free

radicals. *Biochem J* 289, 743-749 (1993)

20. J. M. Gebicki: Protein hydroperoxides as new reactive oxygen species. *Redox Report* 3, 99-110 (1997)

21. S. P. Gieseg, Duggan S. and J. M. Gebicki: Peroxidation of proteins before lipids in U937 cells exposed to peroxyl radicals. *Biochem J* 350, 215-218 (2000)

22. J. M. Gebicki, J. Collins, C. Gay, S. Duggan and S. P. Gieseg: The dissection of oxidative changes in human blood serum and U937 cells exposed to free radicals. *Redox Report* 5, 55-56 (2000)

23. M. B. Hampton, P. E. Morgan and M. J. Davies: Inactivation of cellular caspases by peptide-derived tryptophan and tyrosine peroxides. *FEBS Lett* 527, 289-292 (2002)

24. S. Gebicki and J. M. Gebicki: Crosslinking of DNA and proteins induced by protein hydroperoxides. *Biochem J* 338, 629-636 (1999)

25. M. J. Davies, S. Fu and R. T. Dean: Protein hydroperoxides can give rise to reactive free radicals. *Biochem. J.* 305, 643-649 (1995)

26. C. Luxford, B. Morin, R. T. Dean and M. J. Davies: Histone H1- and other protein- and amino acid-hydroperoxides can give rise to free radicals which oxidize DNA. *Biochem J* 344, 125-34 (1999)

27. C. Smith, M. J. Mitchinson, O. I. Aruoma and B. Halliwell: Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions. *Biochem. J.* 286, 901-905 (1992)

28. N. Stadler, R. A. Lindner and M. J. Davies: Direct detection and quantification of transition metal ions in human atherosclerotic plaques: Evidence for the presence of elevated levels of iron and copper. *Arterioscler Thromb Vasc Biol* 24, 949-954 (2004)

29. B. Fuhrman, J. Oiknine and M. Aviram: Iron induces lipid peroxidation in cultured macrophages, increases their ability to oxidatively modify LDL, and affects their secretory properties. *Atherosclerosis* 111, 65-78 (1994)

30. S. P. Gieseg, J. Whybrow, D. Glubb and C. Rait: Protection of U937 cells from free radical damage by the macrophage synthesized antioxidant 7,8 dihydroneopterin. *Free Radic Res* 35, 311-318 (2001)

31. A. Graham, J. L. Wood, V. J. O'leary and D. Stone: Human (THP-1) macrophages oxidise LDL by a thiol-dependent mechanism. *Free Radic Res* 21, 295-308 (1994)

32. A. Baoutina, R. T. Dean and W. Jessup: Trans-plasma membrane electron transport induces macrophage mediated low density lipoprotein oxidation. *FASEB J* 15, 1580-1582 (2001)

33. S. L. Hazen and J. W. Heinecke : 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *Journal Clinical Investigation* 99, 2075-2081 (1997)

34. J. W. Heinecke, W. Li, H. L. Daehnke III and J. A. Goldstein: Dityrosine, a specific marker of oxidation, is synthesized by the myeloperoxidase-hydrogen peroxide system of human neutrophils and macrophages. *Journal Biol. Chem.* 268, 4069-4077 (1993)

35. S. Sugiyama, Y. Okada, G. K. Sukhova, R. Virmani, J. W. Heinecke and P. Libby: Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in h uman atherosclerosis and implications in a cute coronary syndromes. *Am J Pathol* 158, 879-891 (2001)

36. C. C. Winterbourn, M. B. Hampton, J. H. Livesey and A. J. Kettle: Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome - Implications for microbial killing. *J Biol Chem* 281, 39860-39869 (2006)

37. B. Halliwell: Oxidation of low-density lipoproteins: Questions of initiation, propagation and the effect of antioxidants. *Am. J. Clin. Nutr.* 61, 670s-677s (1995)

38. H. Wachter, D. Fuchs, A. Hausen, G. Reibnegger and E. R. Werner: Neopterin as marker for activation of cellular immunity: immunologic basis and clinical application. *Adv Clin Chem* 27, 81-141 (1989)

39. M. Schneemann, G. Schoedon, S. Hofer, N. Blau, L. Guerrero and A. Schaffner: Nitric-oxide synthase is not a constituent of the antimicrobial armature of human mononuclear phagocytes. *J Infect Dis* 167, 1358-1363 (1993)

40. F. C. Fang and A. S. Vazquez-Torres: Nitric oxide production by human macrophages: There's no doubt about it. *Am J Physiol - Lung Cellular and Molecular Physiology* 282, L941-L943 (2002)

