Barrera G;Pizzimenti S;Ciamporcero ES;Daga M;Ullio C;Arcaro A;Cetrangolo GP;Ferretti C;Dianzani C;Lepore A;Gentile F. Role of 4-Hydroxynonenal-Protein Adducts in Human Diseases.. ANTIOXIDANTS & REDOX SIGNALING. None pp: 1-21.
DOI: 10.1089/ars.2014.6166

FORUM REVIEW ARTICLE

Role of 4-hydroxynonenal-protein adducts in human diseases

Giuseppina Barrera[1], Stefania Pizzimenti[1], Eric Ciamporcero[1], Martina Daga[1], Chiara Ullio[1], Alessia Arcaro[2], Giovanni Paolo Cetrangolo[4], Carlo Ferretti[3] , Chiara Dianzani[3], Alessio Lepore[5], and Fabrizio Gentile[2].

1. Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, 10125 Torino, Italy.

2. Dipartimento di Medicina e Scienze della Salute, Università del Molise, 86010 Campobasso, Italy.

3. Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, 10125 Torino ,Italy

4. Istituto di Biochimica delle Proteine del Consiglio Nazionale delleRicerche, 80010 Napoli, Italy

4. Dipartimento di Medicina Molecolare e Biotecnologie Mediche –Università degli Studi di Napoli Federico II, 80010 Napoli, Italy.

Running title: HNE-protein adduct in diseases

Corresponding Author: Giuseppina Barrera; Departement of Clinical and Biological Sciences; Corso Raffaello 30, 10125 Torino (Italy).

Phone number: +39-011-6707795

Fax Number: +39-011-6707753

e-mail address: giuseppina.barrera@unito.it

Word count: **9647**

Reference numbers: **228**

Greyscale illustrations: **5**

Color illustrations : **2 (online only)**

Abstract

Significance. Oxidative stress provokes the peroxidation of polyunsaturated fatty acids in cellular membranes, leading to the formation of aldheydes that, due to their high chemical reactivity, are considered to act as second messengers of oxidative stress. Among the aldehydes formed during lipid peroxidation, 4-hydroxy-2-nonenal (HNE) is produced at a high level and easily reacts both with low-molecular-weight compounds and macromolecules, such as proteins and DNA. In particular, HNE-protein adducts have been extensively investigated in diseases characterized by the pathogenic contribution of oxidative stress, such as cancer, neurodegenerative, chronic inflammatory and autoimmune diseases.

Recent advances. In this review we describe and discuss recent insights concerning the role played by covalent adducts of HNE with proteins in the development and evolution of those, among the above mentioned disease conditions, in which the functional consequences of their formation have been characterized.

Critical Issue. Results obtained in recent years have shown that the generation of HNE-protein adducts can play important pathogenic roles in several diseases. However, in some cases, the generation of HNE-protein adducts can represent a contrast to the progression of disease or can promote adaptive cell responses, demonstrating that HNE is not only a toxic product of lipid peroxidation, but also a regulatory molecule, involved in several biochemical pathways.

Future directions. In the coming years, the refinement of proteomical techniques, allowing the individuation of novel cellular targets of HNE, will lead to a better understanding the role of HNE in human diseases.

Introduction

Oxidative stress produces reactive intermediates which, in turn, can cause the oxidation of polyunsaturated fatty acids in membrane lipid bilayers, leading eventually to the formation of aldehydes (57). This process can produce changes in the permeability and fluidity of the membrane lipid bilayer and can dramatically alter cell integrity (50). However, LPO can affect cell functions through its end-products endowed with biological activity. Among the products of LPO, 4-hydroxy-2-alkenals represent the most biologically active alkenals and aldehydes, that, due to their prolonged half-lives and their ability to diffuse from their sites of formation, have been considered as second messengers of oxidative stress (14). The peroxidation of n-3 polyunsaturated fatty acids (α-linolenic acid and docosahexaenoic acid) generates 4-hydroxy-hexenal (HHE), which is a mediator of the mitochondrial permeability transition (99), while the peroxidation of n-6 polyunsaturated fatty acids, such as linoleic acid and arachidonic acid, generates 4-hydroxy-2-nonenal (HNE), which is the most intensively studied aldehyde (15, 154), because it is highly electrophilic and easily reacts with low-molecular-weight compounds, such as glutathione (GSH), with proteins and, at higher concentrations, with DNA (57, 204). The mechanism of HNE formation during peroxidation of arachidonic acid is reported in Fig. 1 (159).

Once formed, HNE is able to affect several signalling processes, as well as gene expression pathways and protein functions. Most of these effects depend on the ability of HNE to bind covalently to functional proteins. Indeed, HNE is a γ-hydroxy-α,β-unsaturated electrophilic compound, which preferentially forms 1,4-Michael-type adducts with nucleophiles, such as proteins and DNA. 1,4-Michael addition to 4-HNE occurs readily via the reaction of a nucleophile with C3 of HNE, resulting in the addition of a nucleophile and proton across the HNE carbon–carbon double bond (C=C) (150) (Fig. 2). The addition product subsequently rearranges to a cyclic

hemiacetal (lactol) via the reaction of the 4-hydroxyl group with the aldehydic function. Amino acids known to react with HNE via 1,4-addition are Cys, His, and Lys (150). HNE can also react with lysyl residues through Schiff base formation, leading to pyrrole formation. In addition, HNE modification can result in cross-linking of two lysyl residues through reversibly formed Schiff base Michael adducts (134, 221) (Fig.2).

Due to the high chemical reactivity of aldehydes, mammals have evolved a full set of enzymes converting them to less reactive chemical species and contributing to the control of their steady-state intracellular concentrations, which reflect the equilibria between the rates of formation by LPO and of catabolism into less reactive compounds. The main catabolic reactions are the formation of adducts with GSH, which can occur spontaneously or can be catalysed by glutathione-S-transferases (GSTs), the reduction to alcohols by aldo-keto reductases (AKRs) or alcohol dehydrogenases and the oxidation to acids by aldehyde dehydrogenases (57, 118, 180).

The amphiphilic nature of HNE allows its diffusion across membranes and the covalent modification of cytoplasmic or nuclear compounds far from the site of its origin (135). Similarly, HNE formed outside the cells (i.e., in an inflammatory site or in the plasma), can react with stromal proteins or proteins belonging to adjacent cells, which do not undergo LPO. The targets for HNE are cell-type specific and dependent both on the pattern of proteins expressed by the cell and the aldehyde concentration. Moreover, the modification of specific proteins can have different biological consequences, in relation with the protein function.

In this review we consider some HNE-protein interactions which have been shown to be involved in the development and evolution of some pathological conditions, such as cancer, neurodegenerative, chronic inflammatory and autoimmune diseases.

HNE-protein adducts in cancer cells.

Increases of oxidative stress have been demonstrated in the majority of cancer types, while the concentration of LPO products can vary in relation with cell type. The first experiments in this field demonstrated that, in hepatoma cells, the level of LPO products was lower than in normal liver cells (72, 155) and depended on the degree of deviation from the normal phenotype (166). In accordance with these results, Canuto et al. (28) showed that, during rat liver carcinogenesis, the activities of the enzymes metabolizing the toxic aldehydes increased, thus rendering the cancer cells more protected against the cytotoxic effect of aldehydes. Moreover, in hepatoma cells, the majority of HNE was converted to the HNE-GSH conjugate, which was rapidly and efficiently exported from the cell (197). However, the analysis of HNE-protein adducts in different types of tumors by immunoblotting or immunohistochemistry revealed adducts of this kind in renal (138), and colon cancer cells (88), as well as in astrocytic and ependymal glial tumors, in which the incidence of HNE-immunopositive tumor cells increased with increasing grades of malignancy (89).

Oxidative stress and, consequently, the products of LPO were long considered merely involved in carcinogenesis, due to their reactivity with DNA, while other papers demonstrated that oxidative stress and LPO products, such as HNE, also play important roles in the induction of cell cycle arrest, differentiation and apoptosis in cancer cells (14). Similarly, the presence of HNE-guanosine adducts may not only indicate the mutagenicity of HNE but also its capacity to induce apoptosis in cancer cells. Indeed, the ability to alter DNA is a characteristic of many chemotherapeutic drugs which, through this mechanism, induce apoptosis in actively proliferating cancer cells. Moreover, the concentrations at which HNE can form DNA adducts are rather high and can be achieved only under highly pro-oxidant conditions (216).

In contrast, in several tumor types, the progression of malignancy is accompanied by reductions of oxidative stress, due to the upregulation of antioxidant capacity (199),

and the induction of the Nfr2/Keap1 pathway, which negatively regulates the HNE intracellular concentration (151). On the other hand, despite the reduction of intrinsic oxidative stress, the level of HNE-protein adducts in cancer cells may increase, due to the inflammatory response present in tissues surrounding cancer lesions.

In summary, the divergent results regarding the concentration of HNE in tumor tissues of different origins, and the discrepancies between the levels of oxidative stress and the levels of the products of LPO could have diverse causes , including: the pattern of HNE-metabolizing enzymes in tumor cells; the lipid composition of the cell membranes, with differing levels of peroxidation-susceptible substrates, such as polyunsaturated fatty acids (PUFAs); and the presence of inflammation, which might increase the level of diffusible HNE from neighboring tissues to the tumor cells.

Although the amount of HNE-protein adducts in cancer cells has been often assayed as a means of assessing the level of oxidative stress under diverse experimental conditions, only in some cases the identification and the consequences of HNE-protein adduct formation on cancer cell growth or behavior have been reported. Divergent results obtained in this field document that the formation of HNE adducts can have anti-carcinogenic or pro-carcinogenic effects, depending on the cell type and the specific adduct. In epidermoid carcinoma A431 cells, Liu et al. (119) observed that the signal triggered by the formation and activation of HNE-Epidermal Growth Factor Receptor (EGFR) adducts, detected by immunoblot analysis, followed by phosphorylation/activation of Shc adaptor proteins, ERK and JNK, inhibited DNA synthesis and suggested that this HNE-triggered signal transduction cascade selectively worked to suppress cell growth (119).

In a previous paper, we analyzed the interaction between HNE and α-enolase in HL-60 human leukemic cells (64), using a combination of two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE), immunoblotting and mass

spectrometry. In addition to its enzymatic and transcriptional roles, α-enolase, expressed on the surface of a variety of eukaryotic cells, functions as a strong plasminogen receptor (144)**.** Treatment with HNE strongly inhibited the binding between plasminogen and α-enolase at the surface of HL-60 cells, most probably as a consequence of the formation of HNE adducts with lysyl residues of α-enolase involved in plasminogen binding (7). HL-60 cells, as well as other leukemic cells, display enhanced plasminogen binding, which may contribute to an enhanced fibrinolytic state in leukemic patients (144). The inhibition of plasminogen binding was apparent even at HNE concentrations almost as low as those detected in normal tissues and plasma (1 μM). As a functional consequence, a strong reduction of HL-60 cell adhesion to HUVECs was produced, which might reduce the invasive and metastatic capacity of HL-60 cells (Fig. 3).

In MDA-MB-231 cells, a triple-negative human breast carcinoma cell line, the analysis of HNE-protein adduct formation revealed that HNE could modify, in a dose-dependent way, the enzyme peptidylprolyl cis/trans-isomerase A1, which catalyzes phosphoserine and phosphothreonine-proline conversions from *cis* to *trans* (4). HNE formed Michael adducts with this enzyme, which were detected by matrix-assisted laser desorption ionization / time-of-flight / time-of-flight (MALDI-TOF/TOF) mass spectrometry at the active site residues His157 and Cys113, Cys113 being the primary site of HNE modification. The molecules that covalently modify critical residues in Pin1 catalytic or binding sites have been shown to induce apoptosis and inhibit cell proliferation, possibly due to their inhibition of Pin1 actions on cell cycle. Thus, it was proposed that some antiproliferative effects observed in cancer cells after exposure to HNE might also depend on this enzymatic pathway.

In contrast, in another line of breast cancer cells, MCF-7 cells, and in RKO colon cancer cells, it has been demonstrated that HNE inhibited the AMP-kinase kinase activity of cellular LKB1, a serine/threonine kinase tumor suppressor, which

modulates anabolic and catabolic homeostasis, cell proliferation and organ polarity (209). The authors reasoned that LKB1 would be covalently modified and inactivated by HNE, which may entail increased risks of hypertrophic or neoplastic diseases.

Another HNE effect detected in cancer cells points to an interaction between HNE and Peroxisome Proliferator Activated Receptors (PPARs). PPARs are a superfamily of nuclear receptors, subdivided into three subtypes (α, β/δ and γ), differing for tissue-specific expression, preferential ligand recognition and biological function (95, 207). In HL-60 cells and in U937 leukemic cells, HNE potentiated the effects of PPARγ ligands, suggesting the existence of mutual interactions between HNE- and PPAR-ligand-related pathways in leukemic cell growth and differentiation (153). In addition, it has been reported that HNE directly binds and activates PPAR β/δ which, in the liver, exerts a protective action towards chemically-induced hepatotoxicity (40). This suggests that HNE, as an endogenous modulator of PPARβ/δ activity, might be involved in the protection from liver disease associated with oxidative damage. In this context, it is of interest that HNE stimulated Glutamate Cysteine Ligase (GCL) activity, through post-transcriptional modification of Cys553 in GCL and Cys35 in the modulatory subunit of GCL (GCLM) *in vitro*, detected by MALDI-TOF/TOF mass spectrometry. Since GCL catalyzes the first and rate-limiting step in GSH biosynthesis, these results suggest that the stimulation of GCL activity by HNE may concur to a compensatory cytoprotective response, through an increase of intracellular GSH and GSH-dependent detoxifying potential, during periods of oxidative stress (12**)**. The activation of PPAR β/δ by HNE may have anti-carcinogenic effects in breast cancer too (222). Indeed, Yao et al. recently demonstrated that ligand activation of PPAR β/δ in two human breast cancer cell lines inhibited relative breast cancer tumorigenicity and further advanced the development of ligands of PPAR β/δ able to inhibit specifically breast carcinogenesis (222).

HNE-protein adducts in neurodegenerative diseases.

HNE-protein adducts have been detected in brain tissues and body fluids in several neurodegenerative diseases, such as Alzheimer's Disease (AD), Huntington's Disease (HD), Parkinson's Disease (PD), Amyotrophic Lateral Sclerosis (ALS), and Down Syndrome (DS) (26, 27, 110, 182, 223). Indeed, the brain is one of the major targets of LPO, since it is highly sensitive to oxidative stress (it consumes about 20–30% of inspired oxygen) and contains high levels of PUFAs.

Among neurodegenerative diseases, the formation of HNE-protein adducts in AD has been extensively documented and a number of comprehensive reviews, describing the proteins involved, have been written by our, as well as other research groups (152, 191). The majority of studies in this field adopted proteomic approaches based on the immunochemical detection of HNE-protein adducts with anti-HNE antibodies among cellular proteins separated by 2D-PAGE, followed by Western blotting and identification of immunoreactive spots by mass spectrometry. As seen above, while discussing the studies of HNE-protein adducts in cancer cells, very rare studies proceeded to the non-trivial task of actually demonstrating the oxidative modifications of specific proteins by mass spectrometry. These include the adducts of HNE with regulator of G-protein signaling 4 in PD described below (131). Instead, the pinponting analyses of ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), Cu,Zn-superoxide dismutase (SOD1) and DJ-1 protein in AD and PD conducted by Choi et al. concerned the oxidation of cysteinyl and methionyl residues (35, 36, 37).

Here, we briefly summarize the most important findings and the most recent insights in this field. Cerebral alterations in AD include synaptic loss (176), neurofibrillary tangles (NFTs), and amyloid plaques, whose main protein component is the amyloid β (Aβ) peptide, a molecule of 40-42 amino acids, derived from the proteolytic cleavage of integral membrane Amyloid Precursor Protein (APP), by the action of beta- and gamma-secretases (73). It has been demonstrated that Amyloid β (Aβ) can induce oxidative stress and initiate LPO (27), resulting in the formation of

LPO products, including HNE, malondialdehyde (MDA), and others. HNE, in turn, can directly react with the Aβ peptide, through a covalent cross-linking of Aβ peptides, causing an acceleration in Aβ protofibril formations and an inhibition of the production of straight, mature fibrils (184). Another important target for HNE adduction in AD brain tissue is the Heme Oxygenase Protein-1 (HO-1) (191). The activation of this enzyme is one of the earliest events in AD and plays an important role in the response to oxidative stress (158). HO-1 catalyzes the degradation of heme in a multistep, energy-dependent process and represents the rate-limiting enzyme in bilirubin production (123). Its expression is controlled by the Nrf2 transcription factor, since the HO-1 gene contains in its promoter region the Antioxidant Responsive element (ARE) (63). In AD brains, the increase of oxidative stress leads to increases of Nrf2 activity and, consequently, increases of HO-1 protein levels. At the same time, oxidative stress induces LPO and HNE formation. The increases of HNE and HO-1 lead to increased formation of HNE adducts of HO-1. While, on one hand, HNE adduct formation could impair HO-1 function, the loss of HO-1 function was accompanied, on the other hand, by increased phosphorylation of seryl residues of HO-1, leading to HO-1 functional activation. Moreover, the loss of HO-1 function can increase oxidative stress (Fig. 4) (13).

