[Frontiers in Bioscience 9, 3145-3155, September 1, 2004]

MECHANISMS OF ALCOHOL LIVER DAMAGE: ALDEHYDES, SCAVENGER RECEPTORS, AND AUTOIMMUNITY

Michael J. Duryee [1,2], Monte S. Willis [3], Thomas L. Freeman [1,2], Charles A. Kuszynski [4], Dean J. Tuma [1,2], Lynell W. Klassen [1,2], and Geoffrey M. Thiele [1,2,4]

[1] University of Nebraska Medical Center, Department of Internal Medicine, 988090 Nebraska Medical Center, Omaha, NE 68198-8090, [2] Veterans Administration Alcohol Research Center, Omaha Veterans Administration Medical Center, 4101 Woolworth Avenue, Omaha, NE 68105,. [3] UT Southwestern, Department of Pathology, 5323 Harry Hines Blvd, Dallas, TX 75390-9072, [4] University of Nebraska Medical Center, Department of Pathology and Microbiology, 986495 Nebraska Medical Center, Omaha, NE 68198-6495

TABLE OF CONTENTS

1. ABSTRACT

While most of the investigations into the causative events in the development of alcoholic liver disease (ALD) have been focused on multiple factors, increasing interest has centered around the possible role of immune mechanisms in the pathogenesis and perpetuation of ALD. This is because many of the clinical features of ALD suggest that immune effector mechanisms may be contributing to liver tissue damage, as evidenced by the detection of circulating autoantibodies, and the presence of CD4+ and CD8+ lymphoid cells in the livers of patients with ALD. One mechanism that has been associated with the development of autoimmune responses is the modification (haptenation or adduction) of liver proteins with aldehydes or other products of oxidative stress. This is because it has been shown that these adducted proteins can induce specific immune responses, to the adduct, the adduct plus protein (conformational antigens), as well as the unmodified parts of the protein. More importantly, it is possible to demonstrate that adducted self-proteins can induce reactivity to the normal self-protein and thereby induce autoimmune responses. Therefore, it is the purpose of this manuscript to outline the mechanism(s) by which these modified self proteins can induce autoimmune reactivity, and thus play a role in the development and/or progression of ALD.

2. INTRODUCTION

The underlying mechanisms of alcoholic liver disease (ALD) are not clear. However, recent reviews have outlined several lines of evidence suggesting alterations in the immune response (1,2) including, elevation in serum immunoglobulin levels, alterations in the CD8+/CD4+ T-cell subsets, and changes in the normal cytokine balance. Dysfunction, resulting in immunosuppression, can lead to diseases like pneumonia, tuberculosis, and possibly HIV infections. Chronic alcohol abuse, also can produce diseases with autoimmune components, which include; alcoholic hepatitis, alcoholic cirrhosis, and the presence of circulating autoantibodies specific for lymphocytes, brain, DNA, serum lipoproteins, and liver proteins.

One mechanism that has been associated with the development of these autoimmune responses is the modification of liver proteins with aldehydes or other products of oxidative stress. Acetaldehyde, a product of

ethanol metabolism, has been shown to form stable adducts recognized by the immune system as foreign. Studies done by Israel *et al*, have shown a type I hypersensitivity reaction, yielding IgE antibodies against acetaldehyde adducts, when mice were immunized with plasma proteins modified with acetaldehyde (3). Human studies showed an increase in anti-acetaldehyde-protein IgE antibodies among individuals consuming alcoholic beverages (3). IgG and IgA antibodies against anti-acetaldehyde adducts were found to be elevated in patients with alcoholic liver disease. Also found was IgG antibodies directed against anti-malondialdehyde (MDA) adducts, indicating that both lipid peroxidation and alcohol metabolism are involved, or in the formation of these adducts (4,5).

Malondialdehyde adducts have been implicated in the development of autoimmune diseases resulting from adduct formation. Anti-MDA adducts were induced using trichloroethene (TCE) in MRL +/+ mice (6). TCE causes oxidative stress, which results in lipid peroxidation products and the induction of anti-MDA adducts. These anti-MDA adducts were observed in patients with systemic lupus erythematosus (SLE) and found to correlate with anti-nuclear and anti-cardiolipin antibody markers (6). Also the production of MDA adducts strongly correlates with the development of atherosclerosis. These data suggests a role for oxidative stress and the formation of adducts capable of causing autoimmune disease.

Recently, a new adduct was produced by reacting malondialdehyde and acetaldehyde with proteins to produce hybrid adducts (7). Immune responses to malondialdehyde-acetaldehyde (MAA) adducts have shown correlation with alcoholic liver disease in humans (8). This is of interest because these MAA modified (haptenated) proteins are immunogenic (7,9) and have led to the proposal that immune responses against these proteins may play a role in the initiation and/or propagation of alcoholic liver disease.

Scavenger receptors have been implicated in the onset of autoimmune diseases by their ability to bind aldehyde and other modified proteins. This has been shown in a study using maleylated mouse serum albumin (maley MSA) to break T-cell tolerance, where in the self adducted protein binds scavenger receptors, is internalized, processed and presented in MHC class II. The ability to present self peptide in MHC Class II provides an ideal model for the study of tolerance and autoimmune disease (10). Studies using oxidized LDL in scavenger receptor type A knock out mice have shown similar results. The SR-A negative mice had no proliferative response to oxidized LDL, however, when SR-A positive antigen presenting cells from spleens were added back into the cultures a strong T-cell response was observed (11). SR-A involvement in the processing of antigen and subsequent presentation to T-cells provides a way for damaged macromolecules to become immunogenic. For example, scavenger receptors located on tissue macrophages are involved in the clearance of haemoglobin released from ruptured erythrocytes (12). Intravascular haemolysis is accelerated in patients with autoimmune disease which may be due in part to the up regulation of costimulatory molecules or an alteration in receptor function.

Further evidence suggesting the involvement of scavenger receptors is found in the binding of MAA modified proteins, and their ability to induce cell death (13-15). Recently, apoptosis has been suggested to be involved in the development of autoimmune diseases (17). Apoptosis is important when the immune system has cleared an infection and needs to reduce the rapidly proliferating lymphocytes back to normal levels. If these materials are not removed in the appropriate manner, (i.e. die by necrosis or taken up by dendritic cells) intercellular (18) proteins may become antigenic and possibly lead to autoimmunity (18,19). SLE is one disease that suggests a break down in apoptosis, as patients have been shown to have circulating antibodies directed against dsDNA, and autoantibodies may have been produced by the activation of polyclonal B cells specific for cellular contents following tissue distruction (19). Apoptosis has also been shown to induce protein modifications such as (de)ubiquitination, methylation, and citrullination. These modifications may change the protein structure, which makes them antigenic, and results in the production of autoantibodies to cellular proteins. This could explain the anti-citulline antibodies found in patients with rheumatoid arthritis (RA) (20,21).