41. V. Borutaite and G. C. Brown: Nitric oxide induces apoptosis via hydrogen peroxide, but necrosis via energy and thiol depletion. *Free Rad Biol Med* 35, 1457-1468 (2003)

42. M. M. Zeigler, A. I. Doseff, M. F. Galloway, J. M. Opalek, P. T. Nowicki, J. L. Zweier, C. K. Sen and C. B. Marsh: Presentation of nitric oxide regulates monocyte survival through effects on caspase-9 and caspase-3 activation. *J Biol Chem* 278, 12894-12902 (2003)

43. P. A. Hyslop, D. B. Hinshaw, W. A. S. I. U. Halsey, R. D. Sauerheber, R. G. Spragg, J. H. Jackson and C. G. Cochrane: Mechanisms of oxidant-mediated cell injury. *J Biol Chem* 1665-1675 (1988)

44. Y. Tsuchiya, M. Yamaguchi, T. Chikuma and H. Hojo: Degradation of glyceraldehyde-3-phosphate dehydrogenase triggered by 4-hydroxy-2-nonenal and 4-hydroxy-2-

hexenal. *Arch Biochem Biophys* 438, 217-222 (2005)

45. Y. Sato, S. Kamo, T. Takahshi and Y. Suzuki: Mechanism of free radical induced hemolysis of human erythrocytes: Hemolysis by water soluble radical initiator. *Biochemistry* 34, 8940-8949 (1995)

46. D. M. C. Richards, R. T. Dean and W. Jessup: Membrane proteins are critical targets in free radical mediated cytolysis. *Biochim. Biophys. Acta* 946, 281-288 (1988)

47. J. Du and J. M. Gebicki: Proteins are major initial cell targets of hydroxyl free radicals. *Int. J. Biochem. Cell Biol.* 36, 2334-2343 (2004)

48. R. L. Levine: Oxidative modification of glutamine synthetase. I. Inactivation is due to loss of one histidine residue. *J Biol Chem* 258, 11823-11827 (1983)

49. P. E. Morgan, R. T. Dean and M. J. Davies: Inactivation of cellular enzymes by carbonyls and protein-bound glycation/glycoxidation products. *Arch Biochem Biophys* 403, 259-269 (2002)

50. C. N. Oliver: Inactivation of enzymes and oxidative modification of proteins by stimulated neutrophils. *Arch. Biochem. Biophys.* 253, 62-72 (1987)

51. B. S. Berlett, B. Friguet, M. B. Yim, Chock P.B. and E. R. Stadtman: Peroxynitrite-mediated nitration of tyrosine residues in Escherichia coli glutamine synthetase mimics adenylylation: Relevance to signal transduction. *Proc Natl Acad Sci USA* 93, 1776-1780 (1996)

52. S. K. Kong, M. B. Yim, E. R. Stadtman and P. B. Chock: Peroxynitrite disables the tyrosine phosphylation regulatory mechanism:Lymhocyte-specific tyrosine kinase fails to phosphorylate nitrated cdc2 (6-20)NH_2 peptide. *Proc Natl Acad Sci USA* 93, 3377-3382 (1996)

53. L. Margiloff , L. Chaplia, A. Chow, P. C. Singhal and J. Mattana: Metal-catalyzed oxidation of immunoglobulin G impairs Fc receptor-mediated binding to macrophages. *Free Rad Biol Med* 25, 780-785 (1998)

54. R. Thirumangalathu, S. Krishnan, P. Bondarenko, M. Speed-Ricci, T. W. Randolph, J. F. Carpenter and D. N. Brems: Oxidation of methionine residues in recombinant human interleukin-1 receptor antagonist: Implications of conformational stability on protein oxidation kinetics. *Biochemistry* 46, 6213-6224 (2007)

55. A. Zaidi and M. L. Michaelis: Effects of reactive oxygen species on brain synaptic plasma membrane Ca^{2+}-ATPase . *Free Radicals Biology and Medicine* 27, 810-821 (1999)

56. Y. N. Lee, H. Y. Lee, J. S. Kim, C. Park, Y. H. Choi, T. G. Lee, S. H. Ryu, J. Y. Kwak and Y. S. Bae: The novel phospholipase c activator, M-3m3fbs, induces nonocytic leukemia cell apoptosis. *Cancer Lett* 222, 227-235 (2005)

57. A. Berthier, S. Lemaire-Ewing, C. Prunet, S. Monier, A. Athias, G. Bessede, J. P. P. De Barros, A. Laubriet, P. Gambert, G. Lizard and D. Neel: Involvement of a calcium-dependent dephosphorylation of BAD associated with the localization of TRPC-1 within lipid rafts in 7-ketocholesterol-induced THP-1 cell apoptosis. *Cell Death Differ* 11, 897-905 (2004)