Adducts of HNE with α-enolase have been reported, besides in the HL-60 human leukemic cell line (see the discussion of HNE-protein adducts in cancer cells, above), also in the brain tissue of AD patients, where their level correlated with the reduced glucose metabolism and the upregulation of glycolytic enzymes, necessary for counteracting the mounting energy deficit and hypoxic environment (128). In AD brain tissues, the α-enolase level increased to support the increase of glycolytic activity (190). The oxidative modifications of α-enolase lead to a disruption of neuronal energy metabolism and ATP-dependent ion homeostasis. Conceivably, these alterations might compromise the viability of neurons, rendering them more prone to cytotoxicity and apoptosis (191). Reduced glucose utilization and energy

production in AD may also be related with the formation of HNE adducts with the neuronal glucose transporter GLUT3 (125) and with the mitochondrial ATP synthase α subunit (149). The latter observation is in agreement with previous results demonstrating a decrease of ATP synthase activity in AD brains (175). In view of the possibly important role of α-enolase as plasminogen receptor at the surface of neurons, Sultana and coworkers suggested that the formation of HNE adducts with α-enolase might inhibit the conversion of plasminogen to plasmin, involved in the degradation of oligomeric and fibrillar Aβ, thereby preventing the detoxication of Aβ and facilitating neuronal death (191).

Another HNE target in AD brain tissue is represented by Collapsin Response Mediator Protein 2 (CRMP2) (149). This protein plays an important role in membrane trafficking, cytoskeletal organization, axonogenesis and neurite outgrowth, and neuronal polarity (161, 162). The formation of adducts of HNE with CRMP2 impairs its activity and might be of pathogenic importance for neurite shortening and the loss of synapses, which are early features of AD (75, 175).

In AD brain, Owen et al. have detected HNE adducts with Low Density Lipoprotein (LDL) receptor-related protein 1 (LRP-1), a membrane receptor involved in Aβ peptide removal. The formation of HNE adducts might lead to protein impairment which might contribute, in turn, to the extracellular deposition of amyloid substance (141). Moreover, Perluigi et al. demonstrated that SOD1 is HNE-modified in the inferior parietal lobule of late-stage AD, which results in the formation of protease-resistant protein aggregates, which are considered to be highly toxic and can mediate cell death (149). The multiple HNE-protein adducts found in AD point out the relevance of protein modification by HNE in AD initiation and progression.

Parkinson's disease (PD) is the most common neurodegenerative motion disorder. Hallmarks of PD are the loss of dopaminergic neurons in the *substantia nigra* and the presence of cytoplasmic spherical protein inclusions, named Lewy bodies. These inclusions contain various proteins, including α-synuclein (172).

Immunoistochemically detectable HNE-protein adducts were significantly increased in nigral neurons of patients with PD (223) and stimulated the aggregation of α-synuclein *in vitro* (160). Oxidative modification of α-synuclein and adducts of LPO products with this protein have been found in the dopaminergic neurons of the substantia nigra from PD patients (181). Qin and coworkers (160) demonstrated that incubation of HNE with α-synuclein resulted in the covalent modification of the protein, with up to six HNE molecules per protein molecule incorporated as Michael addition products. The formation of these adducts prevented fibrillation but might result in the formation of toxic oligomers, which might contribute to the demise of neurons subjected to oxidative damage.

The HNE involvement in the pathogenesis of PD has been supported by other observations indicating a pleiotropic role for HNE-protein adducts. HNE-modified glycolytic enzymes (aldolase A, α-enolase, and glyceraldehyde-3-phosphate-dehydrogenase-GAPDH) have been found by a proteomic approach in the frontal cortex of incidental PD, and dementia with Lewy bodies (67), and this has been suggested to be related with the decreases of enzyme activity and the impairment of glucose metabolism and neurological function in the frontal lobe of PD patients. Moreover, HNE protein adduction can affect G-protein-dependent signaling in PD, whose regulation has been implicated as an important pathogenic factor in PD, as well as in other neurodegenerative diseases. HNE was able to impair this signaling pathway by directly modifying $G\alpha_{q/11}$, a subunit of the heterotrimeric G-Protein Coupled Receptor (GPCR), as shown by immunoprecipitation and Western blotting (17). HNE could exert similar effects also by modifying and inactivating the regulator of G-protein signaling 4 (RGS4), which increases the GTPase activity of the Gα subunit, as recently demonstrated in a study by Monroy et al., in which the identification of HNE-RGS4 adducts by immunoprecipitation, Western blot and mass spectrometry was followed by a more refined mass spectrometric analysis, which permitted to detect HNE-modified Cys71, Cys148 and Cys183 (131).

ALS is a motor neuron degenerative disease which occurs both sporadically (sALS) and as a familial disorder (fALS). Although multiple mechanisms likely contribute to the pathogenesis of motor neuron injury in ALS, it has been suggested that oxidative stress may play a significant role in the pathogenesis and amplification of the disease. The levels of HNE and immunochemically detectable HNE-modified proteins were increased in spinal cord motor neurons of ALS patients, indicating that these modifications were associated with motor neuron degeneration in ALS (146). Using proteomic analysis, Perluigi and coworkers (148) detected three proteins significantly modified by HNE in the spinal cord of an animal model of fALS, the G93A-SOD1 transgenic mice: 1) dihydropyrimidinase-Related Protein 2 (DRP-2); 2) Heat-shock protein 70 (Hsp70); and 3) α-enolase. It was suggested that oxidative stress is a major contributing mechanism in the pathogenesis of ALS and that the structural alterations and the losses of functional activity of proteins can contribute to the neurodegenerative process (147).

High levels of oxidized proteins have been found in both Huntington's disease (HD) (reviewed in ref. 24) and Down's syndrome (DS) (48). HD is a dominantly inherited neurodegenerative disorder, caused by the expansion of a CAG repeat in the gene encoding the protein huntingtin (70). It has been suggested that functional defects of mitochondria, which are both important sources of Reactive Oxygen Species (ROS) and targets of ROS-mediated damage, are involved in HD pathogenesis (87). The increase of ROS and the oxidative damage of functional proteins have been associated with pathological neuronal loss in HD. Moreover, a marked increase of HNE adducts has been found by immunohistochemistry in the *nucleus caudatus* and *putamen* of HD brains and in the *corpus striatum* of HD mice, which suggested the therapeutic use of antioxidants to inhibit LPO and protect neurons from oxidative stress-induced cell death, by improving ATP generation and mitochondrial morphology and function (110).

DS is one of the most frequent chromosomal aberrations, resulting from the partial or complete triplication of chromosome 21, characterized by several abnormalities, including premature development of AD neuropathology and by increased oxidative stress, conceivably involved in neurodegeneration (147). Quite recently, Di Domenico *et al.*, by using a redox proteomic approach, have identified various protein targets of HNE in the frontal cortex from DS cases, with and without AD pathology (48). The HNE-modified protein targets identified embraced proteins involved in several biological functions, such as neuronal integrity, axonal transport, cytoskeleton organization, degradative systems, energy metabolism and antioxidant response. The dysfunction determined by the formation of HNE adducts with these proteins might contribute to the progression from DS to AD. Similar repertoires of aldehyde-modified protein targets had been reported in relation with the other neurodegenerative diseases as well (reviewed in ref. 126, 164, 191).

In recent years, the role of autophagy has emerged as an essential antioxidant pathway in neurodegenerative diseases because, by permitting the removal of damaged mitochondria and proteins, it can provide an effective antioxidant strategy, independent of the initiating mechanism (66). It has been proved that the accumulation of toxic oxidation products, such as HNE, is a prevalent feature of neurodegenerative diseases and can promote organelle and protein damage, leading to the induction of autophagy (51). Stimulation of autophagy by HNE has been demonstrated also in rat aortic smooth muscle cells (77). The data obtained in these model cells suggested that the autophagic response to HNE could be attributed, in part, to ER stress, being a component of the cell survival strategy in response to oxidative stress (71). HNE emerges from the sum of the data reported as an important contributor to the pathogenesis of neurodegeneration, whose build-up in the course of disease modifies functionally important proteins, while promoting the autophagic process as a survival-oriented defense mechanism.

HNE-protein adducts in chronic inflammatory diseases

One of the first demonstrations that HNE plays a role in the inflammatory process came by the studies on the effects of HNE on chemotactic oriented migration of neutrophils. When measured in a Boyden chamber, the latter was stimulated by HNE, even at concentrations of 0.1 µM or less (44). In the following years, it became evident that HNE is one of the major biologically active aldehydes produced by membrane LPO, in the course of inflammation and oxidative stress, which can accumulate in certain tissues up to concentrations of 10 µM or more (49, 204). Experimental ischemia or ischemia/reperfusion was shown to induce early generation of HNE and HNE-dependent protein modifications in the lung (43) or in the isolated rat heart (54). High doses of HNE (50 µM) infused into rat lungs caused perivascular edema with vascular compression and early endothelial cells disruption (76). Moreover, in lung inflammatory disorders, HNE induced lung injury and apoptosis (43).

The hyperproduction of HNE in the adipose tissue of obese patients was shown to contribute also to adipose tissue inflammation, by promoting the release of pro-inflammatory cytokines (reviewed in ref. 39). In C57BL/6 mice fed a high-fat diet, body weight gain and epididymal fat expansion were associated with increases of 4-HNE-protein adducts in adipose tissue detected by Western blotting (211). Excess generation of HNE, acting both as a covalent modifier of cell proteins involved in signal transduction, cytoskeletal organization or cell adhesion, and as a cell signal messenger, has been strongly implicated also in endothelial barrier dysfunction and atherosclerosis (112, 205). Evidence for the involvement of LPO-derived aldehydes in the alteration of LDL-receptor binding and in the promotion of atheroma formation came from several immunohistochemical analyses of atherosclerotic lesions from human aorta, using antibodies against such adducts as HNE-histidine (201), N^{ε}-MDA-lysine (203), and N^{ε}-acrolein-lysine (N^{ε}-(3-Formyl-3,4-DehydroPiperidino) lysine, FDP-lysine) (202), in which intense positivities were

associated with cells, primarily macrophages. The role of reactive aldehydes in the pathogenesis of atherosclerosis was also suggested by their increases in plasma, in association with extensive aortic atherosclerosis (142, 169, 170). About 30-40% of the uptake and degradation of oxidized Low Density Lipoprotein (oxLDL) by mouse peritoneal macrophages is mediated by scavenger receptor SR-AI/II, with CD36 accounting for a further 35% (102, 120). LDL modification by aldehydes enhanced their recognition and uptake by macrophages (79, 81). The formation of aldehyde adducts with lysyl residues of Apolipoprotein B (ApoB) in LDL altered the affinity of the latter for the ApoB/E receptor, expressed on most cell types except macrophages, and converted LDL to an atherogenic form that was uptaken by scavenger receptor-bearing cells (macrophages and smooth muscle cells), leading to the formation of foam cells (33, 187, 188, 189). Moreover, modification of human recombinant ApoE with acrolein severely compromised its functional integrity, as for heparin, lipid and LDL receptor binding (196). Acrolein-LDL also induced foam cell formation from macrophages (212).

Phosphatidylcholine γ-hydroxyalkenal, i.e., the γ-hydroxy-α,β-unsaturated core aldehydes still esterified at the *sn*-2 position of phosphatidylcholine, also contribute strongly to the binding of oxLDL by scavenger receptors and to the pathogenesis of atherosclerosis (80, 169). Antibody-based studies revealed the presence of carboxyheptylpyrroles (CHPs) and carboxypropylpyrroles (CPPs) in oxLDL (93), reflecting the presence of protein lysyl adducts in the core aldehydes 9-hydroxy-12-oxo-10-dodecenoyl- acid ester of phosphocholine (HODA-PC), produced by oxidation of 1-palmitoyl-2-linoleoyl-glycero-3-phosphocholine (PL-PC) or linoleoyl-2-arachidonoyl-glycero-3-phosphocholine (LA-PC), and 5-hydroxy-8-oxo-6-octenoyl-acid ester of phosphocholine (HOOA-PC), by oxidation of 1-palmitoyl-2-arachidonoyl-glycero-3-phosphocholine (PA-PC). The CHP immunoreactivity was also significantly higher in the plasma of patients with atherosclerosis and end-stage renal disease than in healthy controls (93). Chemically synthesized HOOA-PC

exhibited properties of a chemical mediator of chronic inflammation. It activated, in a dose-dependent manner, Human Aortic Endothelial Cells (HAEC) to bind monocytes and to secrete increased levels of Monocyte Chemotactic Protein-1 (MCP-1) and interleukin-8 (IL-8), which promoted monocyte entry into chronic lesions. HOOA-PC was found unbound and in pyrrole adducts in lipid extracts of oxLDL and human atheromas (81, 156). The binding of oxLDL to CD36 was mediated partly also by the head group of oxidized, but not native PC, in oxidized phospholipids such as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POV-PC) (18). The scenario emerging from these studies delineated atherogenesis as a result of myeloperoxidase-initiated, free radical-induced production of oxPC, which promoted subendothelial monocyte infiltration and endocytosis of oxLDL by macrophages, accompanied by conversion into foam cells and atheroma formation (169). Thereafter, it was shown that scavenger receptor CD36, another mediator of oxLDL uptake (as well as of recognition and phagocytosis of apoptotic cells) by macrophages, bound oxidized PC derivatives in oxLDL, including HODA-PC and HOOA-PC. These γ-hydroxy-α,β-unsaturated aldehydes, collectively referred to as $oxPC_{CD36}$, were potent activators of the CD36-mediated endocytosis of oxLDL by macrophages, promoting the cytotoxic effects of the adducts of oxidized derivatives of phospholipids and cholesterol with proteins (157, 193). OxLDL and individual $oxPC_{CD36}$ also interfered with the binding of HDL to scavenger SR-B1 receptors of hepatocytes, thus inhibiting the HDL-mediated delivery of cholesteryl esters to the liver (10).

The complexity of this process was illustrated by the report that HNE-histidine adducts bound to Lectin-like oxidized LDL receptor-1 (LOX-1), a class-E scavenger, multiligand receptor also implicated in atherosclerotic plaque formation (100, 121). Cloned as the main receptor for the binding, internalization and degradation of oxLDL by endothelial cells (174), LOX-1 was found to be expressed also in vascular smooth muscle cells, macrophages, fibroblasts and platelets (8, 34, 132, 220). Its C-

type, lectin-like ligand-binding domain was capable of binding such diverse ligands as oxLDL, acetylated LDL (AcLDL), phosphatidylserine, apoptotic bodies, activated platelets, leukocytes and bacteria (90, 133, 140, 183). LOX-1 has been indicated as a pro-inflammatory factor, with a role in atherosclerosis initiation and progression (34, 53, 92, 208). In endothelial cells, LOX-1 was upregulated upon exposure to oxLDL (115). OxLDL binding to LOX-1 induced a decrease in nitric oxide release (42) and the expression of adhesion molecules (116) and monocyte chemoattractant protein-1 (MCP-1) (114), while promoting ROS production, NF-κB activation (41, 42, 127) and apoptosis (115). In macrophages, LOX-1-mediated oxLDL binding stimulated the formation of lipid-laden cells resembling foam cells of atherosclerotic plaques (121, 185) (Fig. 5). As the upregulation of LOX-1 and downregulation of SR-AI/II and CD36 are induced by cytokines, such as TNF-α (101) and TGF-β (52), it is conceivable that LOX-1 play a major role in oxLDL uptake in inflamed atherosclerotic plaques.

In CHO cells stably expressing LOX-1, Bovine Serum Albumine (BSA) modified with HNE, ONE (4-oxo-2-nonenal), or non-hydroxylated alkenals 2-nonenal and 2-hexenal strongly inhibited the uptake of AcLDL (used as an alternative to oxLDL, in order to bypass the variations in the extent of LDL oxidation), with HNE-BSA showing the strongest inhibitory activity. AcLDL uptake was completely inhibited by anti-LOX-1 antibodies and significantly inhibited also by HNE-LDL, but not by native LDL. BSA modified with these aldehydes, unlike native BSA, was taken up by CHO cells transiently expressing LOX-1, in proportion with the level of LOX-1 expression, highlighting LOX-1 as the receptor responsible for the uptake of aldehyde-modified BSA (100). HNE-LDL uptake was inhibited by the substitutions of critical amino acid residues of LOX-1, which had been shown to be crucial for oxLDL binding (139), indicating a shared binding site for oxLDL and HNE-LDL on LOX-1. The binding of oxLDL, HNE-LDL and histidine-LDL to LOX-1 was confirmed with CLTD14, the ligand

recognition domain of LOX-1. Moreover, in HAEC, the binding to LOX-1 of HNE-histidine adducts (HNE-LDL, HNE-N^{α}-acetylhistidine), as well as of oxLDL, but not of LDL and histidine, stimulated ROS formation, an effect which could be inhibited by anti-LOX-1 antibodies. OxLDL, HNE-LDL and HNE-histidine adducts triggered a redox-sensitive signalling cascade, entailing the phosphorylation of ERK 1/2 and NF-κB (100), which resulted in the expression of genes related to endothelial dysfunction and injury (115, 116).

The ability of oxLDL to function as endothelial cell stressors was largely determined by the extent of their oxidative modification. Minimally oxidized LDLs retained their affinity for LDL receptor, activated antiapoptotic signaling and induced inflammatory changes in macrophages and endothelial cells, resulting in the recruitment of inflammatory cells and the secretion of cytokines and chemokines that promoted further oxidation (1). Further LDL LPO and apolipoprotein modification by reactive aldehydes determined the loss of recognition by the LDL receptor, with a shift to recognition by scavenger receptors, leading to foam cell formation from anti-inflammatory M2 macrophages, which were activated and shifted to a pro-inflammatory phenotype (206, 224). Scavenger receptors expressed on DCs (e.g., LOX-1) also mediate oxLDL uptake and the induction of the pro-inflammatory cytokine profile and of differentiation into the mature Dendritic Cell (DC) phenotype (136). Vascular associated DCs (VADCs) thus contribute to the initiation of atherosclerosis (145). Mice receiving DCs pulsed with MDA-LDL exhibited more extensive atherosclerotic lesions, with increased inflammatory signs and antigen-specific immune responses (192) (Fig. 5).