Taken altogether, the observation that MAA modified proteins can induce cell death, upregulate adhesion molecules expression, and induce pro-inflammatory cytokine release, it can be speculated that these modified proteins could play a role in the development of an autoimmune disease. To better understand the role of these adducts and their potential to induce an autoimmune like disease of the liver, would greatly enhance the knowledge of not only alcoholic liver disease, but also other autoimmune diseases as well. Therefore, it is the purpose of this manuscript to review the most recent information with respect to the immune system and alcoholic liver disease.

3. AUTOANTIBODIES AND ALCOHOLIC LIVER DISEASE (ALD)

The onset of ALD has often been associated with circulating antibodies and lymphocytes specific to hepatic antigens (22,23). Antibodies against acetaldehyde adducts (24,25) and hydroxyethyl-free radicals (26) have been implicated in ALD. Recently, malondialdehyde and acetaldehyde have been shown to react synergistically to form a hapten named MAA (Malondialdehyde-Acetaldehdye) (7). The MAA hapten is significant because the constituent aldehydes malondialdehyde (a product of lipid peroxidation) and acetaldehyde (a product of alcohol metabolism) are present during chronic ethanol consumption and react with proteins to a greater extent than either aldehyde alone, and at levels that are near proposed physiologic levels (7).

Evidence for the relationship between the MAA hapten, the immune system, and alcoholic liver disease has

recently been described. Specifically, circulating antibodies against malondialdehyde-acetaldehyde (MAA) haptenated proteins were found to be significantly increased in patients with alcohol-induced cirrhosis and hepatitis, and correlates well with the severity of liver damage (8). In this study, sera from 4 groups of patients were tested against human serum albumin haptenated with malondialdehyde-acetaldehyde (HSA-MAA) in: 1) 50 patients with ALD; 2) 40 patients with non alcoholic liver disease (NALD); 3) 15 heavy drinkers (HD); and 4) 40 healthy individuals (8). The mean optical density (O.D.) for IgG binding to HSA-MAA was found to be: 0.42 (ALD group); 0.18 (NALD group); 0.13 (HD group); and 0.09 (controls) (8). Analysis of the ALD patient samples for isotypes demonstrated that the predominate response to HSA-MAA was IgG, although a weaker IgM response was seen (8). When comparing the Maddrey's DF index (indicating severity of liver damage) for ALD patient's with the amount of IgG antibody generated, there was a higher anti-MAA titer as the amount of liver damage increased (8). Inhibition assays using hexyl-MAA (a synthetic analogue of MAA) found that the IgG response to HSA-MAA was inhibited by 52% in the ALD patients while no inhibition was observed in NALD patients (8). This indicates that antibody response was specific for the MAA hapten and correlated with alcohol induced liver damage.

3.1. Haptens and (Auto)Immunity

Malondialdehyde-acetaldehyde haptenated proteins have been shown to be immunogenic experimentally in animals without the use of adjuvants (9). These antibodies were specific for the carrier protein at relatively low levels, and for the MAA hapten as the amount of protein immunized was increased (9). Interestingly, when another model protein hen egg lysozyme (HEL) was MAA haptenated, the only antibody response induced was IgG1 specific for the carrier protein, but not the hapten itself (27). Taken together, MAA haptenated proteins have the ability to induce immune responses to; the hapten, the site where the hapten meets the carrier protein, and the carrier protein. Thus, the response to the carrier proteins represents a potential mechanism of autoantibody production wherein exogenous proteins could result in the induction of an autoimmune response.

Dendritic cells have been proposed to play a central role in inducing immune responses and have been used experimentally as adjuvants themselves (28). Recently, chemical haptens have been shown to induce dendritic cell activation, upregulate cytokines, and co-stimulatory molecules (29-33). An example of this would be how, 2, 4-dinitrochlorobenzene (DNCB), 2,4,6-trinitrochlorobenzene (TNCB), 2, 4-dinitrofluorobenzene (DNFB), $NiCl_2$, $MnCl_2$, $CoCl_2$, $SnCl_2$, and $CdSO_4$ can augment their expression of CD86 (B7-2) or HLA-DR (33). MAA haptenated proteins have similarly shown an upregulation on antigen presenting cells of the co-stimulatory molecules B7-1 and B7-2. Splenocytes stimulated with 125 micrograms/ml of protein modified with MAA increased B7-1 cell surface expression greater than 4 fold and increased B7-2 expression greater than 2 fold in as little as three hours in vitro (34). Additionally, it appears that these MAA haptenated proteins bind to scavenger receptors on these cells prior to the expression of co-stimulatory molecules and may be one mechanism by which these molecules are modulated (35).

3.2. Haptens, Self tolerance, and Autoimmunity

When proteins are haptenated with maleylated proteins, they are able to induce antibody responses experimentally in a similar manner as has been reported to MAA modified proteins (10,36,37). In fact they have been shown to break tolerance to self proteins in vivo experimentally (36). Other studies in our laboratory have shown that MAA modification is able to break tolerance to mouse serum albumin and mouse cytosol. Interestingly, the antibody responses were primarily to the carrier protein as has been previously reported for exogenous proteins modified with MAA (27). Finally, both maleylated proteins and MAA modified proteins appear to mediate their immunogenicity through scavenger receptors (10,36,37).

3.3. Scavenger Receptors and Haptens

Scavenger receptors were first described in macrophages as alternative receptors to the LDL receptor in the uptake of excessive cholesterol, which leads to the formation of foam cells (38). Since this original description, a broad array of ligands for several classes of scavenger receptors have been described (38). In general, organic acid anhydrides, peroxides, and aldehydes (39) result in the modification of positively charged lysine residues in proteins rendering them highly negatively charged making them ideal scavenger receptor ligands (40).

The binding of aldehyde modified proteins to scavenger receptors has been described by a number of investigators (39,41-44). Receptors specifically recognizing aldehyde modified proteins were first described using formaldehyde modified albumin (F-alb) (41). The specificity of this receptor was demonstrated by inhibiting the binding of albumin modified with a number of other aldehydes (glycolaldehyde, DL-glyceraldehyde, and propionaldehyde) (41).

The glycation of proteins is a complex series of reactions between reducing sugars and amino groups of proteins, resulting in many aldehydes and ketones being formed such as alpha-ketoaldehydes (glyoxal, 3-deoxyglucosone (3-DG), and glucosone) (45). The aldehyde methylglyoxal (MG), a glycolytic product, is produced from the autoxidation of sugars and glycation (45). These alpha-ketoaldehydes respresent highly reactive intermediates in the glycation process and are responsible for the formation of advanced glycation end products (AGE) (45). Experimentally, the binding of AGE modified BSA has been shown to be inhibited with BSA modified formaldehyde and glycoaldehyde, indicating specificity to a scavenger receptor (43). This inhibition shows the broad range of binding the scavenger receptor has to aldehyde modified proteins. Many scavenger receptors have been shown to recognize proteins modified with AGE including

scavenger receptor Class A types I and II (46,47), and Scavenger receptor B (Class I and CD36) (48).