58. D. W. Voehringer and R. E. Meyn: Redox aspects of Bcl-2 function. *Antioxid Redox Signal.* 2, (2000)

59. R. J. Carmody and T. G. Cotter : Signalling apoptosis: a radical approach . *Redox Report* 6, 77-90 (2001)

60. J. S. Armstrong, K. K. Steinauer, B. Hornung, J. M. Irish, P. Lecane, G. W. Birrell, D. M. Peehl and S. J. Knox: Role of glutathione depletion and reactive oxygen species generation in apoptotic signaling in a human B lymphoma cell line. *Cell Death Differ* 9, 252-263 (2002)

61. C. A. Firth and S. P. Yang Y and Gieseg: Lipid oxidation predominates over protein hydroperoxide formation in human monocyte-derived macrophages exposed to aqueous peroxyl radicals. *Free Radic Res* 41, 839-848 (2007)

62. S. K. Baird, M. Hampton and S. P. Gieseg: Oxidised LDL triggers phosphatidylserine exposure in human monocyte cell lines by both caspase-dependent and independent mechanisms. *FEBS Lett* 578, 169-174 (2004)

63. E. S. Wintergerst, J. Jelk, C. Rahner and R. Asmis: Apoptosis induced by oxidized low density lipoprotein in human monocyte-derived macrophages involves CD36 and activation of caspase-3. *Eur J Biochem* 267, 6050-6058 (2000)

64. R. Asmis and J. G. Begley: Oxidized LDL promotes peroxide-mediated mitochondrial dysfunction and cell death in human macrophages - A caspase-3- independent pathway. *Circ Res* 92, E20-E29 (2003)

65. L. Kritharides, W. Jessup, J. Gifford and R. T. Dean: A method for defining the stages of low-density lipoprotein oxidation by the separation of cholesterol and cholesterol ester-oxidation products using HPLC. *Anal Biochem* 213, 79-89 (1993)

66. X. S. Wang, J. Greilberger, G. Ledinski, G. Kager and G. Jurgens: Binding and uptake of differently oxidized low density lipoprotein in mouse peritoneal macrophages and THP-1 macrophages: Involvement of negative charges as well as oxidation-specific epitopes. *J Cell Biochem* 81, 557-569 (2001)

67. H. S. Liao, T. Kodama and Y. J. Geng: Expression of class A scavenger receptor inhibits apoptosis of macrophages triggered by oxidized low density lipoprotein and oxysterol. *Arterioscler Thromb Vasc Biol* 8, 1968-1975 (2000)

68. B. Zhao, Y. F. Li, C. Buono, S. W. Waldo, N. L. Jones,

M. Mori and H. S. Kruth: Constitutive receptor-independent low density lipoprotein uptake and cholesterol accumulation by macrophages differentiated from human monocytes with macrophage-colony-stimulating factor (M-CSF). *J Biol Chem* 281, 15757-15762 (2006)

69. H. Maxeiner, J. Husemann, C. A. Thomas, J. D. Loike, J. El Khoury and S. C. Silverstein: Complementary roles for scavenger receptor a and CD36 of human monocyte-derived macro-phages in adhesion to surfaces coated with oxidized low-density lipoproteins and in secretion of H_2O_2. *J Exp Med* 188, 2257-2265 (1998)

70. C. Rice-Evans, D. Leake, K. R. Bruckdorfer and A. T. Diplock: Practical approaches to low density lipoprotein oxidation: Whys, wherefores and pitfalls. *Free Radic Res* 25, 285-311 (1996)

71. S. K. Baird, L. Reid, M. Hampton and S. P. Gieseg: OxLDL induced cell death is inhibited by the macrophage synthesised pterin, 7,8-dihydroneopterin, in U937 cells but not THP-1 cells. *Biochim Biophys Act* 1745, 361-369 (2005)

72. L. Kritharides, W. Jessup and D. Dean: Marcophages require both iron and copper to oxidise low density lipoprotein in Hanks' balnced salt solution . *Archives of Biochemistry* 323, 127-136 (1995)

73. D. H. Walter , J. Haendeler, J. Galle, A. M. Zeiher and S. Dimmeler: Cyclosporin a inhibits apoptosis of human endothelial cells by preventing release of cytochrome c from mitochondria. *Circulation* 98, 1153-1157 (1998)