Adaptive immune responses contribute to plaque formation and to the maintenance of the atherosclerotis process. HSP-60, which is involved in the delivery of antigens into the MHC-I presentation pathway (218) and the maturation of DCs (59), is a main target of autoimmune cell-mediated responses in atherosclerosis (25, 68, 97, 98, 124, 167). Infiltration of atherosclerotic lesions with HSP60-specific T cells even

appeared to precede the formation of foam cells (96, 129, 219). Intriguingly, HSP-60 is secreted by monocytes (61) and endothelial cells in response to oxLDL (5, 69) and shares with them the LOX-1 receptor (218) (Fig. 5).

Other inflammation-related diseases associated with the presence of HNE-protein adducts are alcoholic liver disorders (113) and chronic alcoholic pancreatitis, in which the increased formation of HNE-protein adducts was evidenced in acinar cells adjacent to interlobular connective tissue (30). In chronic liver injury, it was demonstrated that HNE was involved in the transdifferentiation of hepatic stellate cells into a myofibroblastic phenotype characterized by proliferation and extracellular matrix deposition, leading to fibrosis (225). The exposure of isolated stellate cells to 1–10 μM HNE led to the detection of HNE adducts with Jun terminal kinase. The translocation of protein adducts determined an increased level of c-Jun mRNA, suggesting that HNE was an activating signal for oxidative stress responses.

An anti-inflammatory role for HNE has been demonstrated by studying NF-κB cell signaling. The latter is the major transcription factor associated with inflammation and oxidative stress (111). Inactive NF-κB is localized in the cytosol, bound to its inhibitory protein, IκB. Upon activation, NF-κB dissociates from IκB, after which translocation to the nucleus enables DNA binding and transactivation (91). This process is triggered by sequential phosphorylation and ubiquitination of IκBα, followed by proteasomal digestion. The enzyme that catalyzes the ubiquitination of phosphorylated IκB, IκB kinase (IKK), is constitutively active and, in most cases, represents the key regulator of NF-κB activation (23). Ji *et al.* found covalent adducts of HNE to IKK, by using antibodies against IKK or HNE-protein conjugates in the human colorectal carcinoma cell line (RKO) and the human lung carcinoma cell line (H1299), and demonstrated that HNE binding prevented IκBα degradation and, consequently, inhibited NF-κB activation (86). These authors concluded that, as NF-

κB stimulates transcription in response to oxidative stress, its modification by HNE may limit the magnitude of such transcriptional response.

In another inflammation-related metabolic condition, diabetes mellitus, the increase of oxidative stress and the formation of HNE adducts has been widely reported (45, 200). HNE has been demonstrated to form adducts with some of the proteins involved in the etiopathogenesis of diabetes. Indeed, HNE affected insulin signaling by binding to Insulin Receptor Substrate (IRS)-1/-2 proteins in 3T3-L1 adipocytes, as shown by immunoprecipitation and immunoblotting (47). IRSs are recruited after insulin binding to its receptor and transmit the insulin signal by activating two major pathways: the phosphatidylinositol 3-kinase (PI 3-kinase) cascade for glucose, lipid, and protein metabolism and the mitogen-activated protein kinase (MAPK) cascade for cell proliferation and differentiation (171, 214). HNE-IRS adducts likely impair the function of IRSs and favor their degradation, indicating that this aldehyde plays an important role in insulin resistance development and, therefore, could foster the progression to type 2 diabetes (47).

Moreover, HNE seems to be involved in the etiopathogenesis of diabetic cardiomyopathy. Using immunoblotting with anti-HNE antibodies, Lashin *et al.* demonstrated the presence of HNE adducts with succinyl dehydrogenase (SDH) in the heart of diabetic rats, which contributed to the functional inhibition of mitochondrial complex-II, amplifying the organelle dysfunction and markedly decreasing oxygen consumption in heart mitochondria. (109). In keeping with these results, Mali *et al.*, using immunoprecipitation, showed that 4-HNE formed adducts with myocardial aldehyde dehydrogenase 2 in mice exhibiting metabolic syndrome/type-2 diabetes mellitus, whose formation was associated with a reduction of the enzyme activity, which might contribute to cardiac hypertrophy and dysfunction (122) .

HNE-Protein adducts in autoimmunity: Sjögren's Syndrome (SS) and Systemic Lupus Erythematosus (SLE)

HNE-protein adducts have been involved in both innate and adaptive autoimmune responses. Several oxidation-specific epitopes (OSEs) are recognized as endogenous damage-associate molecular patterns (DAMPs) by innate pattern recognition receptors (PRRs). Such OSEs include the oxidation products of membrane phospholipids and polyunsaturated fatty acids in LDLs and their adducts, as seen in atherosclerosis (107). PRRs involved include Toll-like receptors, scavenger receptors CD36 and SR-B1, C-reactive protein, complement factor H and natural IgM antibodies (213), such as those recognizing the adducts of MDA and HNE with LDLs, detected in the sera of immunodeficient *rag1-/-* mice after reconstitution with B-1 cells (38).

A number of interesting observations were also collected, concerning the adducts of HNE with some autoantigenic targets of antinuclear autoantibodies (ANA) characteristically detected in Sjögren syndrome (SS), SLE and other autoimmune diseases (103). Typical ANA targets in SS include the SS-A/Ro and SS-B/La antigens. The SS-A/Ro antigens comprise a 52-kDa form (SS-A1/Ro52; TRIM21), found both in cytoplasm and nucleus and characterized by a tripartite motif with RING (E3 ubiquitine ligase), B-box and Coiled Coil domains, and a 60-kDa form (SS-A2/Ro60; TROVE2), found mainly in cytoplasm and involved in cell survival to UV damage. Both are components of Ro ribonucleoprotein (RNP) particles, in which they are non-covalently associated with short, non-coding, human cytoplasmic RNAs (hY-RNAs), as in spliceosomal RNPs, and small cytoplasmic RNAs, such as the 5S rRNA precursors of the 60S ribosomal subunit. The 48-kDa SS-B/La antigen is a transcription termination factor forRNA Polymerase III, transiently associated with hY-RNAs in RNPs involved in tRNA processing and histonic mRNA stabilization. Autoantibodies to SS-A2/Ro60 occur in over 60 % of SS patients and 25-40 % of SLE patients, as well as in other autoimmune diseases. SS-Ro and SS-La antigens become

exposed in apoptotic bodies and blebs of variable size at the surface of apoptotic cells (29). Apoptotic cardiocytes from fetuses spontaneously aborted, due to the congenital heart block of neonatal lupus, opsonized by maternal anti-Ro and anti-La antibodies, induced the Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) of co-cultured macrophages (130). Anti-SS-A/Ro antibodies were involved also in the ADCC in damage of keratinocytes in UV-sensitive SLE (62). It was proposed that, in SLE and SS, both an increased susceptibility of leukocytes to apoptosis (55, 65, 165, 227), possibly related with the overexpression of the E3 ubiquitin ligase SS-A1/Ro52 (56), and an impaired clearance of apoptotic cells by macrophages (117, 165) may be triggers of autoimmunity (173). More interestingly from the standpoint of this review, it was proposed that the breaking of tolerance to self antigens at the surface of apoptotic cells might be promoted by oxidative modifications occurring as a consequence of the oxidative stress that characterizes apoptosis (29, 78).

The role of self antigen modification by the formation of HNE adducts in the breaking of immunological tolerance was first documented in an early report (217), in which Murine Serum Albumin (MSA), modified *in vitro* with several unsaturated (MDA, HNE, heptadienal) and saturated aldehydes (butanal, nonanal), induced strong T-cell-dependent antibody responses. Various T-cell hybridomas, established from immunized mice, recognized MDA- and HNE-modified MSA, but not native MSA, in a MHC-restricted manner. All aldehyde-modified MSA preparations induced strong specific antibody responses, while native MSA did not. Of the former, only HNE-MSA and nonanal-MSA induced crossed antibody responses to unmodified MSA, almost as intense as against aldehyde-modified MSA, indicating that the sensitization of T cells to HNE-MSA adducts favored the intramolecular spreading of the immune response to formerly tolerated epitopes of the native self antigen (Fig. 6) (217). Scofield and coworkers hypothesized that modification of SS-A2/Ro60 with HNE might facilitate the breaking of tolerance to the native antigen in Sjögren's Syndrome. After immunizing rabbits with either the HNE-modified or the

unmodified SS-A2/Ro60, they observed that autoimmunity was established faster and more strongly in the animals immunized with HNE-modified SS-A2/Ro60 (106, 178). Later work provided formal proof that the breaking of tolerance to self antigens in the context of apoptosis required the generation of neoepitopes (143). The immunization of A/J mice with late apoptotic thymocytes, expressing the transgenic hSS-B/La antigen of human origin, was followed by the production of anti-SS-B/La antibodies. Immunization with non-apoptotic cells, expressing the transgenic antigen, had similar, although smaller and slower effects. Instead, no responses ensued either the immunization of transgenic mice with syngeneic thymocytes expressing the transgenic antigen, or of wild-type mice with thymocytes expressing autologous SS-B/La (143).

In an extension of this model, an SS-like condition, with anti-SS-A2/Ro60 antibodies, could be induced in BALB/c mice by immunization with a peptide of SS-A2/Ro60 (108). The production of anti-SS-A2/Ro60 and anti-SS-B/La autoantibodies ensued immunization with SS-A2/Ro60, both as such and modified with increasing concentrations of HNE (0.4, 2 or 10 mM). However, antibody production was faster after immunization with low- and, especially, medium-level HNE-modified antigen. The antibodies produced by mice immunized with HNE-modified, but not with unmodified SS-A2/Ro60, included added subpopulations that recognized HNE or HNE-SS-A2/Ro60, but not the unmodified antigen, as well as dsDNA, which induced the authors to imply a SLE-like disease, although they did not provide pathological evidence of it. The occurrence of anti-dsDNA and anti-SS-B/La antibodies, following immunization with SS-A2/Ro60, represents an example of intermolecular epitope spreading. The ability of HNE to form adducts with a large number of biological macromolecules might be of help in understanding the broad range of autoantibody responses in SLE and SS. Moreover, immunization with high-level HNE-modified SS-A2/Ro60 was associated with protein aggregation, lower-level antibody responses to unmodified SS-A2/Ro60 and SS-B/La and a Sjögren-like condition, with reduced

salivary flow and lymphocytic infiltration of salivary glands. These results were interpreted as being due to increased bifunctional cross-linking of SS-A2/Ro60 molecules (108), but a different interpretation could be that large, particulate immunocomplexes of aggregated HNE-SS-A2/Ro60 and autoantibodies stimulated the antigen-presenting activity of macrophages, which skewed the autoimmune response towards a cytotoxic cell-mediated mechanism. The same authors localized the targets of HNE modification within the sequence of Ro60, by using a collection of Multiple Antigenic Peptides (MAPs), chemically synthesized on the base of the sequences of Ro60 targeted by autoantibodies in SLE (82, 177) and anchored in multiple copies to a heptalysine core. Covalent adduct formation, upon exposure to HNE *in vitro*, mostly occurred in sequences participating in the solvent-exposed tertiary structure of Ro60, such as 126-137, 166-172 and 401-195 (105). Quantitative correlations of diagnostic and prognostic interest between markers of LPO, immunological reactivity to lipid-derived reactive aldehydes, and disease activity of SLE were reported. The prevalences and serum titers of MDA- and HNE-specific antibodies were significantly higher in SLE patients than in healthy controls, being also in correlation with the SLE Disease Activity Index (SLEDAI). Analogous correlations were observed between serum levels of MDA and HNE protein adducts and both SLEDAI scores and antibody levels. Such results underscored the pathogenic role of LPO in SLE and the potential usefulness of anti-MDA and anti-HNE antibodies in predicting its progression (210).

The molecular mimicry between the adducts of HNE and its analogs with proteins, on one hand, and DNA, in native or modified form, on the other hand, as a mechanism for the production of anti-DNA autoantibodies in response to aldehyde-modified self protein antigens was investigated by Uchida and coworkers. After raising an anti-HNE monoclonal antibody (anti-*R* mAb 310), which selectively recognized the *R* enantiomer of HNE-histidine Michael adducts (74), these authors found that the sequence of such anti-HNE mAb strictly resembled those of various

clonally related anti-DNA antibodies. Despite this structural similarity, the cross-reactivity of mAb R310 with native dsDNA was limited, but strongly enhanced by the treatment of DNA with ONE, an HNE analog. ONE-2'-deoxynucleoside adducts were identified as alternative epitopes of mAb R310 in ONE-modified DNA. The constituent chemical groups of a common epitope, possibly responsible for the molecular mimicry between the *R*-HNE-histidine configurational isomers and the 1,N^2-etheno-type ONE-2'-deoxyguanosine adducts, and required for the recognition by bispecific antibodies, were highlighted (Fig. 7). On this basis, it was proposed that endogenous electrophilic molecular species, including HNE, may be immunological triggers of autoimmune disease (2). The same authors further investigated the possible role of HNE-modified proteins as the endogenous prompt for the production of anti-DNA antibodies. Having established a murine hybridoma with the splenocytes of BALB/c mice immunized with HNE-modified keyhole limpet hemocyanin (KLH), they found HNE-specific epitopes in the epidermis and dermis of patients with SLE, pemphigus vulgaris and contact dermatitis, as well as antibodies against HNE-modified bovine serum albumin (BSA) both in the sera of patients affected with SLE, SS, rheumatoid arthritis, systemic sclerosis and idiopathic inflammatory myopathies, and in the sera of diseased, lupus-prone MRL/*lpr* mice. Upon repeated immunization with HNE-modified KLH, mice also developed a distinct population of B cell clones, recognizing native DNA, but not HNE-BSA. In accordance with the work previously cited, the reactivity of anti-HNE B cell clones towards DNA was greatly enhanced by DNA modification with ONE. On the other hand, anti-DNA mAbs cross-reacted with ONE-modified BSA. The data suggested that HNE-specific epitopes formed upon HNE generation in cells might serve as sensitizing antigenic determinants for the production of bispecific antibodies against native DNA and ONE-modified proteins (198). Further results in experimental animals and in patients with SLE confirmed that the modification of Human Serum Albumin (HSA) with HNE resulted in the generation of neoepitopes in HSA, which, in

turn, was instrumental for the breaking of tolerance to HSA and was accompanied by cross-reactive responses to similarly modified DNA (58). Moreover, anti-ds-DNA antibodies from 27 out of 40 patients affected by SLE preferentially bound to HNE-modified HSA, with respect to DNA and native HSA. Analogous results were reported, showing that the IgG antibodies raised in rabbits against HNE-modified HSA recognized HSA from SLE patients and cross-reacted with native and ozidized goat liver chromatin, while the anti-native/oxidized chromatin antibodies from 41 out of 74 SLE patients also specifically recognized HNE-HSA (3). These findings strongly supported the pathogenetic role of LPO products in autoimmune disease.

HNE-protein adducts in red blood cell aging and AutoImmune Hemolytic Anemia (AIHA)

In AutoImmune Hemolytic Anemia (AIHA), red blood cells (RBCs) coated with autoantibodies on their surface are destroyed at an accelerated rate by splenic macrophages. Mice of the New Zealand Black strain spontaneously develop AIHA with increasing age and serve as an animal model of the disease. Major membrane proteins of RBCs were identified as autoantigenic targets in NZB mice. Autoantibodies eluted from RBC surfaces and mAbs produced by hybridomas established from NZB mice recognized band 3 protein, the major RBC membrane glycoprotein (32, 46). The breakage of tolerance to band 3 protein apparently resulted from the proteolytic removal of its surface domain or other modifications exposing its membrane-embedded portion (60). More recent studies have provided evidence for the involvement of oxidative modifications of RBC self antigens in the formation of neoepitopes, the loss of tolerance and the triggering of autoimmunity to RBCs (83). A similar phenotype as in NZB mice, i.e., increased production of anti-RBC autoantibodies and accelerated intravascular hemolysis and phagocytic removal of RBCs by Kuppfer cells, together with high levels of reactive oxygen species (ROS) in RBCs, was observed in *sod1*-knockout mice (84, 186). Autoantibodies were

directed against HNE, acrolein and Carbonic Anhydrase II (CAII). Both autoimmune responses and hemolytic anemia were rescued by transgenic expression of human SOD1 in erythroid cells (85). Moreover, immunoblotting and mass spectrometric analyses revealed that exposure of intact human RBCs to HNE resulted in selective HNE-β-spectrin adduct formation and cross-linking of HNE-modified spectrin. Spectrin is the main component of the submembranous cytoskeleton of RBCs and plays a critical role in the stability and strength of RBC plasma membrane. Apparently, local spectrin aggregation might lead to membrane surface area extrusion and loss, by freeing the lipid bilayer from the underlying cytoskeleton (9). As a whole, the observations described above are of relevance both for the physiological destruction of RBCs, in view of the reported accumulation of HNE in aging erythrocytes (6), and for their immune-mediated hemolysis, in conditions of enhanced LPO.

Protein-HNE adducts in autoimmune liver disease and ferritin-induced liver cytotoxicity

Primary biliary cirrhosis (PBC) is a progressive, nonsuppurative, autoimmune cholangiopathy entailing the selective, cell-mediated destruction of small and medium-sized (<100 μm in diameter) intrahepatic bile ducts. The immunochemical detection of HNE-modified proteins in liver biopsies revealed HNE-protein adducts in the cytoplasm of biliary cells of small bile ducts in all of 20 patients with PBC. In 30% of patients, HNE-protein adducts were detected also in periportal hepatocytes, in association with higher serum bilirubin levels and histological stage (stage 3, septal fibrosis), in comparison with patients lacking intrahepatocytic HNE-protein adducts. Thus, hepatic LPO may be an early event in bile duct destruction and contribute to hepatocyte injury and fibrosis during cholestasis in PBC (94).