Oxidized low density lipoprotein (ox-LDL) has been shown to be an important mediator of atherosclerosis, in which the immune system plays a key role (49) and is formed by adduct formation between apolipoprotein and reactive aldehydes such as 4-hydroxynonenal and malondialdehyde (50,51). Polyunsaturated fatty acids in cholesterol esters, phospholipids, and triglycerides are subject to free radical induced oxidation (lipid peroxidation) which leads to the production of smaller fragments 3-9 carbons in length including aldehydes such as malondialdehyde and 4-hydroxyalkenals (45). These aldehyde haptenated proteins appear to generate a wide variety of determinants that lead to cellular and humoral immune responses in vivo (49,52,53). Interestingly, MAA modified proteins have been identified in areas of atherosclerosis in samples where ox-LDL is certainly present (54,55). The mechanism by which these aldehyde modified proteins become immunogenic has been reported to be mediated through the scavenger receptor A in a mouse model (11). The exact pathway, however, as not been clearly delineated.

Acetaldehyde modified proteins have also been shown to bind scavenger receptors. Rats perfused in situ with I^{125}-Alb modified with acetaldehyde demonstrated that binding and degradation occurred in endothelial cells (56). These findings paved the way for the characterization of the physiologically relevant hybrid adduct malondialdehyde-acetaldehyde adduct and its interaction with the scavenger receptor. Recent work in our laboratory has shown that MAA-modified haptens become bound and degraded by both activated peritoneal macrophage and liver sinusoidal endothelial cells (SECs) (9,57,58). Exposure of MAA to activated peritoneal macrophages resulted in the binding of 8.76 nanograms MAA-Alb/10^6 cells and degradation of 14.26 micrograms MAA-Alb/10^6 cells. However, when F-Alb was incubated with these cells binding was 12.3 nanograms MAA-Alb/10^6 cells and degradation was only 6.78 micrograms MAA-Alb/10^6 cells. When 100 X cold F-Alb was used as a specific aldehyde competitor for the binding and degradation of MAA, no inhibition was observed, indicating that receptors on peritoneal macrophage have different specificities or a higher affinity for the MAA epitopes. When LECs were exposed to MAA, binding of 20.4 nanograms MAA-Alb/10^6 cells and degradation of 9.52 micrograms MAA-Alb/10^6 cells was observed (9,57,58). Inhibition studies were performed and both binding and degradation could be inhibited with F-Alb and cold MAA-Alb indicating specificity to a scavenger type receptor for aldehyde modified proteins. When the same experiments were done with rats chronically fed alcohol, SEC degradation of MAA-Alb was impaired by 40-60% (9,58), and this impairment was found to be a result of a defect in the post internalization step of the product, rather than in the binding, internalization, or degradation. While MAA modified proteins appear to bind to immune cells (i.e. macrophages), the affects upon these cells show; enhanced binding, increased co-stimulatory effects, and increased antigen presentation. An increase in cell death also can occur in a dose response manner, (at high doses) to any protein haptenated with MAA.

4. APOPTOSIS AND ALCOHOLIC LIVER DISEASE

Apoptosis plays a role in the liver which can be attributed to four potential causes: 1) physiologic maintenance; 2) toxic substances; 3) viral infections; and 4) carcinoma (7). Toxic substances such as ethanol, acetaminophin, bile salts, cytostatic drugs, copper, and a number of related toxins have been shown to cause apoptosis and result in the initiation of various diseases (59). In vitro studies have demonstrated that the addition of as little as 50mM ethanol to cultured rat hepatocytes increases the number of apoptotic cells (60). This apoptosis does not involve cytotoxic substances from nonparenchymal cells such as TNF-alpha, nitric oxide, or other cytokines (60). Using 4-methylpyrazole (an alcohol dehydrogenase inhibitor) and dimethylthiourea (a membrane permeable hydrogen peroxide scavenger), ethanol induced apoptosis was shown to be blocked (60) suggesting that metabolism of ethanol by alcohol dehydrogenase leads to an excessive build up of hydrogen peroxides, resulting in oxidative stress to hepatocytes and the formation of free radicals capable of cellular damage (60).

Experimentally, apoptosis has been found in models of alcoholic liver injury. In ethanol fed rats, altered hepatocytes (mitochondrial pleomorphism, increased smooth endoplasmic reticulum and deposition of small lipid droplets) and apoptotic bodies observed in the parenchymal cell population have been observed (61). In mice exposed to ethanol vapors, a 57% increase in apoptotic bodies in liver sections over control animals have been observed, and when alcohol exposure was stopped the number of apoptotic bodies decreased significantly compared to controls (62).

Rats fed ethanol intragastrically have significant liver damage including higher levels of apoptosis (63). The most significant levels of apoptosis correlated with liver damage with concomitant ingestion of polyunsaturated fatty acids in corn oil, fish oil, and ethanol (63). These polyunsaturated fatty acids induce lipid peroxidation, which has been implicated in the production of peroxides, free radicals, and malondialdehyde. As discussed below these products have been shown to induce apoptosis and necrosis (63). Thus, alcohol induced liver disease, like other liver disease, may be mediated in part by apoptotic and necrotic cell processes.

5. CELL DEATH AND AUTOIMMUNITY

5.1. Apoptosis, necrosis, and immune responses

There are a number of mechanisms which prevent apoptotic cells from becoming immunogenic including : 1) NF-kappa B activation of apoptotic molecules during inflamation; 2) the release of anti-inflammatory products by phagocytes when apoptotic cells are present; 3) normal phagocytosis by macrophages; and

4) inflammatory mediators affecting the efficiency of the phagocytosis of apoptotic cells. The breakdown of any of these defense mechanisms may result in the emergence of an autoimmune response (64,65). This has been demonstrated experimentally by the ability of dendritic cells to process and present particles of the apoptotic cell in MHC Class I and II to T-cells (65).

The immunogenicity of apoptotic cells has been reported using influenza as a model (66). Using dendritic cells (DCs) co-cultured with influenza infected human monocytes, it has been demonstrated that infected monocytes are recognized by the DCs, processed and presented in MHC Class I to T-cells, thereby inducing a T cytotoxic (CTL) response (66). Dendritic cells phagocytose apoptotic cells by the scavenger receptor CD36, alpha V beta 5, and a phosphatidylserine receptor (66). After DCs bind apoptotic cells, they enter the endocytic pathway where antigens are loaded onto MHC Class II in a regulated manner within non-lysosomal, late endosomal compartments (67). This may be where peptides are loaded onto MHC Class I molecules, or another "phagosome-cytosol" pathway where apoptotic cells enter the cytosol and are processed by the endogenous MHC Class I pathway (68).