74. G. Lizard, C. Miguet, G. Bessede, S. Monier, S. Gueldry, D. Neel and P. Gambert: Impairment with various antioxidants of the loss of mitochondrial transmembrane potential and of the cytosolic release of cytochrome c occuring during 7-ketocholesterol-induced apoptosis. *Free Rad Biol Med* 28, 743-753 (2000)

75. J. W. Zmijewski, D. R. Moellering, C. Le Goffe, A. Landar, A. Ramachandran and V. M. Darley-Usmar: Oxidized LDL induces mitochondrially associated reactive oxygen/nitrogen species formation in endothelial cells. *Am J Physiol-Heart and Circulatory Physiology* 289, H852-H861 (2005)

76. Y. M. Wang, M. Qiao, J. J. Mieyal, L. M. Asmis and R. Asmis: Molecular mechanism of glutathione-mediated protection from oxidized low-density lipoprotein-induced cell injury in human macrophages: Role of glutathione reductase and glutaredoxin. *Free Rad Biol Med* 41, 775-785 (2006)

77. S. M. Colles, J. M. Maxson, S. G. Carlson and G. M. Chisolm: Oxidized LDL-induced injury and apoptosis in atherosclerosis - Potential roles for oxysterols. *Trends Cardiovasc Med* 11, 131-138 (2001)

78. G. Leonarduzzi, B. Vizio, B. Sottero, V. Verde, P. Gamba, C. Mascia, E. Chiarpotto, G. Poli and F. Biasi: Early involvement of ROS overproduction in apoptosis i nduced by 7-ketocholesterol. *Antioxidants & Redox Signaling* 8, 375-380 (2006)

79. I. U. Schraufstatter, K. Browne, A. Harris, P. A. Hyslop, J. H. Jackson, O. Quehenberger and C. G. Cochrane: Mechanisms of hypochlorite injury of target cells. *J Clin Invest* 85, 554-562 (1990)

80. D. I. Pattison and M. J. Davies: Kinetic analysis of the role of histidine chloramines in hypochlorous acid mediated protein oxidation. *Biochemistry* 44, 7378-7387 (2005)

81. C. L. Hawkins and M. J. Davies: Inactivation of protease inhibitors and lysozyme by hypochlorous acid: Role of side-chain oxidation and protein unfolding in loss of biological function. *Chem Res Toxicol* 18, 1600-1610 (2005)

82. M. Whiteman, P. Rose, J. L. Siau, N. S. Cheung, G. S. Tan, B. Halliwell and J. S. Armstrong: Hypochlorous acid-mediated mitochondrial dysfunction and apoptosis in human hepatoma HEPG2 and human fetal liver cells: Role of mitochondrial permeability transition. *Free Rad Biol Med* 38, 1571-1584 (2005)

83. M. Levine, C. Conrycantilena, Y. H. Wang, R. W. Welch, P. W. Washko, K. R. Dhariwal, J. B. Park, A. Lazarev, J. F. Graumlich, J. King and L. R. Cantilena: Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci USA* 93, 3704-3709 (1996)

84. G. R. Buettner and B. A. Jurkiewicz, Chemistry and biochemistry of ascorbic acid, In "Handbook of Antioxidants" 91-115, editor Cadenas, E.a.P.L., New York.: Marcel Dekker, Inc., 1996

85. V. E. Kagan, E. A. Serbinova, T. Forte, G. Scita and L. Packer: Recycling of vitamin-E in human low-density lipoproteins. *J Lipid Res* 33, 385-397 (1992)

86. H. Esterbauer, G. Striegl, H. Puhl and M. Rotheneder: Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radic Res Commun* 6, 67-75 (1989)

87. K. Retsky, M. W. Freeman and B. Frei: Ascorbic acid oxidation product (s) protect human low density lipoprotein against atherogenic modificaton. *J. Biol. Chem.* 268, 1304-1309 (1993)

88. K. L. Retsky and B. Frei: Vitamin-C prevents metal ion-dependent initiation and propagation of lipid-peroxidation in h uman low-density-lipoprotein. *Biochimica Et Biophysica Acta-Lipids and Lipid Metabolism* 1257, 279-287 (1995)

89. S. E. Stait and D. S. Leake: Ascorbic acid can either increase or decrease low density lipoprotein modification. *FEBS Lett* 341, 263-267 (1994)

90. K. L. Retsky, K. Chen, J. Zeind and B. Frei: Inhibition of copper-induced LDL oxidation by vitamin C is associated with decreased copper-binding to LDL and 2-oxo-histidine formation. *Free Radic Biol Med* 26, 90-98 (1999)

91. S. E. Stait and D. S. Leake: The effects of ascorbate and dehydroascorbate on the oxidation of low-density lipoprotein. *Biochem J* 320, 373-381 (1996)