Non-alcoholic Fatty Liver Disease (NAFLD) covers a pathological spectrum of disease, from relatively benign lipid accumulation (simple steatosis, fatty liver), which is devoid of long-term adverse effects, to progressive nonalcoholic steatohepatitis (NASH), which is associated with necrosis, chronic inflammation and fibrosis, leading to liver cirrhosis. Adaptive immunity seems to be involved in the progression of NAFLD from steatosis to NASH, as hepatic oxidative stress markers, such as HNE and 8-hydroxydeoxyguanosine, correlated with the severity of hepatic necrosis, inflammation and fibrosis (31, 179); antibody responses to MDA-modified antigens were associated with increased severity of lobular inflammation or fibrosis (104, 137). In the methionine-choline deficient (MCD) murine model of NASH, autoimmune responses towards aldehyde-modified self antigens contributed to hepatic inflammation, by promoting T_H1 cell differentiation (194). In MCD-fed mice, the severity of hepatocyte damage and lobular inflammation, as revealed by transaminase release and hepatic TNF-α expression, paralleled IgG responses against MDA- and HNE-modified antigens, as well as hepatic infiltration by $CD4^+$ and $CD8^+$ T cells recognizing the same antigens. Immunization with MDA-modified BSA enhanced transaminase release, hepatic TNF-α expression and liver recruitment and differentiation of T_H1 cells. NASH in immunized, MCD-fed mice was also associated with IL-15-mediated expansion of NK T cells (194), which likely contributed to fibrosis by producing osteopontin (195).

A major role of HNE and other reactive aldehydes was implicated also in cell death induced by secreted acidic ferritins (20, 21). These appeared to act as soluble mediators of oxidative stress (19), in spite of the reported ability of human H chain ferritin to serve as a cellular antioxidant and apoptosis inhibitor (16, 228). Pathophysiological interest for these observations comes from the reported increases of serum ferritin levels in various pathological conditions, including acute and chronic inflammation and autoimmunity (163, 226). The cytotoxicity of an

acidic, H-chain-rich isoferritin (FER-CM) secreted by rat primary hepatocytes *in vitro* followed a dose-response relationship, marked by the transition from apoptosis to necrosis at concentrations above 100 ng/mL (22). Pro-apoptotic activity was accompanied by modification of cell proteins with HNE, as revealed by cytosolic accumulation of immunocytochemically detectable HNE-histidine protein (HNE-His-P) adducts, especially in the perinuclear area, and DNA damage, as revealed by the formation of micronuclei. FER-CM-induced apoptosis and HNE-His-P immunoreactivity were partially inhibited by the free radical scavenger 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and, more completely, by the lysosomotropic iron chelator desferrioxamine (DFO), as well as by proliferative stimulation of rat hepatocytes with EGF and insulin, whose mitogenic efficacy was reduced, in turn, in the presence of acidic isoferritins (19). It was suggested that these might act as oxidative stress mediators by promoting ferrous iron loading in lysosomes, ROS production, and lysosomal membrane permeabilization. The latter, in turn, might foster cell damage via the release of ferrous ions, ROS and cathepsins, cytosolic amplification of LPO, aldehyde-mediated protein/DNA modification and mitochondrial outer membrane permeabilization, leading to Fas- and p53-mediated apoptosis or necrosis, depending on the severity of oxidative stress (19). HNE itself was able to trigger p53 and Fas-dependent apoptosis (11). The conclusions drawn in the study cited (19) had some limitations, in that: a) HNE-His-P immunoreactivity varied markedly between different cells treated with FER-CM at the same dosage; b) the protection from FER-CM-induced apoptosis and necrosis provided by trolox was only partial, compared with that afforded by DFO and EGF/insulin, as though HNE hyperproduction were not entirely and directly responsible for the observed effects of FER-CM on cell viability. Ways of addressing these aspects might be the use of: 1) a selective inhibitor, such as nordihydroguaiaretic acid (NDGA) (215), of reticulocyte 15-lipoxygenase (15-LO), the enzyme responsible for the conversion of arachidonic acid to 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HpETE), from which HNE

is produced by a series of non-enzymatic peroxidation reactions; 2) cell transfection/transduction and overexpression of the fatty aldehyde dehydrogenase gene (*ALDH3A2*, *FALDH*), whose product detoxifies HNE by converting it to 4-hydroxynonenoic acid (4-HNA) (39), as already done in 3T3-L1 adipocytes (47).

Conclusions

Results obtained in recent years have shown that the generation of HNE-protein adducts can play important pathogenic roles in several diseases characterized by increases in oxidative stress and, consequently, of LPO and production of reactive aldehydes. However, cancer is peculiar in this respect, as the increases in oxidative stress do not always correlate with increases of LPO, due to differences in membrane lipid composition of cancer cells. Moreover, in cancer cells, the generation of HNE-protein adducts, by leading to apoptosis or to losses of dysregulated functions, can represent a contrast to the progression of disease.

HNE-protein and HNE-DNA adducts can incite autoimmune responses by combined effects on both innate and adaptive immunity. On one hand, they can act as Damage Associated Molecular Patterns (DAMPs) recognized by soluble and cell-associated pattern recognition receptors (PRRs), which may favor the uptake and presentation of self antigens by APCs in the context of enhanced levels of costimulation. Moreover, HNE cross-linking with self antigens can lead to the formation of neoepitopes, which initiate autoimmunity by recruiting T and B cells outside the repertoires of autoreactive T and B cells. Moreover, it has been repeatedly observed, both in the experimental and in the clinical setting, that the breaking of tolerance to a modified self antigen also affected its native counterpart. This effect, which entails the intramolecular spreading of sensitization to other epitopes, reflects both the hapten-carrier relationship linking HNE with its macromolecular targets and the multivalent character of the latter as immunogens. Intermolecular epitope spreading between HNE-modified protein antigens and other proteins or

DNA, either in native form or modified with the HNE analog ONE, has been also reported as a reflection of the molecular mimicry and cross-reaction between structurally related epitopes, as well as of the pleiotropic effects of HNE.

Interestingly, in certain chronic inflammatory and neurodegenerative diseases the presence of HNE adducts can promote adaptive cell responses, by stimulating intracellular GSH synthesis (12), inhibiting the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB) activity (86), inducing HO-1 activation (13) or stimulating autophagy (71). These studies underline the fact that HNE can be considered not only a toxic product of LPO, but also a regulatory molecule, involved in several biochemical pathways.

We believe that, in the coming years, the refinement both of proteomical and of tissue and cell sampling techniques, allowing the individuation of novel cellular targets of HNE, will lead to a better understanding of the mechanisms of HNE action in human diseases.

Acknowledgments

We thank the University of Turin and the Compagnia di San Paolo .

List of Abbreviations

AcLDL: Acetylated Low Density Lipoprotein;
AD: Alzheimer's Disease;
ADCC: Antibody-Dependent Dell-mediated Cytotoxicity;
AIHA: AutoImmune Hemolytic Anemia;
ALS: Amyotrophic Lateral Sclerosis;
ApoB: ApoLipoprotein B;
APP: Amyloid Precursor Protein;
ARE: Antioxidant Responsive Element;
Aβ: Amyloid β;
BCRs: B Cell Receptors;
BSA: Bovine Serum Albumin;
CAII: Carbonic Anhydrase II;
CHPs: carboxyheptylpyrroles;
CPPs: carboxypropylpyrroles;
CRMP2: Collapsin Response Mediator Protein 2;
2D-PAGE: two-Dimensional PolyAcrylamide Gel Electrophoresis;
DAMPs: Damage Associated Molecular Patterns;
DCs: Dendritic Cells;
DFO: DesFerriOxamine;
DRP-2: Dihydropyrimidinase-Related Protein 2;
DS: Down Syndrome;
ECs: Endothelial Cells;
EGFR: Epidermal Growth Factor Receptor;
fALS: familial Amyotrophic Lateral Sclerosis;
FDP-lysine: N^{ε}-(3-Formyl-3,4-DehydroPiperidino) lysine:
FER-CM: H-chain-rich isoferritin;
GAPDH : Glyceraldehyde-3-Phosphate- DeHydrogenase;
GCL: Glutamate Cysteine Ligase;
GCLM: Glutamate Cysteine Ligase Modulatory subunit;
GPCR: G-Protein Coupled Receptor;
GSH: Glutathione;
GSTs: Glutathione-S-Transferases;
HAEC: Human Aortic Endothelial Cells;
HD: Huntington's disease;
HHE: 4-hydroxy-hexenal;
4-HNA: 4 -hydroxynonenoic acid;
HNE: 4-hydroxy-2-nonenal;
HNE-His-P: HNE-histidine protein;
HO-1: Heme Oxygenase Protein-1;

HODA-PC: 9-hydroxy-12-oxo-10-dodecenoyl- acid ester of phosphocholine;
HOOA-PC: 5-hydroxy-8-oxo-6-octenoyl- acid ester of phosphocholine;
15-HpETE: 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid;
HSA: Human Serum Albumin;
Hsp70: Heat-shock protein 70;
HUVECs: Human Venous Endothelial Cells;
hY-RNAs: human cYtoplasmic RNAs
IKK: IκB Kinase;
IL-8: interleukin-8;
IRS: Insulin Receptor Substrate;
KLH: keyhole limpet hemocyanin;
LA-PC: linoleoyl-2-arachidonoyl-glycero-3-phosphocholine;
LDL: Low Density Lipoprotein;
LOX-1: Lectin-like oxidized low density lipoprotein receptor-1
LPO: lipid peroxidation;
LPOs: products of LPO;
LRP-1: Low density lipoprotein receptor-related protein-1;
Mφ: Macrophages;
MALDI-TOF/TOF: matrix-assisted laser desorption ionization / time-of-flight/time-of-flight;
MAPs: Multiple Antigenic Peptides;
MCD: methionine-choline deficient;
MCP-1: Monocyte Chemotactic Protein-1;
MDA: malondialdehyde;
MSA: Murine Serum Albumin;
NAFLD: non-alcoholic fatty liver disease;
NASH: progressive nonalcoholic steatohepatitis;
NDGA: nordihydroguaiaretic acid;
NF-kB: Nuclear Factor kappa-light-chain-enhancer of activated B cells;
NFTs: neurofibrillary tangles;
ONE: 4-oxo-2-nonenal;
OSEs: oxidation-specific epitopes;
oxLDL: oxidized Low Density Lipoprotein;
$oxPC_{CD36}$: specific oxidized phospholipids acting via CD36
PA-PC: 1-palmitoyl-2-arachidonoyl-glycero-3-phosphocholine;
PBC: Primary biliary cirrhosis;
PC: Phosphatidylcholine;
PD: Parkinson's Disease;
PI 3-kinase: PhosphatidylInositol 3-kinase;
PL-PC: 1-palmitoyl-2-linoleoyl-glycero-3-phosphocholine;
POV-PC: 1-Palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine;

PPARs: Peroxisome Proliferator Activated Receptors;
PRRs: cell-associated pattern recognition receptors;
RBCs: red blood cells;
RNP: Ro ribonucleoprotein;
ROS: Reactive Oxygen Species;
sALS: sporadic Amyotrophic Lateral Sclerosis;
SDH: succinyl dehydrogenase;
SLE: Systemic Lupus Erythematosus;
SLEDAI: SLE Disease Activity Index;
SOD1: Cu, Zn-superoxide dismutase;
SS: Sjögren Syndrome;
TCR: T Cell Receptor;
UCH-L1: ubiquitin carboxyl-terminal hydrolase L1;
VADCs: Vascular Associated Dendritic Cells.

References

1. Ait-Oufella H, Taleb S, Mallat Z, and Tedgui A. Recent advances on the role of cytokines in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 31: 969-979, 2011.
2. Akagawa M, Ito S, Toyoda K, Ishii Y, Tatsuda E, Shibata T, Yamaguchi S, Kawai Y, Ishino K, Kishi Y Adachi T, Tsubata T, Takasaki Y, Hattori N, Matsuda T and Uchida K. Bispecific Abs against modified protein and DNA with oxidized lipids. *Proc Natl Acad Sci U S A* 103: 6160-6165, 2006.
3. Al-Shobaili HA, Al Robaee AA, Alzolibani AA, and Rasheed Z. Antibodies against 4-hydroxy-2-nonenal modified epitopes recognized chromatin and its oxidized forms: role of vhromatin, oxidized forms of chromatin and 4-hydroxy-2-nonenal modified epitopes on the etiopathogenesis of SLE. *Disease Markers* 33: 19-34, 2012.
4. Aluise CD , Rose K, Boiani M, Reyzer ML, Manna JD, Tallman K, Porter NA, and Marnett LJ. Peptidyl-prolyl cis/trans-Isomerase A1 (Pin1) is a target for modification by lipid electrophiles. *Chem Res Toxicol* 26: 270-279, 2013.
5. Amberger A, Maczek C, Jürgens G, Michaelis D, Schett G, Trieb K, Eberl T, indal S, Xu Q, Wick G. Co-expression of ICAM-1, VCAM-1, ELAM-1 and Hsp60 in human arterial and venous endothelial cells in response to cytokines and oxidized low-density lipoproteins. *Cell Stress & Chaperones* 22: 94-103, 1997.
6. Ando K, Beppu M, and Kitagawa K. Evidence for the accumulation of lipid hydroperoxides during the aging of human red blood cells in the circulation. *Biol Pharmacol Bull* 18: 659-663, 1995.
7. Andronicos NM, Ranson M, Bognacki J, and Baker MS. The human ENO1 gene product (recombinant human alpha-enolase) displays characteristics required for a plasminogen binding protein. *Biochim Biophys Acta* 1337: 27-39, 1997.
8. Aoyama T, Chen M, Fujiwara H, Masaki T, and Sawamura T. LOX-1 mediates lysophosphatidylcholine-induced oxidized LDL uptake in smooth muscle cells. *FEBS Lett* 467: 217-220, 2000.
9. Arashiki N, Otsuka Y, Ito D, Komatsu T, Sato K, and Inaba M. The covalent modification of spectrin in red cell membranes by the lipid peroxidation product 4-hydroxy-2-nonenal. *Biochem Biophys Res Commun* 391: 1543-1547, 2010.

10. Ashraf MZ, Kar NS, Chen X, Choi J, Salomon RG, Febbraio M, and Podrez EA. Specific oxidized phospholipids inhibit scavenger receptor bi-mediated selective uptake of cholesteryl esters. *J Biol Chem* 283: 10408-10414, 2008.
11. Awasthi YC, Sharma R, Sharma A, Yadav S, Singhal SS, Chaudhary P, and Awasthi S. Self-regulatory role of 4-hydroxynonenal in signaling for stress induced programmed cell death. *Free Radic Biol Med* 45: 111-118, 2008.
12. Backos DS, Fritz KS, Roede JR, Petersen DR, and Franklin CC. Post-Translational modification and regulation of glutamate cysteine ligase by the α,β-unsaturated aldehyde 4-Hydroxy-2-Nonenal (4-HNE). *Free Radic Biol Med* 50: 14-26, 2011.
13. Barone E, Di Domenico F, Sultana R, Coccia R, Mancuso C, Perluigi M, and Butterfield DA. Heme oxygenase-1 posttranslational modifications in the brain of subjects with Alzheimer disease and mild cognitive impairment. *Free Radic Biol Med* 52: 2292-2301, 2012.
14. Barrera G, Pizzimenti S, and Dianzani MU. Lipid peroxidation: control of cell proliferation, cell differentiation and cell death. *Mol Aspects Med* 29: 1-8, 2008.
15. Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN Oncol* 2012: 137289, 2012.
16. Berberat PO, Katori M, Kaczmarek E, Anselmo D, Lassman C, Ke B, Shen X, Busuttil RW, Yamashita K, Csizmadia E, Tyagi S, Otterbein LE, Brouard S, Tobiasch E, Bach FH, Kupiec-Weglinski JW, and Soares MP. Heavy chain ferritin acts as an antiapoptotic gene that protects livers from ischemia reperfusion injury. *FASEB J* 17: 1724-1726, 2003.
17. Blanc EM, Kelly JF, Mark RJ, Waeg G, and Mattson MP. 4-Hydroxynonenal, an aldehydic product of lipid peroxidation, impairs signal transduction associated with muscarinic acetylcholine and metabotropic glutamate receptors: possible action on G alpha(q/11*). J Neurochem* 69: 570-580, 2007.
18. Boullier A, Friedman P, Harkewicz R, Hartvigsen K, Green SR, Almazan A, Dennis EA, Steinberg D, Witztum JL, and Quehenberger O. Phosphocholine as a pattern recognition ligand for CD36. *J Lipid Res* 46: 969-976, 2005.
19. Bresgen N, Jaksch H, Lacher H, Ohlenschläger I, Uchida K, and Eckl PM. Iron-mediated oxidative stress plays an essential role in ferritin-induced cell death. *Free Radic Biol Med*. 48: 1347-1357, 2010.