Necrosis results in the leakage of cellular contents as a result of exposure to stressful stimuli including environmental factors (toxic substances) and infections. The release of cellular contents, such as heat shock proteins (HSP), has been shown to result in the infiltration of inflammatory cells (69). It has been demonstrated that HSPs released after infections are related to autoimmune disease (70). The maturation of dendritic cells, the main antigen presenting cell in inducing primary responses, by HSP proteins has been demonstrated (25). Immature dendritic cells exposed to cell lysates with hsp70 demonstrated a 4 fold increase in uptake of cell lysates over cell lysates without hsp70 (25). Additionally, MHC class I and class II expression was increased in dendritic cells when cell lysates were given in the presence of hsp70 (25). The activation of dendritic cells by this intracellular protein may allow dendritic cells to enhance their capacity to capture antigen at times when large amounts of tumor antigen or cell debris are present. Another study has demonstrated that necrotic, but not apoptotic cells release these heat shock proteins and deliver maturation signals which activate the NF-kappa B pathway (71). HSPs such as gp96, calreticulin, hsp90 and hsp70 increase the levels of TNF-alpha, IL-12, IL-1beta and may represent conserved molecules (bacteria to mammals) that activate the adaptive immune system in vivo (71).

The cross-presentation of antigen (exogenous antigen presented in MHC Class I) acquired from dying cells has been demonstrated in immature dendritic cells (72). The expression of CD83, DC-LAMP (a maturation-associate markers), CD86 (co-stimulatory markers), and class I and II increased after exposure to necrotic human tumor cell lines (72). The release of a molecule in the supernatant (most likely TNF-alpha or IL-1beta) was implicated in the maturation of DCs and subsequent proliferation of CD8+ T cells (72). It is in this way that necrotic cells might induce high levels of mRNA for heat shock proteins that target antigens to immature DCs (25,73,74). The potential then exists for HSP bound self peptide(s) to be released from a cell, be taken up by a DC, mature, and present this peptide in MHC Class I with the appropriate co-stimulatory signals to T-cells, resulting in an autoimmune disease.

5.2. Acetaldehyde, Malondialdehyde, 4-Hydroxynonenal (4-HNE), and Cell death

A number of studies have implicated proteins haptenated with aldehydes associated with alcohol consumption (acetaldehyde) and lipid peroxidation (malondialdehyde and 4-HNE) in the induction of cell death (apoptosis and necrosis) in several cell types such as lymphocytes, macrophage/monocytes, and hepatic cells (75-78). Wickramasinghe and colleagues have reported that cytotoxic proteins are generated in the serum of healthy volunteers consuming ethanol and can be mimicked by acetaldehyde haptenated serum proteins, mainly albumin (79-81). Additionally, it has been shown that macrophage have the ability to form acetaldehyde haptens when exposed to ethanol and therefore produce cytotoxic serum proteins (76-78,82-87), implicating a role of macrophage in potentiating cytotoxicity. In other studies it has been shown that acetaldehyde haptenated proteins have the ability to inhibit IL-2 secretion in Con A stimulated murine splenocytes (88,89), which indicates that these haptens might be able to inhibit the proliferation of T cells in vivo. Damage and hapten formation mediated by 4-HNE has been correlated in murine alveolar macrophage and other cells found in the lung (90,91). The cytotoxicity and inhibition of proliferation of malondialdehyde adducts has currently been unreported.

5.3. Oxidized LDL

Oxidized low density lipoprotein (ox-LDL) has been shown to be an important mediator of atherosclerosis and is characterized by adduct formation between apolipoprotein and reactive aldehydes such as 4-hydroxynonenal and malondialdehyde, which are products of lipid peroxidation (50,51). The distinct recognition of ox-LDL has been determined to be complex because it is a heterogeneous ligand. Pyrrole adducts on ox-LDL are recognized by scavenger receptor A (SR-A) and CD36 while the pyridinium adducts on ox-LDL are recognized by CD36 but not SR-A. Oxidized LDL exposure leads to apoptosis (92,93) mediated by partial lysosomal rupture (93). Normal content of lysosomal iron may play an important role in oxLDL-induced cell damage, presumably by catalyzing intralysosomal fragmentation of lipid peroxides and the formation of toxic aldehydes and oxygen-centered radicals (93).

5.4. MAA Haptenated Proteins

Malondialdehyde-acetaldehyde (MAA) haptenated proteins have been shown to induce a dose dependent cell death in antigen presenting cells, lymphocytes, and hepatocytes in vitro (13,14,94). The exposure of splenocytes to MAA haptenated protein demonstrated significant levels of necrosis compared to

controls in as little as 5 hours of exposure (13). DNA degradation in T cell and B cells demonstrated that apoptosis occurred after 24 hours at relatively lower doses of MAA haptenated protein as demonstrated by the JAM test, TUNNEL assays, and DNA ladders (13). These data demonstrate that lower levels of HEL-MAA incubated for shorter time points will cause cells to undergo apoptosis, however increases in concentration or longer incubation times will cause cells to undergo necrosis.

5.5. Scavenger Receptors and Cell Death

Scavenger receptors have the ability to bind and internalize proteins with a modification of their positively charged amino acids to form clusters of negative charges which then act as ligands. There have been many functions found for these receptors such as a role in antigen processing and presentation and subsequent immunogenicity (11). Other functions include the uptake of apoptotic cells (36,65,80,81,95-97). Experimentally, Class A scavenger receptors on macrophages (thymic and peritoneal) have been shown to bind apoptotic thymocytes (36). Furthermore, macropahges from SR-A deficient mice had a reduction of uptake of apoptotic cells (50%), indicating involvement of multiple scavenger receptor types (36).

The scavenger receptor Class B has also been shown to take up apoptotic cells. Chinese hamster ovary cells transfected with the SRB-1 receptor on their surface are involved in the binding of apoptotic cells by phosphatidylserine (PS) found on the lipid membrane (80). These data demonstrate that SRB-1 is involved in the binding of PS, which is a common tag on apoptotic cells (80). Another SRB receptor, CD36, has also been shown to bind and take up apoptotic cells by PS on the cell membrane (98). In studies involving CD36 transfected cells, a significant increase in the uptake of apoptotic neutophils, lymphocytes, and fibroblasts was observed (98). Additionally, CD36 has been shown to play a role in human peripheral macrophage which act as a cofactor for phagocytosis using the phosphatidylserine moieties of the apoptotic membrane (99). These studies indicate that CD36 is only partially responsible for the binding of apoptotic cells, as a blocking antibody against CD36 could not completely inhibit their binding (99).