92. L. K. Harris, G. E. Mann, E. Ruiz, S. Mushtaq and D. S. Leake: Ascorbate does not protect macrophages against apoptosis induced by oxidised low density lipoprotein. *Arch Biochem Biophys* 455, 68-76 (2006)

93. J. M. May, J. J. Huang and Z. C. Qu: Macrophage uptake and recycling of ascorbic acid: Response to activation by lipopolysaccharide. *Free Rad Biol Med* 39, 1449-1459 (2005)

94. Siow R.C.M., J. P. Richards, K. C. Pedley, D. S. Leake and G. E. Mann: Vitamin C protects human vascular smooth muscle cells against apoptosis induced by moderately oxidized LDL containing high levels of lipid hydroperoxides. *Arterioscler Thromb Vasc Biol* 19, 2387-2394 (1999)

95. M. C. M. Vissers, W. G. Lee and M. B. Hampton: Regulation of apoptosis by vitamin C - Specific protection of the apoptotic machinery against exposure to chlorinated oxidants. *J Biol Chem* 276, 46835-46840 (2001)

96. V. M. Darley-Usmar, A. Severn, V. J. O'Leary and M. Rogers: Treatment of macrophages with oxidized low-density lipoprotein increases their intracellular glutathione content. *Biochem J* 278, 429-434 (1991)

97. R. C. M. Siow, H. Sato, D. S. Leake, J. D. Pearson, S. Bannai and G. E. Mann: Vitamin C protects human arterial smooth muscle cells against atherogenic lipoproteins - effects of antioxidant vitamins C and E on oxidized LDL-induced adaptive increases in cystine transport and glutathione. *Arterioscler Thromb Vasc Biol* 18, 1662-1670 (1998)

98. A. M. Jenner , J. E. Ruiz, C. Dunster, B. Halliwell, G. E. Mann and R. C. M. Siow: Vitamin C protects against hypochlorous acid-induced glutathione depletion and DNA base and protein damage in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 22, 574-580 (2002)

99. V. W. Bowry, K. U. Ingold and S. Stocker: Vitamin E in human low-density lipoprotein, When and how this antioxidant becomes a pro-oxidant. *Biochem J* 288, 341-344 (1992)

100. P. M. Abuja and H. Esterbauer : Simulation of lipid-peroxidation in low-density-lipoprotein by a basic skeleton of reactions. *Chem Res Toxicol* 8, 753-763 (1995)

101. B. Pekiner and J. F. Pennock: Oxidation of human red blood cells by a free radical initiator and effects of radical scavengers. *Biochemistry & Molecular Biology International* 33, 1159-1167 (1994)

102. J. M. May, Z. Qu and Mendiratta. S.: Protection and recycling of α-tocopherol in human erythrocytes by intracellular ascorbic acid. *Archieves of Biochemistry and Biophysics* 349, 281-289 (1998)

103. M. Kappler, Gerry A.J, E. Brown, L. Reid, D. S. Leake and S. P. Gieseg: Aqueous peroxyl radical exposure to THP-1 cells causes glutathione loss followed by protein oxidation and cell death without increased caspase-3 activity. *Biochemica Et Biophysica Acta* 1773, 945-953 (2007)

104. R. Asmis and J. Jelk: Vitamin E supplementation of human macrophages prevents neither foam cell formation nor increased susceptibility of foam cells to lysis by oxidized LDL. *Arterioscler Thromb Vasc Biol* 20, 2078-2086 (2000)

105. O. Cachia, J. El Benna, E. Pedruzzi, B. Descomps, M. A. Gougerot-Pocidalo and C. L. Leger: Alpha-Tocopherol inhibits the respiratory burst in human monocytes - Attenuation of P47 (Phox) membrane translocation and phosphorylation. *J Biol Chem* 273, 32801-32805 (1998)

106. J. W. Baty, M. B. Hampton and C. C. Winterbourn: Detection of oxidant sensitive thiol proteins by fluorescence labeling and two-dimensional electrophoresis. *Proteomics* 2, 1261-1266 (2002)

107. M. B. Hampton, I. Stamenkovic and C. C. Winterbourn: Interaction with substrate sensitises caspase-3 to inactivation by hydrogen peroxide. *FEBS Lett* 517, 229-232 (2002)

108. A. Balcerczyk and G. Bartosz: Thiols are main determinants of total antioxidant capacity of cellular homogenates. *Free Radic Res* 37, 537-541 (2003)

109. S. E. Boggs, T. S. Mccormick and E. G. Lapetina: Glutathione levels determine apoptosis in macrophages. *Biochem Biophys Res Commun* 247, 229-233 (1998)