20. Bresgen N, Ohlenschlager I, Fiedler B, Wacht N, Zach S, Dunkelmann B, Arosio P, Kuffner E, Lottspeich F, and Eckl PM. Ferritin - a mediator of apoptosis? *J Cell Physiol* 212: 157-164, 2007.
21. Bresgen N, Ohlenschlager I, Wacht N, Afazel S, Ladurner G, and Eckl PM. Ferritin and FasL (CD95L) mediate density dependent apoptosis in primary rat hepatocytes. *J Cell Physiol* 217: 800-808, 2008.
22. Bresgen N, Rolinek R, Hochleitner E, Lottspeich F, and Eckl PM. Induction of apoptosis by a hepatocyte conditioned medium. *J Cell Physiol* 198: 452-460, 2004.
23. Brown K, Gerstberger S, Carlson L, Franzoso G, and Siebenlist U. Control of I kappa B-alpha proteolysis by site-specific, signal-induced phosphorylation. *Science* 267: 1485-1488, 1995.
24. Browne SE, and Beal MF. Oxidative damage in Huntington's disease pathogenesis *Antioxid Redox Signal* 8: 2061-2073, 2006.
25. Bussone G, Dib H, Tamby MC, Broussard C, Federici C, Woimant G, Camoin L, Guillevin L, and Mouthon L. Identification of new autoantibody specificities directed at proteins involved in the transforming growth factor β pathway in patients with systemic sclerosis. *Arthr Res Ther* 13: 74, 2011.
26. Butterfield DA, Drake J, Pocernich C, and Castegna A. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. *Trends Mol Med* 7: 548-554, 2001.
27. Butterfield DA, Reed T, and Sultana R. Roles of 3-nitrotyrosine- and 4-hydroxynonenal-modified brain proteins in the progression and pathogenesis of Alzheimer's disease. *Free Radic Res* 45: 59-72, 2011.
28. Canuto RA, Muzio G, Maggiora M, Biocca ME, and Dianzani MU: Glutathione-S-transferase, alcohol dehydrogenase and aldehyde reductase activities during diethylnitrosamine-carcinogenesis in rat liver. *Cancer Lett* 68: 177-183, 1993.
29. Casciola-Rosen LA, Anhalt G, and Rosen A. Autoantigens targeted in sistemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J Exp Med* 179: 1317-1330, 1994.
30. Casini A, Galli A, Pignalosa P, Frulloni L, Grappone C, Milani S, Pederzoli P, Cavallini G, and Surrenti C. Collagen type I synthesized by pancreatic periacinar stellate cells

(PSC) co-localizes with lipid peroxidation-derived aldehydes in chronic alcoholic pancreatitis. *J Pathol* 192: 81-89, 2000.

31. Chalasani N, Deeg MA, and Crabb DW. Systemic lipid peroxidation and its metabolic and dietary correlates in patients with non-alcoholic steatohepatitis. *Am J Gastroenterol* 99: 1497-1502, 2004.
32. Chang NH, MacLeod R, and Wither JE. Autoreactive B cells in lupus-prone New Zealand black mice exhibit aberrant survival and proliferation in the presence of self-antigen in vivo. *J Immunol* 172: 1553-1560, 2004.
33. Chen M, Kakutani M, Minami M, Kataoka H, Kume N, Narumiya S, Kita T, Masaki T, and Sawamura T. Increased expression of lectin-like oxidized low density lipoprotein receptor-1 in initial atherosclerotic lesions of Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol* 20: 1107-1115, 2000.
34. Chen M, Kakutani M, Naruka T, Ueda M, Narumiya S, Masaki T, and Sawamura T. Activation-dependent surface expression of LOX-1 in human platelets. *Biochem Biophys Res Commun* 282: 153-158, 2001.
35. Choi J, Levey AI, Weintraub ST, Rees HD, Gearing M, Chin LS, and Li L. Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. *J Biol Chem* 279: 13256–13264, 2004.
36. Choi J, Rees HD, Weintraub ST, Levey AI, Chin LS, and Li L. Oxidative modifications and aggregation of Cu,Zn-superoxide dismutase associated with Alzheimer and Parkinson diseases. *J Biol Chem* 280: 11648–11655, 2005.
37. Choi J, Sullards MC, Olzmann JA, Rees HD, Weintraub ST, Bostwick DE, Gearing M, Levey AI, Chin L-S, and Li L. Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. *J Biol Chem* 281: 10816–10824, 2006.
38. Chou MY, Fogelstrand L, Hartvigsen K, Hansen LF, Woelkers D, Shaw PX, Choi J, Perkmann T, Bäckhed F, Miller YI, Hörkkö S, Corr M, Witztum JL, and Binder CJ. Oxidation-specific epitopes are dominant targets of innate natural antibodies in mice and humans. *J Clin Invest* 119: 1335-1349, 2009.
39. Cohen G , Riahi Y , and Sasson S . Lipid peroxidation of poly-unsaturated fatty acids in normal and obese adipose tissues. *Arch Physiol Biochem* 117: 131-139, 2011.

40. Coleman JD, Prabhu KS, Thompson JT, Reddy PS, Peters JM, Peterson BR, Reddy CC, and Vanden Heuvel JP. The oxidative stress mediator 4-hydroxynonenal is an intracellular agonist of the nuclear receptor peroxisome proliferator-activated receptor-β/δ (PPARβ/δ). *Free Radic Biol Med* 42: 1155-1164, 2007.
41. Cominacini L, Pasini AF, Garbin U, Davoli A, Tosetti ML, Campagnola M, Rigoni A, Pastorino AM, Lo Cascio V, and Sawamura T. Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-kappaB through an increased production of intracellular reactive oxygen species. *J Biol Chem* 275: 12633-12638, 2000.
42. Cominacini L, Rigoni A, Pasini AF, Garbin U, Davoli A, Campagnola M, Pastorino AM, Lo Cascio V, and Sawamura T. The binding of oxidized low density lipoprotein (ox-LDL) to ox-LDL receptor-1 reduces the intracellular concentration of nitric oxide in endothelial cells through an increased production of superoxide. *J Biol Chem* 276: 13750-13755, 2001.
43. Compton CN, Franko AP, Murray MT, Diebel LN, and Dulchavsky SA. Signaling of apoptotic lung injury by lipid hydroperoxides. *J Trauma* 44: 783-788, 1998.
44. Curzio M, Esterbauer H, DiMauro C, Cecchini G, and Dianzani MU. Chemotactic activity of the lipid peroxidation product 4-hydroxynonenal and homologous hydroxyalkenals and consequences neutrophil motility. *Free Rad Res Communs* 5: 55-66, 1988.
45. Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, and Nicotera T. Oxidative damage to DNA in diabetes mellitus. *Lancet* 347: 444-445, 1996.
46. de Sá Oliveira GG, Izui S, Ravirajan CT, Mageed RA, Lydyard PM, Elson CJ, and Barker RN. Diverse antigen specificity of erythrocyte-reactive monoclonal autoantibodies from NZB mice. *Clin Exp Immunol* 105: 313-320, 1996.
47. Demozay D, Mas JC, Rocchi S, and Van Obberghen E. FALDH reverses the deleterious action of oxidative stress induced by lipid peroxidation product 4-hydroxynonenal on insulin signaling in 3T3-L1 adipocytes. *Diabetes* 57: 1216-1226, 2008.
48. Di Domenico F, Pupo G, Tramutola A, Giorgi A, Schininà ME, Coccia R, Head E, Butterfield DA, and Perluigi M. Redox proteomics analysis of HNE-modified proteins

in Down syndrome brain: clues for understanding the development of Alzheimer disease. *Free Radic Biol Med* 71C: 270-280, 2014.

49. Dianzani MU. 4-hydroxynonenal from pathology to physiology. *Mol Aspects Med* 24: 263-272, 2003.
50. Dix TA, and Aikens J. Mechanisms and biological relevance of lipid peroxidation initiation. *Chem Res Toxicol* 6: 2-18, 1993.
51. Dodson M, Liang Q, Johnson MS, Redmann M, Fineberg N, Darley-Usmar VM, and Zhang J. Inhibition of glycolysis attenuates 4-hydroxynonenal-dependent autophagy and exacerbates apoptosis in differentiated SH-SY5Y neuroblastoma cells. *Autophagy* 9: 1996-2008, 2013.
52. Draude G, and Lorenz RL. TGF-beta1 downregulates CD36 and scavenger receptor A but upregulates LOX-1 in human macrophages. *Am J Physiol Heart Circ Physiol* 278: 1042-1048, 2000.
53. Dunn S, Vohra RS, Murphy JE, Homer-Vanniasinkam S, Walker JH, and Ponnambalam S. The lectin-like oxidized low-density–lipoprotein receptor: a pro-inflammatory factor in vascular disease. *Biochem J* 409: 349-355, 2008.
54. Eaton P, Li JM, Hearse DJ, and Shattock MJ. Formation of 4-hydroxy-2-nonenal-modified proteins in ischemic rat heart. *Am J Physiol* 276: 935-943, 1999.
55. Emlen W, Niebur J, and Kadera R. Accelerated in vitro apoptosys of lymphocytes from patients with systemic lupus erythematosus. *J Immunol* 152: 3685-3692, 1994.
56. Espinosa A, Zhou W, Ek M, Hedlund M, Brauner S, Popovic K, Horvath L, Wallerskog T, Oukka M, Nyberg F, Kuchroo VK, and Wahren-Herlenius M. The Sjögren's Sydrome-associated autoantigen Ro52 is an E3 ligase that regulates proliferation and cell death. *J Immunol* 176: 6277-6285, 2006.
57. Esterbauer H, Schaur RJ, and Zollner H. Chemistry and Biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 11: 81-28, 1991.
58. Farzana Khatoon M, and Khursheed Alam AA. Physicochemical and immunological studies on 4-hydroxynonenal modified HSA: Implications of protein damage by lipid peroxidation products in the etiopathogenesisi of SLE. *Hum Immunol* 73: 1132-1139, 2012.

59. Flohé SB, Bruggemann J, Lendemans S, Nikulina M, Meierhoff GS, Flohé S, and Kolb H. Human heat shock protein 60 induces maturation of dendritic cells versus a Th1-promoting phenotype. *J Immunol* 170: 2340–2348, 2003.

60. Fossati-Jimack L, Azeredo da Silveira S, Moll T, Kina T, Kuypers FA, Oldenborg PA, and Reininger L, Izui S. Selective increase of autoimmune epitope expression on aged erythrocytes in mice: implications in anti-erythrocyte autoimmune responses. *J Autoimmun* 18: 17-25, 2002.

61. Frostegard J, Kjellman B, Gidlund M, Andersson B, Jindal S, and Kiessling R. Induction of a heat-shock protein in monocytic cells by oxidized low density lipoprotein. *Atherosclerosis* 121: 93-103, 1996.

62. Furukawa F, Itoh T, Wakita H, Yagi H, Tokura Y, Norris DA, and Takigawa M. Keratinocytes form patients with lupus erythematosus show enhanced cytotoxicity to ultraviolet radiation and to antibody-mediated cytotoxicity. *Clin Exp Immunol* 118: 164-170, 1999.

63. Gan L, and Johnson JA. Oxidative damage and the Nrf2-ARE pathway in neurodegenerative diseases. *Biochim Biophys Acta* 1842: 1208-1218, 2014.

64. Gentile F, Pizzimenti S, Arcaro A, Pettazzoni P, Minelli R, D'Angelo D, Mamone G, Ferranti P, Toaldo C, Cetrangolo G, Formisano S, Dianzani MU, Uchida K, Dianzani C, and Barrera G. Exposure of HL-60 human leukaemic cells to 4-hydroxynonenal promotes the formation of adduct(s) with alpha-enolase devoid of plasminogen binding activity. *Biochem J* 422: 285-294, 2009.

65. Georgescu L, Vakkalanka RK, Elkon KB, and Crow MK. Interleukin-10 promotes activation-induced cell death of SLE lymphocytes mediated by Fas ligand. *J Clin Invest* 100: 2622-2633, 1997.

66. Giordano S, Darley-Usmar V, and Zhang J. Autophagy as an essential cellular antioxidant pathway in neurodegenerative disease. *Redox Biol* 2: 82-90, 2013.

67. Gómez A, and Ferrer I. Increased oxidation of certain glycolysis and energy metabolism enzymes in the frontal cortex in Lewy body diseases. *J Neurosci Res* 87: 1002-1013, 2009.

68. Grundtman C, and Wick G. The autoimmune concept of atherosclerosis. *Curr Opin Lipidol* 22: 327-334, 2011.

69. Grundtman C, Kreutmayer SB, Almanzar G, Wick MC, Wick G. Heat shock protein 60 and immune inflammatory responses in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*. 31: 960-968, 2011.

70. H.s.D.C.R. Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* 72: 971-983, 1993.

71. Haberzett P and Hill BG. Oxidized lipids activate autophagy in a JNK-dependent manner by stimulating the endoplasmic reticulum stress response. *Redox Biology* 1: 56-64, 2013.

72. Hammer A, Ferro M, Tillian HL Tatzber F, Zollner H, Schauenstein E, and Schaur RJ. Effect of oxidative stress by iron on 4-hydroxynonenal formation and proliferative activity in hepatomas of different degrees of differentiation. *Free Radic Biol Med* 23: 26-33, 1997.

73. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science 297*: 353-356, 2002. Erratum in: Science 297:2209, 2002.

74. Hashimoto M, Shibata T, Wasada H, Toyokuni S, Uchida K. Structural basis of protein-bound endogenous aldehydes. Chemical and immunochemical characterization of configurational isomers of a 4-hydroxy-2-nonenal-histidine adduct. *J Biol Chem* 278: 5044-5051, 2003.

75. Hensley K, Venkova K, Christov A, Gunning W, and Park J. Collapsin response mediator protein-2: an emerging pathologic feature and therapeutic target for neurodisease indications. *Mol Neurobiol* 43: 180-91, 2011.

76. Herbst U, Toborek M, Kaiser S, Mattson MP, and Hennig B. 4-Hydroxynonenal induces dysfunction and apoptosis of cultured endothelial cells. *J Cell Physiol* 181: 295-303, 1999.

77. Hill BG, Haberzettl P, Ahmed Y, Srivastava S, and Bhatnagar A. Unsaturated lipid peroxidation-derived aldehydes activate autophagy in vascular smooth-muscle cells. *Biochem J* 410: 525-534, 2008.

78. Hockenberry DM, Oltvai ZN, Yin X-M, Milliman CL, and Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75: 241-251, 1993.

79. Hoff HF, and O'Neil JA. Modification of LDL with 4-hydroxynonenal and malondialdehyde as a model for soluble oxidized LDL. *J Lipid Res* 34: 1209-1217, 1993.

80. Hoff HF, O'Neil J, Wu Z, Hoppe G. and Salomon RL. Phospholipid hydroxyalkenals. Biological and chemical properties of specific oxidized lipids present in atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 23, 275-282, 2003.

81. Hoff HF, O'Neil J, Chisolm GM, Cole TB, Quebenberger O, Esterbauer H, and Jürgens G. Modification of LDL with 4-hydroxynonenal induces uptake of LDL by macrophages. *Arteriosclerosis* 9: 538-549, 1989.

82. Huang SC, Scofield RH, Kurien BT, and Harley JB. Human anti-Ro autoantibodies bind multiple conformational epitopes of 60-kD Ro autoantigen. *J Clin Immunol* 17: 212-219, 1997.

83. Iuchi Y, Kibe N, Tsunoda S, Suzuki S, Mikami T, Okada F, Uchida K, and Fujii J. Implication of oxidative stress as a cause of autoimmune hemolytic anemia in NZB mice. *Free Radic Biol Med* 48: 935-944, 2010.

84. Iuchi Y, Okada F, Onuma K, Onoda T, Asao H, Kobayashi M, and Fujii J. Elevated oxidative stress in erythrocytes due to an SOD1 deficiency causes anemia and triggers autoantibody production. *Biochem J* 402: 219-227, 2007.

85. Iuchi Y, Okada F, Takamiya R, Kibe N, Tsunoda S, Nakajima O, Toyoda K, Nagae R, Suematsu M, Soga T, Uchida K, and Fujii J. Rescue of anemia and autoimmune responses in SOD1-deficient mice by transgenic expression of human SOD1 in erythrocytes. *Biochem J* 422: 313-320, 2009.

86. Ji C, Kozak KR, and Marnett LJ. IkappaB kinase, a molecular target for inhibition by 4-hydroxy-2-nonenal. *J Biol Chem* 276: 18223-18228, 2001.

87. Johri A, Chandra A, and Beal MF. PGC-1α, mitochondrial dysfunction, and Huntington's disease. *Free Radic Biol Med* 62: 37-46, 2013.

88. Jung KA, and Kwak MK. Enhanced 4-hydroxynonenal resistance in KEAP1 silenced human colon cancer cells. *Oxid Med Cell Longev* 2013: 423965, 2013.

89. Juric-Sekhar G, Zarkovic K, Waeg G, Cipak A, and Zarkovic N. Distribution of 4-hydroxynonenal-protein conjugates as a marker of lipid peroxidation and parameter of malignancy in astrocytic and ependymal tumors of the brain. *Tumori* 95: 762-768, 2009.

90. Kakutani M, Masaki T, and Sawamura T. A platelet-endothelium interaction mediated by lectin-like oxidized low-density lipoprotein receptor-1. *Proc Natl Acad Sci U S A* 97: 360-364, 2000.

91. Karin M. The beginning of the end: IkappaB kinase (IKK) and NF-kappaB activation. *J Biol Chem* 274: 27339-27342, 1999.

92. Kataoka H, Kume N, Miyamoto S, Minami M, Moriwaki H, Murase T, Sawamura T, Masaki T, Hashimoto N, and Kita T. Expression of lectin-like oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. *Circulation* 99: 3110-3117, 1999.

93. Kaur K, Salomon RG, O'Neil J, and Hoff HF. (Carboxyalkyl)pyrroles in human plasma and oxidized low-density lipoproteins. *Chem Res Toxicol* 10, 1387-1396, 1997.

94. Kawamura K, Kobayashi Y, Kageyama F, Kawasaki T, Nagasawa M, Tokoyuni S, Uchida K, and Nakamura I. Enhanced hepatic lipid peroxidation in patients with primary biliary cirrhosis. *Am J Gastroenterol* 95: 3597-3602, 2000.

95. Khan SA, and Vanden Heuvel JP. Role of nuclear receptors in the regulation of gene expression by dietary fatty acids. *J Nutr Biochem* 14: 554-567, 2003.