6. ALCOHOLISM: IMMUNE SUPPRESSION AND AUTOIMMUNITY

There are several lines of evidence that alcohol abuse suppresses the immune system including increases in pneumonia, tuberculosis, HIV, and hepatitis C in chronic alcoholics as recently reviewed (100). Alcoholics have an increased susceptibility to pneumonia and septicemia than non-alcoholic individuals (101,102). Infection with tuberculosis is also higher in alcoholic individuals, with rates as high as 15 to 200 times more than control individuals (103). The severity of HIV infection among alcoholics as been complex and some groups have reported abnormalities in T-cell subsets particularly an increase in CD8 + T cells (104). Hepatitis C infection is also more prevalent in individuals who are alcoholics.

However, there exists a dichotomous view to the role of the immune system in ALD. This is due to observations that alcoholics have been shown to be immunosuppressed (increased infections), yet they have also been shown to have elevated immunoglobulin levels, altered T-cell subsets, persistent activation of immune cells, and increased levels of cytokines (100). Elevated IgA levels in patients with ALD are observed and have been associated with immune complexes, which become deposited in the skin, liver, and kidney tissues (105). Patients with ALD have autoantibodies to lymphocytes, brain, DNA, serum lipoproteins, and various liver proteins are observed, again indicating many autoimmune phenomena as a result of alcohol consumption (23,80,106,107). Thus, it appears further investigation will be required to determine why autoimmune-like aspects occur in a setting of immunosuppression. One possible suggestion is that acute ethanol ingestion results in immunosuppression, but as the alcohol is metabolized the immune response begins to "rebound" resulting in an enhanced autoimmune response to altered self proteins.

7. DISCUSSION

The mechanisms underlying alcohol induced liver disease (ALD) are not clear and several clinical features of ALD suggest that autoimmune effector mechanism(s) may be contributing to this damage (23,106-108). Recently, circulating antibodies against Malondialdehyde-Acetaldehyde (MAA) haptenated proteins have been shown to be increased in patients with alcohol induced cirrhosis and hepatitis, associated with the severity of liver disease, and has been proposed as a mechanism by which self liver proteins are made immunogenic (8). The hypothesis that MAA haptenated self proteins are involved in the induction and/or progression of ALD is a hypothesis that brings together clincial and experimental evidence of autoimmunity, toxicity, and lipid peroxidation.

Haptens, including MAA, have been shown to induce immune responses experimentally in animals, (9) and make self proteins immunogenic without the use of adjuvants (36). Several mechanisms have been implicated in this immunogenicity and include: 1) Increase in co-stimulatory molecules on key antigen presenting cells; 2) Increased receptor mediated uptake by antigen presenting cells; and, 3) Increased cell death by apoptosis and/or necrosis. The upregulation of co-stimulatory molecules (B7) and activation/maturation of DCs (including the upregulation of cytokine production) has been shown to occur in response to chemical haptens (including MAA) (29-33). Thus taken together, it appears that chemical haptens may act to initiate immunity in similar ways as traditional adjuvants.

MAA haptenated proteins have been shown to be taken up by scavenger receptors (9,57) in a similar fashion as other haptens by antigen presenting cells (10,11,36,37). The blocking of scavenger receptor families has demonstrated a decrease or modification in the resulting immune response, indicating a pivotal role that these

receptors may play in the immunogenicity induced by haptenated proteins (10,11,109).

Many cell types undergo apoptosis when exposed to lower levels of MAA haptenated proteins incubated for short time periods (13). Increases in necrosis occur when the concentration of the MAA hapten or the incubation time is increased (13). In addition to malondialdehyde-acetaldehyde (MAA) haptenated proteins, acetaldehyde (75-78), ox-LDL (93), and 4-HNE (90,91) have been shown to induce cell death (dose dependent apoptosis and necrosis) and induce immune responses. These findings are interesting as hepatitis B and autoimmune hepatitis have been shown to be mediated by apoptosis (59) and ethanol consumption (of which MAA is a product) has been shown to induce apoptosis in vivo (60). Additionally in influenza models of immunogenicity, it has been demonstrated that apoptotic cells are taken up by DCs, processed and presented in the MHC Class I pathway, and the lack of apoptosis negates effective immune responses (66). Cellular fragments from necrotic cells have also been shown to present peptides in MHC Class II (110). The release of heat shock proteins during necrosis has been shown to; correlate with autoimmune disease (69), increase the amount of cytokines released (111), be related to their immunogenicity in vivo (112), and induce the maturation of dendritic cells (25). The cross-presentation of antigens acquired from dying cells has been demonstrated in immature dendritic cells, inducing co-stimulatory, MHC Class I/II, and cytokine expressions (73). The potential then exists for HSP bound self peptides to activate dendritic cells to initiate an immune response. Lastly, MAA haptenated proteins appear to cause lysosomal integrity to be compromised, causing exogenous antigens to be released into the cytoplasm of antigen presenting cells (13) and into the Class I pathway.

Several studies have demonstrated that scavenger receptors bind haptenated proteins and are related to their immunogenicity (10) as has been demonstrated with ox-LDL (11). The role that cell death plays in ox-LDL and its immunogenicity has not been investigated to this point. We propose two mechanisms by which MAA modified proteins are immunogenic: 1) They are taken up by antigen presenting cells (dendritic cells) by their scavenger receptors, inducing maturation, and the up-regulation of co-stimulatory molecules which initiate immune responses; and 2) MAA modified proteins may cause cell death in vivo leading to increased uptake of liver self proteins by scavenger receptors. We propose that this is not a common occurrence as normal regulatory mechanisms that remove apoptotic and/or necrotic cells must be overcome and that may be why ALD is not common among alcoholics.

Although antibody titers to aldehyde haptenated proteins correlate with ALD in humans, these patients demonstrate immune suppression. This duality of alcoholic liver disease has been noted by several reviewers (2,22,100,113). Certainly other co-factors; such as viral infections (hepatitis C) and hepatic toxins play an important role in the initiation and/or progression of ALD. However, the potential roles of aldehyde-modified proteins, scavenger receptors, and apoptosis in the breaking of self tolerance (autoimmunity) are becoming more evident as potential mechanism(s) involved in the development and/or progression of ALD.

8. ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health Grant R29 AA10435, R01 AA07818 and R01 AA04961. Also supported by the Department of Veterans Affairs Alcohol Research Center. The authors would like to thank the members of the Experimental Immunology Laboratory and Liver Study Unit at the Omaha VA Medical Center.