110. S. Duggan, C. Rait, J. M. Gebicki and S. P. Gieseg: Inhibition of protein oxidation by the macrophage synthesised antioxidant 7,8-dihydroneopterin. *Redox Report* 6, 188-190 (2001)

111. N. Gotoh, A. Graham, E. Niki and V. M. Darley-Usmar: Inhibition of glutathione synthesis increases the toxicity of oxidized low-density lipoprotein to human monocytes and macrophages. *Biochem J* 296, 151-154 (1993)

112. G. Jurgens, J. Lang and H. Esterbauer: Modification of human low-density lipoprotein by the lipid peroxidation product 4-hydroxynonenal. *Biochim Biophys Acta* 875, 103-114 (1986)

113. R. S. Rosenson-Schloss, E. Chnari, T. A. Brieva, A.

Dang and P. V. Moghe: Glutathione preconditioning attenuates Ac-LDL-induced macrophage apoptosis via protein kinase c-dependent Ac-LDL trafficking. *Exp Biol Med* 230, 40-48 (2005)

114. M. Qiao, M. Kisgati, J. M. Cholewa, W. F. Zhu, E. J. Smart, M. S. Sulistio and R. Asmis: Increased expression of glutathione reductase in macrophages decreases atherosclerotic lesion formation in low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 27, 1375-1382 (2007)

115. A. V. Peskin and C. C. Winterbourn: Kinetics of the reactions of hypochlorous acid and amino acid chloramines with thiols, methionine, and ascorbate. *Free Rad Biol Med* 30, 572-579 (2001)

116. M. C. Vissers, A. C. Carr and A. L. P. Chapman: Comparison of human red cell lysis by hypochlorous and hypobromous acids: Insights into the mechanism of lysis. *Biochem J* 330, 131-138 (1998)

117. C. L. Hawkins, B. E. Brown and M. J. Davies: Hypochlorite- and hypobromite-mediated radical formation and its role in cell lysis. *Arch Biochem Biophys* 395, 137-145 (2001)

118. J. M. Pullar, M. C. M. Vissers and C. C. Winterbourn: Glutathione oxidation by hypochlorous acid in endothelial cells produces glutathione sulfonamide as a major product but not glutathione disulfide. *J Biol Chem* 276, 22120-22125 (2001)

119. S. Sugiyama, K. Kugiyama, M. Aikawa, S. Nakamura, H. Ogawa and P. Libby: Hypochlorous acid, a macrophage product, induces endothelial apoptosis and tissue factor expression - Involvement of myeloperoxidase-mediated oxidant in plaque erosion and thrombogenesis. *Arterioscler Thromb Vasc Biol* 24, 1309-1314 (2004)

120. M. A. Cassatella, V. Dellabianca, G. Berton and F. Rossi: Activation by gamma-interferon of human macrophage capability to produce toxic oxygen molecules is accompanied by decreased Km of the superoxide-generating NADPH oxidase. *Biochem Biophys Res Commun* 132, 908-914 (1985)

121. H. Kikuchi, T. Fujinawa, F. Kuribayashi, A. Nakanishi, S. Imajohohmi, M. Goto and S. Kanegasaki: Induction of essential components of the superoxide generating-system in human monoblastic leukemia U937 cells. *J Biochem (Tokyo)* 116, 742-746 (1994)

122. S. P. Gieseg, E. M. Crone, Flavall E.A. and Z. Amit: Potential to inhibit growth of atherosclerotic plaque development through modulation of macrophage neopterin/7,8-dihydroneopterin synthesis. *Br J Pharmacol* 153, 627-635 (2007)

123. M. M. Muller, H. Curtis, M. Herold and C. H. Huber: Neopterin in clinical practice. *Clin Chim Acta* 201, 1-16 (1991)

124. Wachter, H., Fuchs, D., Hausen, A., Reibnegger, G., Weiss, G., Werner, E.R. and Werener-Felmayer, G. (1992) Neopterin: Biochemistry Methods Clinical Applications, Walter de Gruyter, Berlin and New York.