96. Kleindienst R, Xu Q, Willeit J, Waldenberger FR, Weimann S, Wick G. Immunology of atherosclerosis. Demonstration of heat shock protein 60 expression and T lymphocytes bearing alpha/beta or gamma/delta receptor in human atherosclerotic lesions. *Am J Pathol* 142: 1927-1937, 1993.

97. Knoflach M, Bernhard D, and Wick G. Anti-HSP60 immunity is already associated with atherosclerosis early in life. *Ann N Y Acad Sci* 1051: 323-331, 2005.

98. Knoflach M, Kiechl S, Mayr B, Kind M, Gaston JSH, van der Zee R, Faggionato A, Mayr A, Willeit J, and Wick G. T-cell reactivity against HSP60 relates to early but not advanced atherosclerosis. *Atherosclerosis* 195: 333-338, 2007.

99. Kristal BS, Park BK, and Yu BP. 4-Hydroxyhexenal is a potent inducer of the mitochondrial permeability transition. *J Biol Chem* 271: 6033-6038, 1996.

100. Kumano-Kuramochi M, Shimozu Y, Wakita C, Ohnishi-Kameyama M, Shibata T, Matsunaga S, Takano-Ishikawa Y, Watanabe J, Goto M, Xie Q, Komba S, Uchida K, and Machida S. Identification of 4-hydroxy-2-nonenal-histidine adducts that serve as ligands for human lectin-like oxidized LDL receptor-1. *Biochem J* 442: 171-80, 2012.

101. Kume N, Moriwaki H, Kataoka H, Minami M, Murase T, Sawamura T, Masaki T, and Kita T. Inducible expression of LOX-1, a novel receptor for oxidized LDL, in macrophages and vascular smooth muscle cells. *Ann N Y Acad Sci* 902: 323-327, 2000.

102. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, and Freeman MW. Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. *J Biol Chem* 277: 49982-49988, 2002.

103. Kurien BT, and Scofield RH. Autoantibody determination in the diagnosis of systemic lupus erythematosus. *Scand J Immunol* 64: 227-235, 2006.

104. Kurien BT, and Scofield RH. Autoimmunity and oxidatively modified autoantigens. *Autoimmunity Rev* 7: 567-573, 2008.

105. Kurien BT, D'Souza A, Terzyan S, and Scofield RH. Putative sequences on Ro60 three-dimensional structure accessible for 4-hydroxy-2-nonenal (HNE) modification compared to in vitro HNE modification of Ro60 sequences. *Mol Immunol* 50: 185-192, 2012.

106. Kurien BT, Hensley K, Bachmann M, and Scofield RH. Oxidatively modified autoantigens in autoimmune diseases. *Free Radic Biol Med* 41: 549-556, 2006.

107. Kurien BT, Scofield RH. Lipid peroxidation in systemic lupus erythematosus. *Indian J Exp Biol* 44:349-56, 2006.

108. Kurien T, Porter A, Dorri Y, Iqbal S, D'Souza A, Singh A, Asfa S, Cartellieri M, Mathias K, Matsumoto H, Bachmann M, Hensley K, and Scofield RH. Degree of modification of Ro60 by the lipid peroxidation by-product 4-hydroxy-2-nonenal may differentially induce Sjögren's syndrome or sistemic lupus erythematosus in BALB/c mice. *Free Radic Biol Med* 50: 1222-1233, 2011.

109. Lashin OM, Szweda PA, Szweda LI, and Romani AM . Decreased complex II respiration and HNE-modified SDH subunit in diabetic heart. *Free Radic Biol Med* 40: 886-896, 2006.

110. Lee J, Kosaras B, Del Signore SJ, Cormier K, McKee A, Ratan RR, Kowall NW, and Ryu H. Modulation of lipid peroxidation and mitochondrial function improves

neuropathology in Huntington's disease mice. *Acta Neuropathol* 121: 487-498, 2011.

111. Lenardo MJ, and Baltimore D. NF-kappa B: a pleiotropic mediator of inducible and tissue-specific gene control. *Cell* 58: 227-229, 1989.
112. Leonarduzzi G , Chiarpotto E , Biasi F , and Poli G . 4-Hydroxynonenal and cholesterol oxidation products in atherosclerosis . *Mol Nutr Food Res* 49: 1044-1049, 2005.
113. Li CJ , Nanji AA, Siakotos AN, and Lin RC. Acetaldehyde-modified and 4-hydroxynonenal-modified proteins in the livers of rats with alcoholic liver disease. *Hepatology* 26: 650-657, 1997.
114. Li D, and Mehta JL. Antisense to LOX-1 inhibits oxidized LDL-mediated upregulation of monocyte chemoattractant protein-1 and monocyte adhesion to human coronary artery endothelial cells. *Circulation* 101: 2889-2895, 2000.
115. Li D, and Mehta JL. Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells: evidence from use of antisense LOX-1 mRNA and chemical inhibitors. *Arterioscler Thromb Vasc Biol*. 20:1116-22, 2000.
116. Li D, Chen H, Romeo F, Sawamura T, Saldeen T, and Mehta JL. Statins modulate oxidized low-density lipoprotein-mediated adhesion molecule expression in human coronary artery endothelial cells: role of LOX-1. *J Pharmacol Exp Ther* 302: 601-605, 2002.
117. Licht R, Dieker JWC, Jacobs CWM, Tax WJM, and Berden JHM. Decreased phagocytosis of apoptotic cells in diseased SLE mice. *J Autoimmun* 22: 139-145, 2004.
118. Lin P, Danielson H, and Mannervik B. 4-Hydroxy-2-enals are substrates for glutathione transferase. *FEBS Lett* 179: 267-270, 1985.
119. Liu W, Akhand AA, Kato M, Yokoyama I, Miyata T,Kurokawa K, Uchida K, and Nakashima I. 4-Hydroxynonenal triggers an epidermal growth factor receptor-linked signal pathway for growth inhibition. *J Cell Sci* 112: 2409-2417, 1999.
120. Lougheed M, Ming Lum C, Ling W, Suzuki H, Kodama T, and Steinbrecher UP. High-affinity saturable uptake of oxidized low density lipoprotein by macrophages from

mice lacking the scavenger receptor class A type I/I. *J Biol Chem* 272: 12938-12944, 1997.

121. Lusis AJ. Atherosclerosis. *Nature* 407: 233-241, 2000.
122. Mali VR, Ning R, Chen J, Yang XP, Xu J, and Palaniyandi SS. Impairment of aldehyde dehydrogenase-2 by 4-hydroxy-2-nonenal adduct formation and cardiomyocyte hypertrophy in mice fed a high-fat diet and injected with low-dose streptozotocin. *Exp Biol Med* 239: 610-618, 2014
123. Mancuso C, and Barone E, The hemeoxygenase/biliverdin reductase pathway in drug research and development. *Curr Drug Metab* 10: 579-594, 2009.
124. Mandal K, Jahangiri M, and Xu Q. Autoimmunity to heat shock proteins in atherosclerosis. *Autoimmunity Rev* 3: 31-37, 2004.
125. Mark RJ, Pang Z, Geddes JW, Uchida K, and Mattson MP. Amyloid beta-peptide impairs glucose transport in hippocampal and cortical neurons: involvement of membrane lipid peroxidation. *J Neurosci* 17: 1046-1054, 1997.
126. Martínez A, Portero-Otin M, Pamplona R, and Ferrer I. Protein targets of oxidative damage in human neurodegenerative diseases with abnormal protein aggregates. *Brain Pathol* 20: 281-297, 2010.
127. Matsunaga T, Hokari S, Koyama I, Harada T, and Komoda T. NF-kappa B activation in endothelial cells treated with oxidized high-density lipoprotein. *Biochem Biophys Res Commun* 303: 313-319, 2003.
128. Mielke R, Schroder R, Fink G.R, Kessler J, Herholz K, and Heiss WD. Regional cerebral glucose metabolism and postmortem pathology in Alzheimer's disease. *Acta Neuropathol* 91: 174-179, 1996.
129. Millonig G, Malcom GT, Wick G. Early inflammatory-immunological lesions in juvenile atherosclerosis from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY)-study. *Atherosclerosis* 160: 441-448, 2002.
130. Miranda-Carús M-E, Askanase AD, Clancy RM, Di Donato F, Chou T-M, Libera MR, Chan EKL, and Buyon JP. Anti-SSA/Ro and anti-SSB/La autoantibodies bind the surface of apoptotic fetal cardiocytes and promote secretion of TNF-a by macrophages. *J Immunol* 165: 5345-5351, 2000.

131. Monroy CA, Doorn JA, and Roman DL. Modification and functional inhibition of regulator of G-protein signaling 4 (RGS4) by 4-hydroxy-2-nonenal. *Chem Res Toxicol* 26: 1832-1839, 2013.
132. Moriwaki H, Kume N, Sawamura T, Aoyama T, Hoshikawa H, Ochi H, Nishi E, Masaki T, and Kita T. Ligand specificity of LOX-1, a novel endothelial receptor for oxidized low density lipoprotein. *Arterioscler Thromb Vasc Biol* 18: 1541-1547, 1998.
133. Murphy JE, Tacon D, Tedbury PR, Hadden JM, Knowling S, Sawamura T, Peckham M, Phillips SE, Walker JH, and Ponnambalam S. LOX-1 scavenger receptor mediates calcium-dependent recognition of phosphatidylserine and apoptotic cells. *Biochem J* 393: 107-115, 2006.
134. Nadkarni DV, and Sayre LM. Structural definition of early lysine and histidine adduction chemistry of 4-hydroxynonenal. *Chem Res Toxicol* 8: 284-291, 1995.
135. Negre-Salvayre A, Coatrieux C, Ingueneau C, and Salvayre R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Br J Pharmacol* 153: 6-20, 2008.
136. Nickel T, Schmauss D, Hanssen H, Hanssen H, Sicic Z, Krebs B, Jankl S, Summo C, Fraunberger P, Walli AK, Pfeiler S, and Weis M. oxLDL uptake by dendritic cells induces upregulation of scavenger-receptors, maturation and differentiation. *Atherosclerosis* 205: 442-450, 2009.
137. Nobili V, Parola M, Alisi A, Marra F, Piemonte F, Mombello C, Sutti S, Povero D, Maina V, Novo E, Albano E. Oxidative stress parameters in paediatric non-alcoholic fatty liver disease. *Int J Molec Med* 26: 471-476, 2010.
138. Oberley TD, Toyokuni S, and Szweda LI. Localization of hydroxynonenal protein adducts in normal human kidney and selected human kidney cancers. *Free Radic Biol Med* 27: 695-703, 1999.
139. Ohki I, Ishigaki T, Oyama T, Matsunaga S, Xie Q, Ohnishi-Kameyama M, Murata T, Tsuchiya D, Machida S, Morikawa K, and Tate S. Crystal structure of human lectin-like, oxidized low-density lipoprotein receptor 1 ligand binding domain and its ligand recognition mode to OxLDL. *Structure* 13: 905-917, 2005.
140. Oka K, Sawamura T, Kikuta K, Itokawa S, Kume N, Kita T, and Masaki T. Lectin-like oxidized low-density lipoprotein receptor 1 mediates phagocytosis of

aged/apoptotic cells in endothelial cells. *Proc Natl Acad Sci U S A* 95: 9535-9540, 1998.

141. Owen JB, Sultana R, Aluise CD, Erickson MA, Price TO, Bu G, Banks WA, and Butterfield DA. Oxidative modification to LDL receptor-related protein 1 in hippocampus from subjects with Alzheimer disease: implications for Aβ accumulation in AD brain. *Free Radic Biol Med* 49: 1798-1803, 2010.
142. Palinski W, Ylä-Herttuala S, Rosenfeld ME, Butler SW, Socher SA, Parthasarathy S, Curtiss LK, and Witztum JL. Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 10: 325-335, 1990.
143. Pan Z-J, Davis K,Maier S, Bachmann MP, Kim-Howard XR, Keech C, Gordon TP, McCluskey J, and Farris AD. Neo-epitopes are required for immunogenicity of La/SS-B nuclear antigen in the context of late apoptotic cells. *Clin Experim Immunol* 143: 237-248, 2006.
144. Pancholi V. Multifunctional alpha-enolase: its role in diseases. *Cell Mol Life Sci* 58: 902-920, 2001.
145. Paulson KE, Zhu SN, Chen M, Nurmohamed S, Jongstra-Bilen J, and Cybulsky MI. Resident intimal dendritic cells accumulate lipid and contribute to the initiation of atherosclerosis. *Circ Res* 106: 383-390, 2010.
146. Pedersen WA, Fu W, Keller JN, Markesbery WR, Appel S, Smith RG, Kasarskis E, and Mattson MP. Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients. *Ann Neurol* 44: 819-824, 1998.
147. Perluigi M, and Butterfield DA. Oxidative stress and Down syndrome: a route toward Alzheimer-like dementia. *Curr Gerontol Geriatr Res* 2012: 724904, 2012.
148. Perluigi M, Fai Poon H, Hensley K, Pierce WM, Klein JB, Calabrese V, De Marco C, and Butterfield DA. Proteomic analysis of 4-hydroxy-2-nonenal-modified proteins in G93A-SOD1 transgenic mice--a model of familial amyotrophic lateral sclerosis. *Free Radic Biol Med* 38: 960-968, 2005.
149. Perluigi M, Sultana R, Cenini G, Di Domenico F, Memo M, Pierce WM, Coccia R, and Butterfield DA. Redox proteomics identification of 4-hydroxynonenal-modified brain

proteins in Alzheimer's disease: Role of lipid peroxidation in Alzheimer's disease pathogenesis. *Proteomics Clin Appl* 3: 682-693, 2009.

150. Petersen DR, and Doorn JA. Reactions of 4-hydroxynonenal with proteins and cellular targets. *Free Radic Biol Med* 37: 937-945, 2004.
151. Pettazzoni P, Ciamporcero E, Medana C, Pizzimenti S, Dal Bello F, Minero VG, Toaldo C, Minelli R, Uchida K, Dianzani MU, Pili R, and Barrera G. Nuclear factor erythroid 2-related factor-2 activity controls 4-hydroxynonenal metabolism and activity in prostate cancer cells. *Free Radic Biol Med* 51: 1610-1618, 2011.
152. Pizzimenti S, Ciamporcero E, Daga M, Pettazzoni P, Arcaro A, Cetrangolo G, Minelli R, Dianzani C, Lepore A, Gentile F, and Barrera G. Interaction of aldehydes derived from lipid peroxidation and membrane proteins. *Front Physiol* 4: 242, eCollection, 2013.
153. Pizzimenti S, Laurora S, Briatore F, Ferretti C, Dianzani MU, and Barrera G. Synergistic effect of 4-hydroxynonenal and PPAR ligands in controlling human leukemic cell growth and differentiation. *Free Radic Biol Med* 32: 233-245, 2002.
154. Pizzimenti S, Toaldo C, Pettazzoni P, Dianzani MU, and Barrera G. The “Two-Faced” effects of reactive oxygen species and the lipid peroxidation product 4-hydroxynonenal in the hallmarks of cancer. *Cancers* 2: 338-363, 2010.
155. Player TJ, Mills DJ, Horton AA. Lipid peroxidation of the microsomal fraction and extracted microsomal lipids from DAB-induced hepatomas. *Br J Cancer* 39: 773-778, 1979
156. Podrez EA, Poliakov E, Shen Z, Zhang R, Deng Y, and Sun M. A novel family of atherogenic oxidized phospholipids promotes macrophage foam cell formation via the scavenger receptor CD36 and is enriched in atherosclerotic lesions. *J Biol Chem* 277: 38517-38523, 2002.
157. Podrez EA, Poliakov E, Shen Z, Zhang R, Deng Y, Sun M, Finton PJ, Shan L, Gugiu B, Fox PL, Hoff HF, Salomon RG, and Hazen SL. Identification of a novel family of oxidized phospholipids that serve as ligands for the macrophage scavenger receptor CD36. *J Biol Chem* 277: 38503-38516, 2002.
158. Poon HF, Calabrese V, Scapagnini G, and Butterfield DA. Free radicals: key to brain aging and heme oxygenase as a cellular response to oxidative stress. *J Gerontol A Biol Sci Med Sci* 59: 478-493, 2004.

159. Pryor WA, and Porter NA. Suggested mechanisms for the production of 4-hydroxy-2-nonenal from the autoxidation of polyunsaturated fatty acids. *Free Radic Biol Med* 8: 541-543, 1990.

160. Qin Z, Hu D, Han S, Reaney SH, Di Monte DA, and Fink AL. Effect of 4-hydroxy-2-nonenal modification on alpha-synuclein aggregation. *J Biol Chem* 282: 5862-5870, 2007.

161. Quach TT, Duchemin AM, Rogemond V, Aguera M, Honnorat J, Belin MF, and Kolattukudy PE. Involvement of collapsin response mediator proteins in the neurite extension induced by neurotrophins in dorsal root ganglion neurons. *Mol Cell Neurosci* 25: 433-443, 2004.

162. Rahajeng J, Giridharan SS, Naslavsky N, and Caplan S. Collapsin response mediator protein-2 (Crmp2) regulates trafficking by linking endocytic regulatory proteins to dynein motors. *J Biol Chem* 285: 31918-31922, 2010.

163. Recalcati S, Invernizzi P, Arosio P, and Cairo G. New functions for an iron storage protein: the role of ferritin in immunity and autoimmunity. *J Autoimmun* 30: 84-89, 2008.

164. Reed TT. Lipid peroxidation and neurodegenerative disease. *Free Radic Biol Med* 51: 1302-1319, 2011.

165. Ren Y, Tang J, Mok MY, Chan AW, Wu A, and Lau CS. Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. *Arthritis Rheum* 48: 2888-2897, 2003.