9. REFERENCES

1. Cook R.T., T.J. Waldschmidt, B.L. Cook, D.R. LaBrecque, & K. McLatchie: Loss of the CD5+ and CD45RAhi B cell subsets in alcoholics. *Clin Exp Immunol* 103, 304-310 (1996)
2. Szabo G: Monocytes, alcohol use, and altered immunity. *Alcohol Clin Exp Res* 22, 216S-219S (1998)
3. Israel Y, A. MacDonald, O. Niemela, D. Zamel, E. Shami, & M. Zywulko: Hypersensitivity to acetaldehyde-protein adducts. *Mol Pharmacol* 42, 711-717 (1992)
4. Viitala K., Y. Israel, J.E. Blake, & O. Niemela: Serum IgA, IgG, and IgM antibodies directed against acetaldehyde-derived epitopes: relationship to liver disease severity and alcohol consumption. *Hepatology* 25,1418-1424 (1997)
5. Viitala K., K. Makkonen, Y. Israel, T. Lehtimaki, O. Jaakkola , & T. Koivula: Autoimmune responses against oxidant stress and acetaldehyde-derived epitopes in human alcohol consumers. *Alcohol Clin Exp Res* 24, 1103-1109 (2000)
6. Khan M.F., X. Wu, & G.A. Ansari: Anti-malondialdehyde antibodies in MRL+/+ mice treated with trichloroethene and dichloroacetyl chloride: possible role of lipid peroxidation in autoimmunity. *Toxicol Appl Pharmacol* 170, 88-92 (2001)
7. Tuma D.J., G.M. Thiele, D. Xu, L.W. Klassen, & M.F. Sorrell: Acetaldehyde and malondialdehyde react together to generate distinct protein adducts in the liver during long-term ethanol administration. *Hepatology* 23, 872-880 (1996)
8. Rolla R., D. Vay, E. Mottaran, M. Parodi, N. Traverso, & S. Arico: Detection of circulating antibodies against malondialdehyde-acetaldehyde adducts in patients with alcohol-induced liver disease. *Hepatology* 31, 878-884 (2000)
9. Thiele G.M., D.J. Tuma, M.S. Willis, J.A. Miller, T.L. McDonald, & M.F. Sorrell: Soluble proteins modified with acetaldehyde and malondialdehyde are immunogenic in the absence of adjuvant. *Alcohol Clin Exp Res* 22, 1731-1739 (1998)
10. Abraham R., A. Choudhury, S.K. Basu, V. Bal, & S. Rath: Disruption of T cell tolerance by directing a self antigen to macrophage-specific scavenger receptors. *J Immunol* 158, 4029-4035 (1997)
11. Nicoletti A., G. Caligiuri, I. Tornberg, T. Kodama, S. Stemme, & G.K. Hansson: The macrophage scavenger receptor type A directs modified proteins to antigen presentation. *Eur J Immunol* 29, 512-521 (1999)
12. Kristiansen M., J.H. Graversen, C. Jacobsen, O. Sonne, H.J. Hoffman, & S. K. Law: Identification of the

haemoglobin scavenger receptor. *Nature* 409, 198-201 (2001)
13. Willis M.S., L.W. Klassen, D.J. Tuma, & G.M. Thiele: Malondialdehyde-acetaldehyde-haptenated protein induces cell death by induction of necrosis and apoptosis in immune cells. *Int Immunopharmacol* 2, 519-535 (2002)
14. Willis M.S., C.C. Miller, D.J. Tuma, L.W. Klassen, & G.M. Thiele: Immune cell death is induced by a malondialdehyde-acetaldehyde (MAA) adducted protein. *Alcohol Clin Exp Res* 22, 174A-174A (1998)
15. Willis M.S., D.J. Tuma, L.W. Klassen, & G.M. Thiele: Lymphocyte and macrophage cell death induced by a malondialdehyde-acetaldehyde (MAA) adducted protein. *Alcohol Clin Exp Res* 22, 22A-22A (1998)
16. Xu D., G.M. Thiele, J.L. Beckenhauer, L.W. Klassen, M.F. Sorrell, & D.J. Tuma: Detection of circulating antibodies to malondialdehyde-acetaldehyde adducts in ethanol-fed rats. *Gastroenterology* 115, 686-692 (1998)
17. Higuchi H., I. Kurose, S. Kato, S. Miura, & H. Ishii: Ethanol-induced apoptosis and oxidative stress in hepatocytes. *Alcohol Clin Exp Res* 20, 340A-346A (1996)
18. Andrade F., L. Casciola-Rosen, & A. Rosen: Apoptosis in systemic lupus erythematosus. Clinical implications. *Rheum Dis Clin North Am* 26, 215-27 (2000)
19. Lorenz H.M., M. Herrmann, T. Winkler, U. Gaipl, & J.R. Kalden: Role of apoptosis in autoimmunity. *Apoptosis* 5, 443-449 (2000)
20. Levine J.S. & J.S. Koh: The role of apoptosis in autoimmunity: immunogen, antigen, and accelerant. *Semin Nephrol* 19, 34-47 (1999)
21. Rodenburg R.J., J.M. Raats, G.J. Pruijn, & W.J. van Venrooij: Cell death: a trigger of autoimmunity? *Bioessays* 22, 627-636 (2000)
22. Klassen L.W., D. Tuma, & M.F. Sorrell: Immune mechanisms of alcohol-induced liver disease. *Hepatology* 22, 355-357 (1995)
23. Paronetto F.: Immunologic reactions in alcoholic liver disease. *Semin Liver Dis* 13, 183-195 (1993)
24. Deaciuc I.V., M. Nikolova-Karakashian, F. Fortunato, E.Y. Lee, D.B. Hill, & C.J. McClain: Apoptosis and dysregulated ceramide metabolism in a murine model of alcohol-enhanced lipopolysaccharide hepatotoxicity. *Alcohol Clin Exp Res* 24, 1557-1565 (2000)
25. Nicholls R., J. de Jersey, S. Worrall, & P. Wilce: Modification of proteins and other biological molecules by acetaldehyde: adduct structure and functional significance. *Int J Biochem* 24, 1899-1906 (1992)26. Clot P., G. Bellomo, M. Tabone, S. Arico, & E. Albano: Detection of antibodies against proteins modified by hydroxyethyl free radicals in patients with alcoholic cirrhosis. *Gastroenterology* 108, 201-207 (1995)
27. Thiele G.M., D.J. Tuma, M.S. Willis, J.A. Miller, T.L. McDonald, & M.F. Sorrell: Soluble proteins modified with acetaldehyde and malondialdehyde are immunogenic in the absence of adjuvant. *Alcohol Clin Exp Res* 22,1731-1739 (1998)
28. Esche C., M.R. Shurin, & M.T. Lotze: The use of dendritic cells for cancer vaccination. *Curr Opin Mol Ther* 1, 72-81 (1999)
29. Aiba S: Maturation of dendritic cells induced by cytokines and haptens. *Tohoku J Exp Med* 184, 159-172 (1998)
30. Aiba S. & H. Tagami: Dendritic cell activation induced by various stimuli, e.g. exposure to microorganisms, their products, cytokines, and simple chemicals as well as adhesion to extracellular matrix. *J Dermatol Sci* 20, 1-13 (1998)
31. Aiba S. & H. Tagami: Dendritic cells play a crucial role in innate immunity to simple chemicals. *J Investig Dermatol Symp Proc* 4, 158-163 (1999)
32. Aiba S., A. Terunuma, H. Manome, & H. Tagami: Dendritic cells differently respond to haptens and irritants by their production of cytokines and expression of co-stimulatory molecules. *Eur J Immunol* 27, 3031-3038 (1997)
33. Manome H., S. Aiba, & H. Tagami: Simple chemicals can induce maturation and apoptosis of dendritic cells. *Immunology* 98, 481-490 (1999)
34. Thiele G.M., L.W. Klassen, D.J. Tuma, & M.S. Willis: The Increased Binding of MAA (Malondialdehyde-Acetaldehyde) Adducted Protein to Scavenger Receptors and the Up-regulation of B7 May Play a Role in Immunogenicity. *Hepatology* 30, 328A-328A (1999)
35. Willis M.S., L.W. Klassen, D.J. Tuma, M.F. Sorrell, & G.M. Thiele: Binding and Antibody Responses to Malondialdehyde-Acetaldehyde (MAA) Adducted Protein are Mediated by Scavenger Receptor A (SRA). *Alcohol Clin Exp Res* 24, 5S (2000)
36. Abraham R., N. Singh, A. Mukhopadhyay, S.K. Basu, V. Bal, & S. Rath: Modulation of immunogenicity and antigenicity of proteins by maleylation to target scavenger receptors on macrophages. *J Immunol* 154, 1-8 (1995)
37. Singh N., S. Bhatia, R. Abraham, S.K. Basu, A. George, & V. Bal: Modulation of T cell cytokine profiles and peptide-MHC complex availability in vivo by delivery to scavenger receptors via antigen maleylation. *J Immunol* 160, 4869-4880 (1998)
38. Terpstra V., E.S. van Amersfoort, A.G. Van Velzen, J. Kuiper, & T.J. Van Berkel: Hepatic and extrahepatic scavenger receptors: function in relation to disease. *Arterioscler Thromb Vasc Biol* 20, 1860-1872 (2000)
39. Brown M.S., S.K. Basu, J.R. Falck, Y.K. Ho, & J.L. Goldstein: The scavenger cell pathway for lipoprotein degradation: specificity of the binding site that mediates the uptake of negatively-charged LDL by macrophages. *J Supramol Struct* 13, 67-81 (1980)
40. Alaiz M., M. Beppu, K. Ohishi, & K. Kikugawa: Modification of delipidated apoprotein B of low density lipoprotein by lipid oxidation products in relation to macrophage scavenger receptor binding. *Biol Pharm Bull* 17, 51-57 (1994)
41. Horiuchi S., M. Murakami, K. Takata, & Y. Morino: Scavenger receptor for aldehyde-modified proteins. *J Biol Chem* 261, 4962-4966 (1986)
42. Steinbrecher U.P., M. Lougheed, & W.C. Kwan: Dirks M. Recognition of oxidized low density lipoprotein by the scavenger receptor of macrophages results from derivatization of apolipoprotein B by products of fatty acid peroxidation. *J Biol Chem* 264, 15216-15223 (1989)
43. Takata K., S. Horiuchi, N. Araki, M. Shiga, M. Saitoh, & Y. Morino: Endocytic uptake of nonenzymatically glycosylated proteins is mediated by a scavenger receptor for aldehyde-modified proteins. *J Biol Chem* 263, 14819-14825 (1988)