125. E. R. Werner, G. Bitterlich, D. Fuchs, A. Hausen, G. Reibnegger, G. Szabo, M. P. Dierich and H. Wachter: Human macrophages degrade tryptophan upon induction by interferon gamma. *Life Science* 41, 273-280 (1987)

126. G. Werner-Felmayer, E. R. Werner, D. Fuchs, A. Hausen, G. Reibnegger and H. Wachter: Neopterin formation and tryptophan degradation by a human myelomonocytic cell line (THP-1) upon cytokine treatment. *Cancer Res* 50, 2863-2867 (1990)

127. R. Margreiter, D. Fuchs, A. Hausen, C. Huber, G. Reibnegger, M. Spielberger and H. Wachter: Neopterin as a new biomarker for the diagnosis of allograft rejection. Experience based upon evaluation of 100 consecutive cases. *Transplant* 36, 650-653 (1983)

128. I. Yuksekol , M. Ozkan, O. Akgul, E. Tozkoparan, M. Al-Rashed, A. Balkan, K. Hatipoglu, H. Bilgic, K. Erbil and N. Demirci: Urinary neopterin measurement as a non-invasive diagnostic method in pulmonary tuberculosis. *Int J Tuberc Lung Dis* 7, 771-776 (2003)

129. G. Reibnegger, D. Egg, D. Fuchs, R. Gunther, A. Hausen, E. R. Werner and H. Wachter: Urinary neopterin reflects clinical activity in patients with rheumtoid arthritis. *Arthritis Rheum* 29, 1063-1070 (1986)

130. M. Schumacher, B. Eder, F. Tatzber, P. Kaufmann, H. Esterbauer and W. Klein: Neopterin levels in patients with coronary artery disease. *Atherosclerosis* 94, 87-88 (1992)

131. B. Wirleitner, G. Obermoser, G. Bock, G. Neurauter, H. Schennach, N. Sepp and D. Fuchs: Induction of apoptosis in human blood T cells by 7,8-dihydroneopterin: The difference between healthy controls and patients with systemic lupus erythematosus. *Clin Immunol* 107, 152-159 (2003)

132. J. Rieder, P. Lirk and G. Hoffmann: Neopterin as a potential modulator of tumor cell growth and proliferation. *Med Hypotheses* 60, 531-534 (2003)

133. G. Hoffmann , S. Kenn, B. Wirleitner, C. Deetjen, S. Frede, M. Smolny, J. Rieder, D. Fuchs, G. Baier-Bitterlich and W. Schobergsberger: Neopterin induces nitric oxide-dependent apoptosis in rat vascular smooth muscle cells. *Immunobiology* 199, 63-73. (1998)

134. K. Oettl, S. Dikalov, H. J. Freisleben, W. Mlekusch and G. Reibnegger: Spin trapping study of antioxidant properties of neopterin and 7,8-dihydroneopterin. *Biochemical and Biophysical Research Commmunications* 234, 774-778 (1997)

135. S. P. Gieseg, S. Duggan, C. Rait and A. Platt: Protein and thiol oxidation in cells exposed to peroxyl radicals, is

inhibited by the macrophage synthesised pterin 7,8-dihydroneopterin. *Biochim. Biophys. Acta* 1591, 139-145 (2002)

136. S. P. Gieseg, Maghzal G. and D. Glubb: Inhibition of haemolysis by the macrophage synthesized antioxidant, 7,8-dihydroneopterin. *Redox Report* 5, 97-100 (2000)

137. B. Widner, C. Mayr, B. Wirleitner and D. Fuchs: Oxidation of 7,8-Dihydroneopterin by hypochlorous acid yields neopterin. *Biochem Biophys Res Commun* 275, 307-311 (2000)

138. S. Christen, E. Peterhans and R. Stocker: Antioxidant activities of some tryptophan metabolities:Possible implication for inflammatory diseases. *Proc Natl Acad Sci USA* 87, 2506-2510 (1990)

139. S. Christen, S. R. Thomas, B. Garner and R. Stocker: Inhibition by interferon-gamma of human mononuclear cell-mediated low density lipoprotein oxidation: Participation of tryptophan metabolism along the kynurenine pathway. *J Clin Invest* 93, 2149-2158 (1994)

140. S. R. Thomas, P. K. Witting and R. Stocker: 3-Hydroxyanthranilic acid is an efficient, cell-derived co-antioxidant for alpha-tocopherol, inhibiting human low density lipoprotein and plasma lipid peroxidation. *J Biol Chem* 271, 32714-32721 (1996)

141. G. Leipnitz , C. Schumacher, K. B. Dalcin, K. Scussiato, A. Solano, C. Funchal, C. S. Dutra-Filho, A. T. S. Wyse, C. M. D. Wannmacher, A. Latini and M. Wajner: *In vitro* evidence for an antioxidant role of 3-hydroxykynurenine and 3-hydroxyanthranilic acid in the brain. *Neurochem Int* 50, 83-94 (2007)

142. G. Weiss, A. Diez-Ruiz, C. Murr, I. Theurl and D. Fuchs: Tryptophan metabolites as scavengers of reactive oxygen and chlorine species. *Pteridines* 13, 140-144 (2002)