166. Rossi MA, Cecchini G. Lipid peroxidation in hepatomas of different degrees of deviation. *Cell Biochem Funct.* 1:49-54, 1983

167. Rossmann A, Henderson B, Heidecker B, Seiler R, Fraedrich G, Singh M, Parson W, Keller M, Grubeck-Loebenstein B, and Wick G. T-cells from advanced atherosclerotic lesions recognize hHSP60 and have a restricted T-cell receptor repertoire. *Exp Gerontol.* 43: 229-237, 2008.

168. Sajdel-Sulkowska EM, and Marotta CA. Alzheimer's disease brain: alterations in RNA levels and in a ribonuclease-inhibitor complex. *Science* 225: 947-949, 1984.

169. Salomon RG, and Gu X. Critical insights into cardiovascular disease from basic research on the oxidation of phospholipids: the γ-hydroxyalkenal phospholipid hypothesis. *Chem Res Toxicol* 24: 1791-1802, 2011.

170. Salomon RG, Kaur K, Podrez E, Hoff HF, Krushinsky AV, and Sayre LM. HNE-derived 2-pentylpyrroles are generated during oxidation of LDL, are more prevalent in blood plasma from patients with renal disease or atherosclerosis, and are present in atherosclerotic plaques. *Chem Res Toxicol*: 557-64, 2000.

171. Saltiel AR, and Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414: 799-806, 2001.

172. Sanders LH, and Greenamyre JT. Oxidative damage to macromolecules in human Parkinson disease and the rotenone model. *Free Radic Biol Med* 62: 111-120, 2013.

173. Savill J, Dransfield I., Gregory C, and Haslett C. A blast from the past: clearance of apoptotic cells regulates immune responses. *Nat Rev Immunol* 2: 965-975, 2002.

174. Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y, Tanaka T, Miwa S, Katsura Y, Kita T, and Masaki T. An endothelial receptor for oxidized low-density lipoprotein. *Nature* 386: 73-77, 1997.

175. Schägger H, and Ohm TG. Human diseases with defects in oxidative phosphorylation. 2. F1F0 ATP-synthase defects in Alzheimer disease revealed by blue native polyacrylamide gel electrophoresis. *Eur J Biochem* 227: 916-921, 1995.

176. Scheff SW, Price DA, Schmitt FA, Scheff MA, and Mufson EJ. Synaptic loss in the inferior temporal gyrus in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis* 24: 547-57, 2011.

177. Scofield RH, Kurien BT, and Reichlin M. Immunologically restricted and inhibitory anti-Ro/SSA in monozygotic twins. *Lupus* 6: 395-398, 1997

178. Scofield RH, Kurien BT, Ganick S, McClain MT, Pye Q, James JA, Schneider RI, Broyles RH, Bachmann M, and Hensley K. Modification of lupus-associated 60-kDa Ro protein with the lipid oxidation product 4-hydroxy-2-nonenal increases antigenicity and facilitates epitope spreading. *Free Radic Biol Med* 38: 719-28, 2005.

179. Seki S, Kitada T, Yamada T, Sakaguchi H, Nakatani K, and Wakasa K. In situ detection of lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver disease. *J Hepatol* 37: 56-62, 2002.

180. Sellin S, Holmquist B, Mannervik B, and Vallee BL. Oxidation and reduction of 4-hydroxyalkenals catalyzed by isozymes of human alcohol dehydrogenase. *Biochemistry* 30: 2514-2518, 1991.

181. Shamoto-Nagai M, Maruyama W, Hashizume Y, Yoshida M, Osawa T, Riederer P, and Naoi M. In parkinsonian substantia nigra, alpha-synuclein is modified by acrolein, a lipid-peroxidation product, and accumulates in the dopamine neurons with inhibition of proteasome activity. *J Neural Transm* 114: 1559-1567, 2007.

182. Shichiri M, Yoshida Y, Ishida N, Hagihara Y, Iwahashi H, Tamai H, and Niki E. α-Tocopherol suppresses lipid peroxidation and behavioral and cognitive impairments in the Ts65Dn mouse model of Down syndrome. *Free Radic Biol Med* 50: 1801-1811, 2011.

183. Shimaoka T, Kume N, Minami M, Hayashida K, Sawamura T, Kita T, and Yonehara S. LOX-1 supports adhesion of Gram-positive and Gram-negative bacteria. *J Immunol* 166: 5108-5114, 2001.

184. Siegel SJ, Bieschke J, Powers ET, and Kelly JW. The oxidative stress metabolite 4-hydroxynonenal promotes Alzheimer protofibril formation. *Biochemistry* 46: 1503-1510, 2007.

185. Smirnova IV, Kajstura M, Sawamura T, and Goligorsky MS. Asymmetric dimethylarginine upregulates LOX-1 in activated macrophages: role in foam cell formation. *Am J Physiol Heart Circ Physiol* 287: 782-790, 2004.

186. Starzyński RR, Canonne-Hergaux F, Willemetz A, Gralak MA, Woliński J, Styś A, Olszak J, and Lipiński P. Hemolytic anemia and alterations in hepatic iron metabolism in aged mice lacking Cu,Zn-superoxide dismutase. *Biochem J* 420: 383-390, 2009.

187. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, and Wittztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 320: 915-924, 1989.

188. Steinberg D. Role of oxidized LDL and antioxidants in atherosclerosis. *Adv Exp Med Biol* 369: 39-48, 1995.

189. Steinbrecher UP. Receptors for oxidized low density lipoprotein. *Biochim Biophys Acta* 1436: 279-298, 1999.

190. Sultana R, Boyd-Kimball D, Cai J, Pierce WM, Klein JB, Merchant M, and Butterfield DA. Proteomics analysis of the Alzheimer's disease hippocampal proteome. *J Alzheimers Dis* 11: 153-164, 2007.

191. Sultana R, Perluigi M, and Butterfield AD. Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. *Free Radic Biol Med* 62: 157-169, 2013.

192. Sun J, Hartvigsen K, Chou MY, Zhang Y, Sukhova GK, Zhang J, Lopez-Ilasaca M, Diehl CJ, Yakov N, Harats D, George J, Witztum JL, Libby P, Ploegh H, and Shi GP. Deficiency of antigen-presenting cell invariant chain reduces atherosclerosis in mice. *Circulation* 122: 808-820, 2010.

193. Sun M, Deng Y, Batyreva E, Sha W, and Salomon RG. Novel bioactive phospholipids: practical total syntheses of products from the oxidation of arachidonic and linoleic esters of 2-lysophosphatidylcholine. *J Biol Chem* 67: 3575-3584, 2002.

194. Sutti S, Jindal A, Vacchiano M, Gigliotti L, Bozzola C, and Albano E. Adaptive immune responses triggered by oxidative stress contribute to hepatic inflammation in NASH. *Hepatology* 59: 886-897, 2014.

195. Syn WK, Agboola KM, Swiderska M, Michelotti GA, Liaskou E, Pang H, Xie G, Philips G, Chan IS, Karaca GF, Pereira Tde A, Chen Y, Mi Z, Kuo PC, Choi SS, Guy CD, Abdelmalek MF, and Diehl AM. NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. *Gut* 61: 1323-1329, 2012.

196. Tamamizu-Kato S, Wong JY, Jairam V and Uchida K. Modification by acrolein, a component of tobacco smoke and age-related oxidative stress, mediates functional impairment of human apolipoprotein E. *Biochemistry* 46: 8392-8400, 2007.

197. Tjalkens RB, Cook LW, and Petersen DR. Formation and export of the glutathione conjugate of 4-hydroxy-2,3-E-nonenal (4-HNE) in hepatoma cells. *Arch Biochem Biophys* 361: 113-119, 1999.

198. Toyoda K, Nagae R, Akagawa M, Ishino K, Shibata T, Ito S, Shibata N, Yamamoto T, Kobayashi M, Takasaki Y, Matsuda T, and Uchida K. Protein-bound 4-hydroxy-2-nonenal. An endogenous triggering antigen of anti-DNA response. *J Biol Chem* 282: 25769-25778, 2007.

199. Trachootham D, Alexandre J, and Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov* 8: 579-591, 2009.

200. Traverso N, Menini S, Odetti P, Pronzato MA, Cottalasso D, and Marinari UM. Lipoperoxidation in hepatic subcellular compartments of diabetic rats. *Free Radic Biol Med* 26: 538-547, 1999.

201. Uchida K, Itakura K, Kawakishi S, Hiai H, Toyokuni S, and Stadtman ER. Characterization of epitopes recognized by 4-hydroxy-2-nonenal specific antibodies. *Arch Biochem Biophys* 324: 241-248, 1995.

202. Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Mizuno Y, Suzuki D, Miyata T, Noguchi N, Niki E, and Osawa T. Protein-bound acrolein: potential markers for oxidative stress. *Proc Natl Acad Sci U S A* 95: 4882-4887, 1998.

203. Uchida K, Sakai K, Itakura K, Osawa T, and Toyokuni S. Protein modification by lipid peroxidation products: formation of malondialdehyde-derived Ne-(2-propenal)lysine in proteins. *Arch Biochem Biophys* 346: 45-52, 1997.

204. Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog Lipid Res* 42: 318-343, 2003.

205. Usatyuk PV, and Natarajan V. Hydroxyalkenals and oxidized phospholipids modulation of endothelial cytoskeleton, focal adhesion and adherens junction proteins in regulating endothelial barrier function. *Microvasc Res* 83: 45-55, 2012.

206. van Tits LJ, Stienstra R, van Lent PL, Netea MG, Joosten LA, and Stalenhoef AF. Oxidized LDL enhances pro-inflammatory responses of alternatively activated M2 macrophages: a crucial role for Kruppel-like factor 2. *Atherosclerosis* 214: 345-349, 2011.

207. Vanden Heuvel JP. Peroxisome proliferator-activated receptors (PPARS) and carcinogenesis. *Toxicol Sci* 47: 1-8, 1999.

208. Vohra RS, Murphy JE, Walker JH, Ponnambalam S, and Homer-Vanniasinkam S. Atherosclerosis and the Lectin-like OXidized low-density lipoprotein scavenger receptor. *Trends Cardiovasc Med* 16: 60-64, 2006.

209. Wagner TM, Mullally JE, and Fitzpatrick FA. Reactive lipid species from cyclooxygenase-2 inactivate tumor suppressor LKB1/STK11: cyclopentenone prostaglandins and 4-hydroxy-2-nonenal covalently modify and inhibit the amp-kinase kinase that modulates cellular energy homeostasis and protein translation. *J Biol Chem* 281: 2598-2604, 2006.

210. Wang G, Pierangeli SS, Papalardo E, Ansari GAS, and Khan F. Markers of oxidative and nitrosative stress in systemic lupus erythematosus: correlation with disease activity. *Arthr Rheum* 62: 2064-2072, 2010.

211. Wang Z, Dou X, Gu D, Shen C, Yao T, Nguyen V, Braunschweig C, and Song Z. 4-hydroxynonenal differentially regulates adiponectin gene expression and secretion via activating PPAR and accelerating ubiquitin-proteasome degradation. *Mol Cell Endocrinol* 349: 222-231, 2012.

212. Watanabe K, Nakazato Y, Saiki R, Igarashi K, Kitada M, and Ishii I. Acrolein-conjugated low-density lipoprotein induces macrophage foam cell formation. *Atherosclerosis* 227: 51-57, 2013.

213. Weismann D, and Binder CJ. The innate immune response to products of phospholipid peroxidation. *Biochim Biophysica Acta* 1818: 2465-2475, 2012.

214. White MF. IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab* 283: 413-422, 2002.

215. Whitman S, Gezginci M, Timmermann BN, Holman TR. Structure-activity relationship studies of nordihydroguaiaretic acid inhibitors toward soybean, 12-human, and 15-human lipoxygenase. *J Med Chem* 45: 2659-2661, 2002

216. Winter CK, Segall HJ, Haddon WF. Formation of cyclic adducts of deoxyguanosine with the aldehydes trans-4-hydroxy-2-hexenal and trans-4-hydroxy-2-nonenal in vitro. *Cancer Res* 46: 5682-5686, 1986

217. Wuttge DM, Bruzelius M, and Stemme S. T-cell recognition of lipid peroxidation products breaks tolerance to self proteins. *Immunology* 98: 273-279, 1999.

218. Xie J, Zhu H, Guo L, Ruan Y, Wang L, Sun L, Zhou L, Wu W, Yun X, Shen A, and Gu J. Lectin-like oxidized low-density lipoprotein receptor-1 delivers heat shock protein-60 fused antigen into the MHC class I presentation pathway. *J Immunol* 185: 2306-2313, 2010.

219. Xu G, Liu Y, Kansal MM, and Sayre LM. Rapid cross-linking of proteins by 4-ketoaldehydes and 4-hydroxy-2-alkenals does not arise from the lysine-derived monoalkylpyrroles. *Chem Res Toxicol* 12: 855-861, 1999.

220. Xu QB, Oberhuber G, Gruschwitz M, Wick G Immunology of atherosclerosis cellular composition and major histocompatibility complex class II antigen expression in

aortic intima, fatty streaks, and atherosclerotic plaques in young and aged human specimens. *Clin. Immunol. Immunopathol*. 56: 344-359, 1990.

221. Yamada S, Kumazawa S, Ishii T, Nakayama T, Itakura K, Shibata N, Konayashi M, Sakai K, Osawa T, and Uchida K. Immunochemical detection of a lipofuscin-like fluorophore derived from malondialdehyde and lysine. *J Lipid Res* 42: 1187-1196, 2001.
222. Yao PL1, Morales JL, Zhu B, Kang BH, Gonzalez FJ, Peters JM. Activation of peroxisome proliferator-activated receptor-β/δ (PPAR-β/δ) inhibits human breast cancer cell line tumorigenicity. *Mol Cancer Ther* 13:1008-1017, 2014
223. Yoritaka A, Hattori N, Uchida K, Tanaka M, Stadtman ER, and Mizuno Y. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. *Proc Natl Acad Sci U S A* 93: 2696-2701, 1996.
224. Yoshida H, Kondratenko N, Green S, Steinberg D, and Quehenberger O. Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. *Biochem J* 334: 9-13, 1998.
225. Zamara E, Novo E, Marra F, Gentilini A, Romanelli RG, Caligiuri A, Robino G, Tamagno E, Aragno M, Danni O, Autelli R, Colombatto S, Dianzani MU, Pinzani M, and Parola M. 4-Hydroxynonenal as a selective pro-fibrogenic stimulus for activated human hepatic stellate cells. *J Hepatol* 40: 60-68, 2004.
226. Zandman-Goddard G, and Shoenfeld Y. Ferritin in autoimmune diseases. *Autoimmun Rev* 6: 457-463, 2007.
227. Zeher M, Szodoray P, Gyimesi E, and Szondy Z. Correlation of increased susceptibility to apoptosis of $CD4^+$ T cells with lymphocyte activation and activity of disease in patients with primary Sjögren's syndrome. *Arthritis Rheum* 42: 1673-1681, 1999.
228. Zhao G, Arosio P, and Chasteen ND. Iron(II) and hydrogen peroxide detoxification by human H-chain ferritin. An EPR spin-trapping study. *Biochemistry* 45: 3429-3436, 2006.

Figures legends

Figure 1. Mechanism of HNE formation during peroxidation of arachidonic acid.

Figure 2. Reaction of HNE with cysteinyl, histidyl, and lysyl residues via 1,4-Michael addition.

Figure 3. The inhibitory effect of covalent modification by HNE on the binding of plasminogen to α-enolase leads to the inhibition of adhesion of HL-60 human leukemic cells to Human Venous Endothelial Cells (HUVECs).

Figure 4. The formation of adducts of HNE with heme oxygenase (HO-1) might impair HO-1 function. In turn, the loss of HO-1 function might determine an increase of oxidative stress, resulting in increased HNE production from LPO.

Figure 5. Contribution of the products of LPO (LPOs) in LDL to the pathogenesis of atherosclerosis. LPOs implicated include the lysyl- adducts of MDA and the lysyl- and histidyl- adducts of HNE with apolipoproteins, as well as the 4-hydroxy-2,3-unsaturated core aldehydes ($oxPC_{CD36}$), like HODA-PC and HOOA-PC, and other aldehydes (e.g., POV-PC) produced by the oxidation of phosphatidylcholine (PC). Myeloperoxidase-initiated, ROS-dependent LDL oxidation in plasma impairs LDL binding to LDL-R and improves their binding to scavenger receptors CD36 and LOX-1 in endothelial cells and macrophages, while also upregulating them. This has two consequences: 1) increased ROS production by macrophages, with conversion into dysfunctional, lipid-laden foam cells; 2) endothelial cell dysfunction, with increased ROS and chemokine MCP-1 release, increased expression of adhesion molecules (which promotes monocyte infiltration), and NF-KB-induced apoptosis. By binding to CD36 and LOX-1, oxLDL also promote the maturation of vascular-associated DCs (VADCs), activate platelets, and stimulate the production of HSP-60 by monocytes and endothelial cells. HSP-60, which also binds to LOX-1, ushers the inflammatory response into a cell-mediated adaptive response, being itself a prominent target of

it, by inducing the maturation of APCs and delivering self antigens of apoptosed cells into their MHC-I-associated presentation pathway.