44. Takata K., S. Horiuchi, N. Araki, M. Shiga, M. Saitoh, & Y. Morino: Scavenger receptor of human monocytic leukemia cell line (THP-1) and murine macrophages for nonenzymatically glycosylated proteins. *Biochim Biophys Acta* 986, 18-26 (1989)
45. Uchida K: Role of reactive aldehyde in cardiovascular diseases. *Free Radic Biol Med* 28, 1685-1696 (2000)
46. Araki N., T. Higashi, T. Mori, R. Shibayama, Y. Kawabe, & T. Kodama: Macrophage scavenger receptor mediates the endocytic uptake and degradation of advanced glycation end products of the Maillard reaction. *Eur J Biochem* 230, 408-415 (1995)
47. Suzuki H., Y. Kurihara, M. Takeya, N. Kamada, M. Kataoka, & K. Jishage: A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. *Nature* 386, 292-296 (1997)
48. Ohgami N., R. Nagai, A. Miyazaki, M. Ikemoto, H. Arai, & S. Horiuchi: Scavenger receptor class B type I-mediated reverse cholesterol transport is inhibited by advanced glycation end products. *J Biol Chem* 276, 13348-13355 (2001)
49. Horkko S., C.J. Binder, P.X. Shaw, M.K. Chang, G. Silverman, & W. Palinski: Immunological responses to oxidized LDL. *Free Radic Biol Med* 28, 1771-1779 (2000)
50. Hoff H.F. & J. O'Neil: Structural and functional changes in LDL after modification with both 4-hydroxynonenal and malondialdehyde. *J Lipid Res* 34, 1209-1217 (1993)
51. Palinski W., S. Yla-Herttuala , M.E. Rosenfeld, S.W. Butler, & S.A. Socher: Parthasarathy S et al. Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 10, 325-335 (1990)
52. Palinski W., M.E. Rosenfeld, S. Yla-Herttuala, G.C. Gurtner, S.S. Socher, & S.W. Butler: Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci U S A* 86, 1372-1376 (1989)
53. Yla-Herttuala S., W. Palinski, M.E. Rosenfeld, S. Parthasarathy, T.E. Carew, & S. Butler: Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest* 84, 1086-1095 (1989)
54. Hill G.E., J.A. Miller, B.T. Baxter, L.W. Klassen, M.J. Duryee, & D.J. Tuma: Association of malondialdehyde-acetaldehyde (MAA) adducted proteins with atherosclerotic-induced vascular inflammatory injury. *Atherosclerosis* 141, 107-116 (1998)
55. Yamada S., S. Kumazawa, T. Ishii, T. Nakayama, K. Itakura, & N. Shibata: Immunochemical detection of a lipofuscin-like fluorophore derived from malondialdehyde and lysine. *J Lipid Res* 42, 1187-1196 (2001)
56. Thiele G.M., J.A. Miller, L.W. Klassen, & D.J. Tuma: Long-term ethanol administration alters the degradation of acetaldehyde adducts by liver endothelial cells. *Hepatology* 24, 643-648 (1996)
57. Thiele G.M., J.A. Miller, L.W. Klassen, & D.J. Tuma: Chronic ethanol consumption impairs receptor-mediated endocytosis of formaldehyde-treated albumin by isolated rat liver endothelial cells. *Hepatology* 29,1511-1517 (1999)
58. Duryee M.J., L.W. Klassen, T.L. Freeman, M.S. Willis, D.J. Tuma, & G.M. Thiele: Chronic ethanol consumption impairs receptor-mediated endocytosis of MAA-modified albumin by liver endothelial cells. *Biochem Pharmacol* 66, 1045-1054 (2003)
59. Chancerelle Y.& J.F. Kergonou: Immunologic relevance of malonic dialdehyde. *Ann Pharm Fr* 53, 241-250 (1995)
60. Rush G.F., J.R. Gorski, M.G. Ripple, J. Sowinski, P. Bugelski, & W.R. Hewitt: Organic hydroperoxide-induced lipid peroxidation and cell death in isolated hepatocytes. *Toxicol Appl Pharmacol* 78, 473-483 (1985)
61. Kruman I., A.J. Bruce-Keller, D. Bredesen, G. Waeg, & M.P. Mattson: Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. *J Neurosci* 17, 5089-5100 (1997)
62. Chancerelle Y., C. Alban, R. Viret, F. Tosetti, & J.F. Kergonou: Immunological relevance of malonic dialdehyde (MDA): IV. Further evidences about the epitope recognized by antibodies obtained from rabbits immunized with MDA-modified lysozyme. *Biochem Int* 24, 157-163 (1991)
63. Poot M., H. Esterbauer, P.S. Rabinovitch, & H. Hoehn: Disturbance of cell proliferation by two model compounds of lipid peroxidation contradicts causative role in proliferative senescence. *J Cell Physiol* 137, 421-429 (1988)
64. Allen P.M., D.J. Strydom, & E.R. Unanue: Processing of lysozyme by macrophages: identification of the determinant recognized by two T-cell hybridomas. *Proc Natl Acad Sci U S A* 81, 2489-2493 (1984)
65. Kergonou J.F., I. Pennacino, C. Lafite, & R. Ducousso: Immunological relevance of malonic dialdehyde (MDA): III. Immuno- enzymatic determination of human immunoglobulin binding to MDA- crosslinked proteins. *Biochem Int* 16, 845-852 (1988)
66. Suthanthiran M., M.E. Anderson, V.K. Sharma, & A. Meister: Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. *Proc Natl Acad Sci U S A* 87, 3343-3347 (1990)
67. Pierre P., S.J. Turley, E. Gatti, M. Hull, J. Meltzer, & A. Mirza: Developmental regulation of MHC class II transport in mouse dendritic cells. *Nature* 388, 787-792 (1997)
68. Rock K.L: A new foreign policy: MHC class I molecules monitor the outside world. *Immunol Today* 17, 131-137 (1996)
69. Savolainen E.R., M..A. Leo, R. Timpl, & C.S. Lieber: Acetaldehyde and lactate stimulate collagen synthesis of cultured baboon liver myofibroblasts. *Gastroenterology* 87, 777-787 (1984)
70. Lumeng L. & R.C. Lin: Formation of a 37 kilodalton liver protein-acetaldehyde adduct in vivo and in liver cell culture during chronic alcohol exposure. *Ann N Y Acad Sci* 625:793-801:793-801 (1991)
71. Basu S., R.J. Binder, R. Suto, K.M. Anderson, & P.K. Srivastava: Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. *Int Immunol* 12, 1539-1546 (2000)
72. Krokan H., R.C. Grafstrom, K. Sundqvist, H. Esterbauer, & C.C. Harris: Cytotoxicity, thiol depletion and inhibition of O6-methylguanine-DNA methyltransferase by various aldehydes in cultured human bronchial fibroblasts. *Carcinogenesis* 6, 1755-1759 (1985)