143. S. R. Thomas, D. Mohr and R. Stocker: Nitric-oxide inhibits indoleamine 2,3-dioxygenase activity in interferon-gamma primed mononuclear phagocytes. *J Biol Chem* 269, 14457-14464 (1994)

144. G. H. W. Wong and D. V. Goeddel: Induction of manganous superoxide-dismutase by tumor necrosis factor - Possible protective mechanism. *Science* 242, 941-944 (1988)

145. A. Y. Gracey, J. Logue, P. E. Tiku and A. R. Cossins, Adaptation of biological membranes to temperature: biophysical perspectives and molecular mechanisms, In "Animals and Temperature. Phenotypic and Evolutionary Adaptation" 1-21, Editors Johnston, I.A. and Bennett, A.F., Cambridge, England: Cambridge University Press, 1996

146. G. A. Visner, W. C. Dougall, J. M. Wilson, I. A. Burr and H. S. Nick: Regulation of manganese superoxide-dismutase by lipopolysaccharide, lnterleukin-1, and tumor necrosis factor - Role in the acute inflammatory response. *J Biol Chem* 265, 2856-2864 (1990)

147. D. Schlittenhardt, A. Schober, J. Strelau, G. A. Bonaterra, W. Schmiedt, K. Unsicker, J. Metz and R. Kinscherf: Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxldl-induced apoptosis of human macrophages *in vitro* and in arteriosclerotic lesions. *Cell Tissue Res* 318, 325-333 (2004)

148. X. M. Yuan, W. Li, U. T. Brunk, H. Dalen, Y. H. Chang and A. Sevanian: Lysosomal destabilization during macrophage damage induced by cholesterol oxidation products. *Free Rad Biol Med* 28, 208-218 (2000)

149. R. Kinscherf, H. P. Deigner, C. Usinger, J. Pill, M. Wagner, H. Kamencic, D. M. Hou, M. Chen, W. Schmiedt, M. Schrader, G. Kovacs, K. Kato and J. Metz: Induction of mitochondrial manganese superoxide dismutase in macrophages by oxidized LDL: its relevance in atherosclerosis of humans and heritable hyperlipidemic rabbits . *FASEB J* 11, 1317-1328 (1997)

150. V. A. Shatrov and B. Brune: Induced expression of manganese superoxide dismutase by non-toxic concentrations of oxidized low-density lipoprotein (oxLDL) protects against oxLDL-mediated cytotoxicity. *Biochem J* 374, 505-511 (2003)

151. P. Holvoet, P. C. Davey, D. De Keyzer, M. Doukoure, E. Deridder, M. L. Bochaton-Piallat, G. Gabbiani, E. Beaufort, K. Bishay, N. Andrieux, N. Benhabiles and G. Marguerie: Oxidized low-density lipoprotein correlates positively with toll-like receptor 2 and interferon regulatory factor-1 and inversely with superoxide dismutase-1 expression - Studies in h ypercholesterolemic swine and THP-1 cells. *Arterioscler Thromb Vasc Biol* 26, 1558-1565 (2006)

152. R. Kinscherf, R. Claus, M. Wagner, C. Gehrke, H. Kamencic, D. Hou, O. Nauen, W. Schmiedt, G. Kovacs, J. Pill, J. Metz and H. P. Deigner: Apoptosis caused by oxidized LDL is manganese superoxide dismutase and p53 dependent. *FASEB J* 12, 461-467 (1998)

153. Y. Higashi, T. Peng, J. Du, S. Sukhanov, Y. X. Li, H. Itabe, S. Parthasarathy and P. Delafontaine: A Redox-sensitive pathway mediates oxidized LDL-induced downregulation of insulin-like growth factor-1 receptor. *J Lipid Res* 46, 1266-1277 (2005)

154. S. P. Gieseg and H. Esterbauer: Low density lipoprotein is saturable by pro-oxidant copper. *FEBS Lett* 343, 188-194 (1994)

155. T. Mosmann: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immun. Meth.* 65, 55-63 (1983)

Abbreviations: 2,2'-azobis (2-amidinopropane) dihydrochloride AAPH; 3,4-dihydroxyphenylalanine, DOPA; glutathione, GSH; 3-hydroxyanthranilic acid, 3HAA; indoleamine

2,3-dioxygenase, IDO; low density lipoprotein, LDL; myeloperoxidase, MPO; oxidised low density lipoprotein, oxLDL; protein bound DOPA, PB-DOPA; superoxide dismutase, SOD; polyunsaturated fatty acid, PUFA

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