Figure 6. Proposed mechanisms for the breaking of tolerance to self antigens upon formation of adducts of HNE. A) Neoepitopes are generated by the covalent modification of macromolecular self antigens with HNE. B) HNE-protein adducts stimulate differentiation and maturation of macrophages (Mφ), dendritic cells (DCs) and endothelial cells (ECs), with upregulation of scavenger receptors, which facilitates their uptake, and expression of costimulatory molecules, which permit their efficient presentation to neoepitope-recognizing $CD4^+$ T cells. These are selected outside the repertoire of autoreactive T cells, which were either clonally deleted or put under intrinsic or extrinsic regulatory control. B1) Once differentiated in effector T_H2 cells, these cooperate in the differentiation of cognate non-autoreactive B cells, recognizing neoformed epitopes with their B Cell Receptors (BCRs), into memory B and plasma cells. B2) Cooperation from neoepitope-specific T_H2 cells is provided also to B cells which internalize HNE-modified macromolecular antigens via BCRs recognizing native self epitopes, but present both these and HNE-related neoepitopes at their surface. This leads to the differentiation of plasma cells secreting true autoantibodies. C) APCs which uptake and process HNE-modified antigens also present the entire repertoire of HNE-modified and native self epitopes, thus recruiting into the adaptive response T cells with autoreactive T cell receptors (TCRs), as well. Reinforcement to the expression of costimulatory molecules provided to these APCs from non-autoreactive T cells recognizing HNE-related neoepitopes helps them to overcome the anergy of autoreactive T cells recognizing native self epitopes, leading to the differentiation of autoreactive effector T_H2 cells and autoantibody-secreting plasma cells.

Figure 7. Molecular mimicry between the *R*-HNE-histidine and the 7-(2-oxo-heptyl)-substituted 1,N^2-etheno-type ONE-2'-deoxyguanosine adducts. Background shades of grey highlight shared or closely resembling functional groups implicated as the

constituents of a common epitope, responsible for the molecular mimicry between the two adducts and required for recognition by bispecific antibodies. Color-code: *light grey*, 2'-deoxyribose-like tetrahydrofuran rings; *dark grey*, hydroxyl groups; *dotted grey*, nitrogen-containing heterocyclic groups (histidine and guanine). Likely, also the shared alkyl (pentyl) groups of the HNE-histidine and ONE-2'-deoxynucleoside adducts (in bold) are involved in the recognition by antibodies (2).

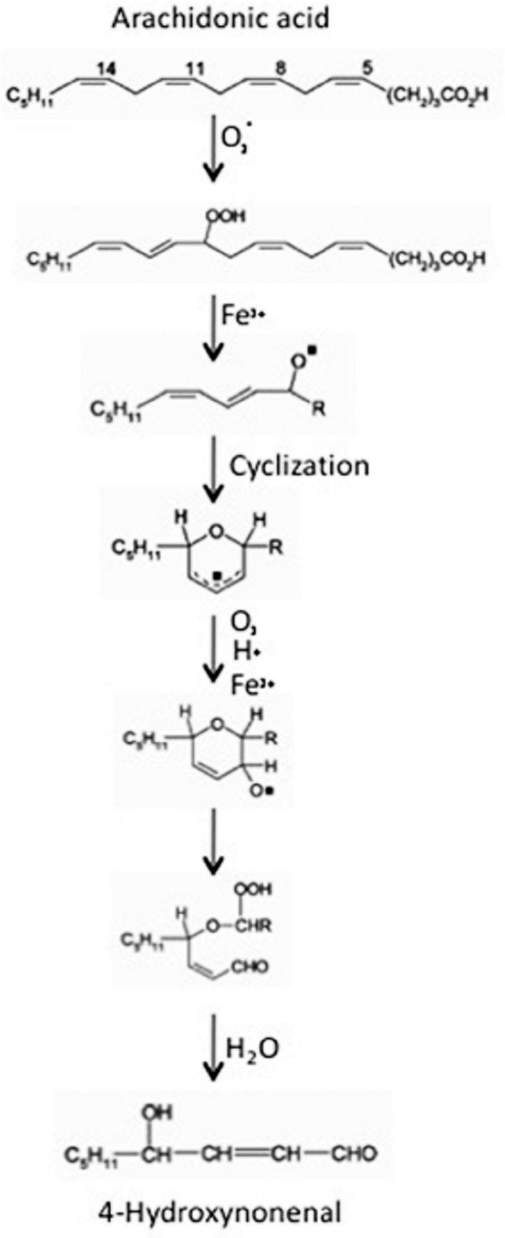
Arachidonic acid
14
11
8
5
C_5H_{11}
$(CH_2)_3CO_2H$
$O_2^{\cdot}$
OOH
Fe^{3+}
O
R
Cyclization
H
O
O_2
H^+
Fe^{3+}
OOH
CHR
CHO
H_2O
OH
CH—CH═CH—CHO
4-Hydroxynonenal

Fig 1

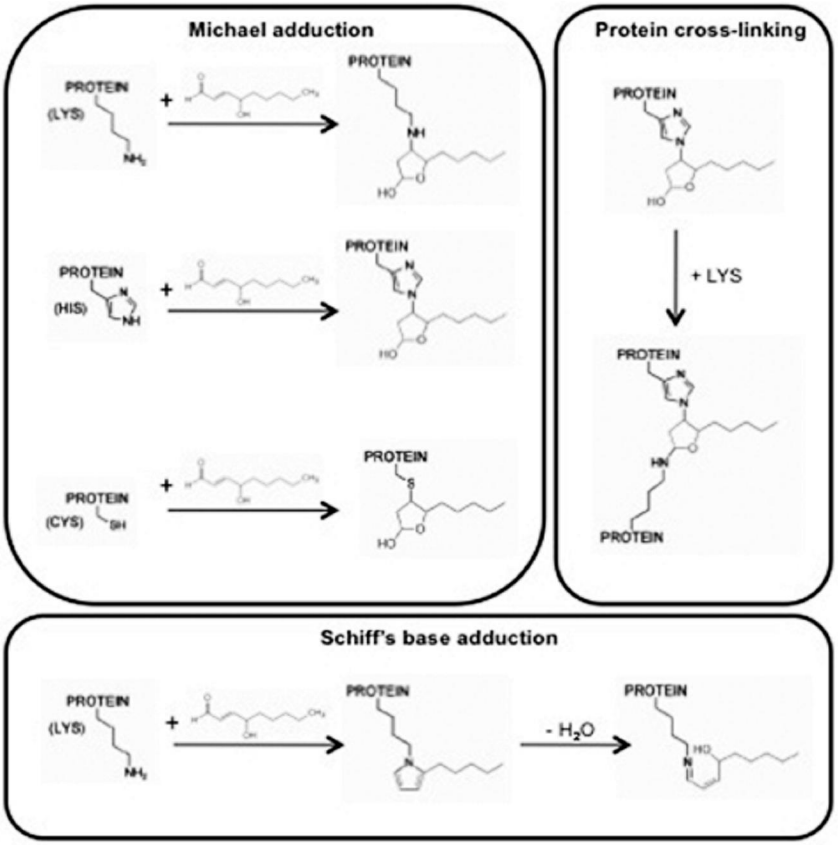
Michael adduction
PROTEIN
(LYS)
PROTEIN
PROTEIN
(HIS)
PROTEIN
PROTEIN
(CYS)
SH
PROTEIN
Protein cross-linking
PROTEIN
+ LYS
PROTEIN
PROTEIN
Schiff's base adduction
PROTEIN
(LYS)
PROTEIN
- H2O
PROTEIN

Fig 2

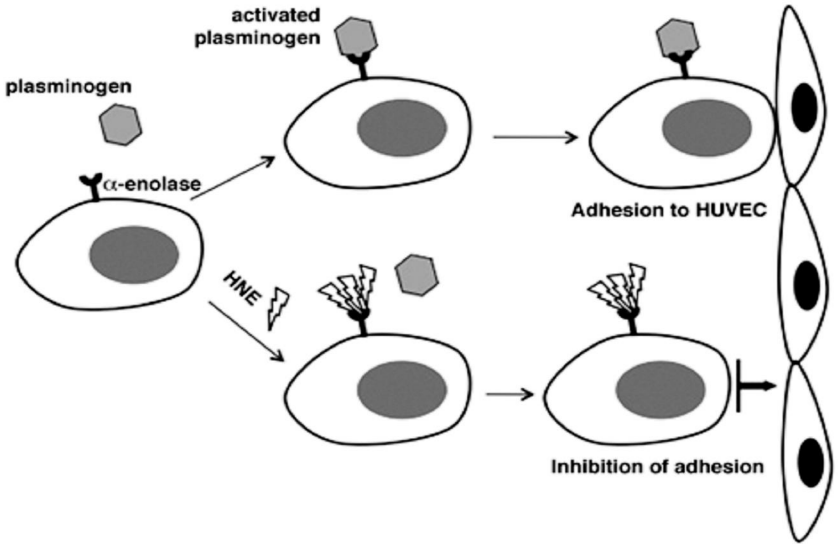
activated
plasminogen
plasminogen
α-enolase
HNE
Adhesion to HUVEC
Inhibition of adhesion

Fig 3

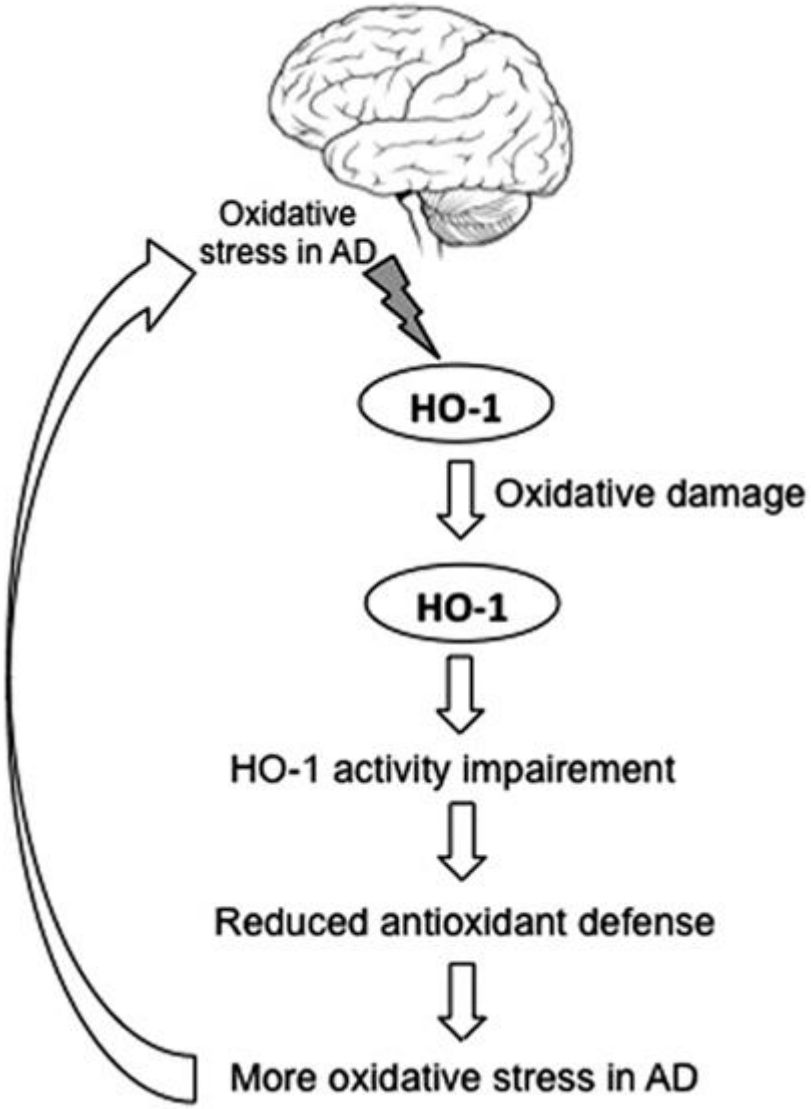
Oxidative
stress in AD
HO-1
Oxidative damage
HO-1
HO-1 activity impairement
Reduced antioxidant defense
More oxidative stress in AD

FIG 4

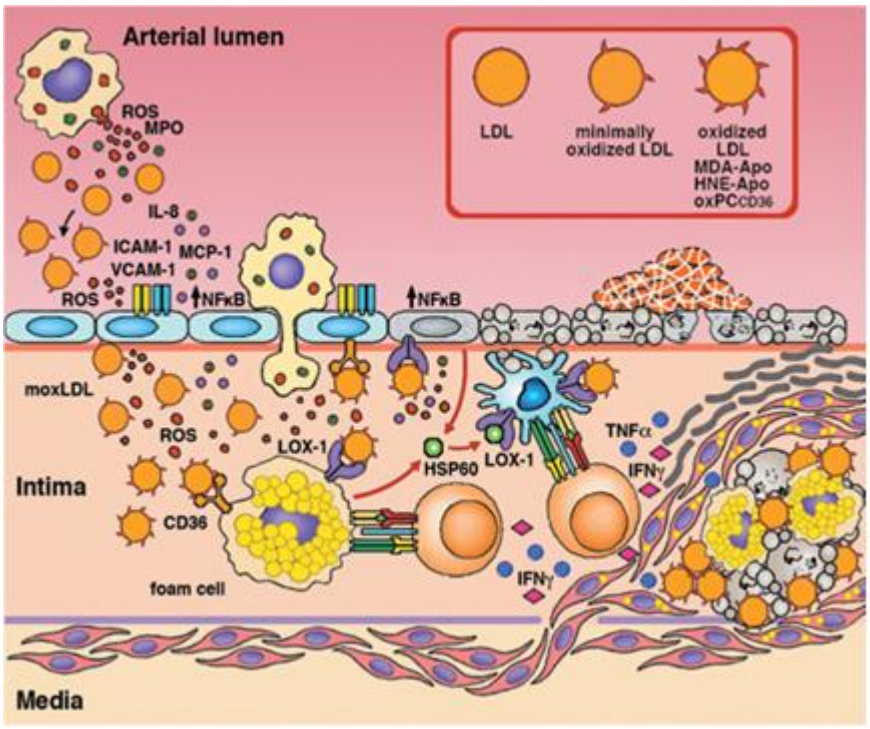
Arterial lumen
ROS
MPO
IL-6
ICAM-1
MCP-1
VCAM-1
ROS
NFκB
NFκB
LDL
minimally oxidized LDL
oxidized LDL
MDA-Apo
HNE-Apo
oxPCCD36
moxLDL
ROS
LOX-1
HSP60
LOX-1
TNFα
IFNγ
Intima
CD36
foam cell
IFNγ
Media

FIG 5

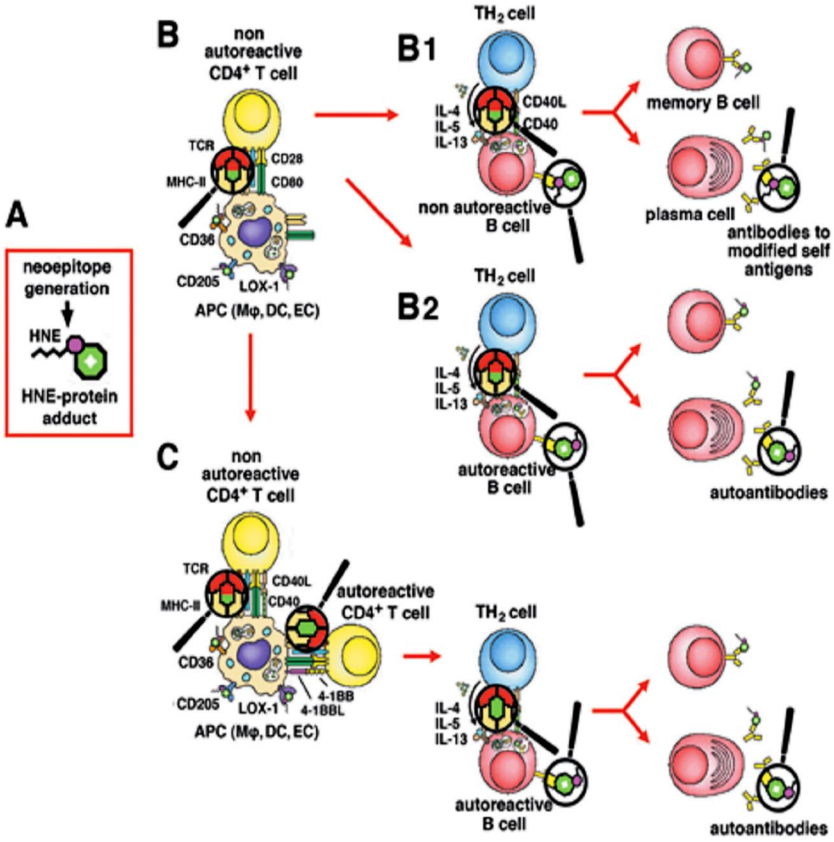
A
neoepitope generation
HNE
HNE-protein adduct
B
non autoreactive CD4+ T cell
TCR
CD28
MHC-II
CD80
CD36
CD205
LOX-1
APC (Mφ, DC, EC)
B1
TH2 cell
IL-4
IL-5
IL-13
CD40L
CD40
non autoreactive B cell
memory B cell
plasma cell
antibodies to modified self antigens
B2
TH2 cell
IL-4
IL-5
IL-13
autoreactive B cell
autoantibodies
C
non autoreactive CD4+ T cell
TCR
CD40L
MHC-II
CD40
autoreactive CD4+ T cell
CD36
CD205
LOX-1
4-1BB
4-1BBL
APC (Mφ, DC, EC)
TH2 cell
IL-4
IL-5
IL-13
autoreactive B cell
autoantibodies

Fig 6

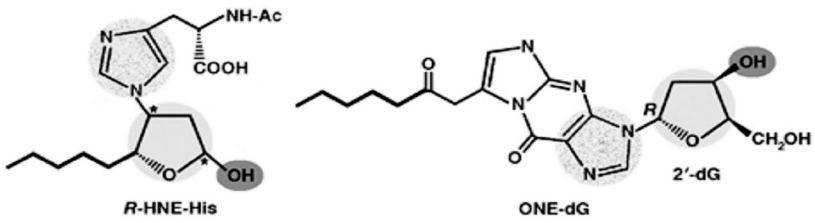
NH-Ac
COOH
OH
R-HNE-His
O
N
OH
CH2OH
2'-dG
ONE-dG

FIG 7