73. Mihas A.A., G. Saccomani, & J.G. Spenney: LSA--a new liver-specific antigen in the rat. II: Immunological and biological properties. *J Med* 25, 285-303 (1994)
74. Tuma D.J., M.R. Newman, T.M. Donohue, & M.F. Sorrell: Covalent binding of acetaldehyde to proteins: participation of lysine residues. *Alcohol Clin Exp Res* 11, 579-584 (1987)
75. Wickramasinghe S.N.: Evidence of drug metabolism by macrophages: possible role of macrophages in the pathogenesis of drug-induced tissue damage and in the activation of environmental procarcinogens. *Clin Lab Haematol* 9, 271-280 (1987)
76. Wickramasinghe S.N., G. Barden, & L. Levy: The capacity of macrophages from different murine tissues to metabolise ethanol and generate an ethanol-dependent non-dialysable cytotoxic activity in vitro. *Alcohol Alcohol* 22, 31-39 (1987)
77. Wickramasinghe S.N. & R. Hasan: Comparison of the generation of albumin-associated cytotoxic activity in supernatants from ethanol-containing cultures of human blood monocyte-derived macrophages and of two human hepatoma cell lines. *Alcohol Alcohol* 26, 147-153 (1991)
78. Wickramasinghe S.N., F. Mawas, R. Hasan, I.N. Brown, & R.D. Goldin: Macrophages are a major source of acetaldehyde in circulating acetaldehyde-albumin complexes formed after exposure of mice to ethanol. *Alcohol Clin Exp Res* 18, 1463-1467 (1994)
79. Wickramasinghe S.N. & G. Barden: Acetaldehyde-modified serum proteins inhibit mitogen-stimulated human lymphocyte transformation. *Br J Haematol* 73, 23-28 (1989)
80. Wickramasinghe S.N., B. Gardner, & G. Barden: Cytotoxic protein molecules generated as a consequence of ethanol metabolism in vitro and in vivo. *Lancet* 2, 823-826 (1986)
81. Wickramasinghe S.N., B. Gardner, & G. Barden: Circulating cytotoxic protein generated after ethanol consumption: identification and mechanism of reaction with cells. *Lancet* 2, 122-126 (1987)
82. Davies D.R., S. Sheriff, & E.A. Padlan: Antibody-antigen complexes. *J Biol Chem* 263, 10541-10544 (1988)
83. de Rijke Y.B., E.M. Hessels , & T.J. Van Berkel: Recognition sites on rat liver cells for oxidatively modified beta-very low density lipoproteins. *Arterioscler Thromb* 12, 41-49 (1992)
84. Kleinherenbrink-Stins M.F., J.H. van de Boom, D. Schouten, P.J. Roholl, vd.H. Niels, & A. Brouwer: Visualization of the interaction of native and modified lipoproteins with parenchymal, endothelial and Kupffer cells from human liver. *Hepatology* 14, 79-90 (1991)
85. Padlan E.A., E.W. Silverton, S. Sheriff , G.H. Cohen, S.J. Smith-Gill & D.R. Davies: Structure of an antibody-antigen complex: crystal structure of the HyHEL-10 Fab-lysozyme complex. *Proc Natl Acad Sci U S A* 86, 5938-5942 (1989)
86. Wickramasinghe S.N.: Supernatants from ethanol-containing macrophage cultures have cytotoxic activity. *Alcohol Alcohol* 21, 263-268 (1986)
87. Wu R., R. Giscombe , G. Holm & A.K. Lefvert : Induction of human cytotoxic T lymphocytes by oxidized low density lipoproteins. *Scand J Immunol* 43, 381-384 (1996)
88. Braden B.C., B.A. Fields, X. Ysern, F.A. Goldbaum, W. Dall'Acqua, & F.P. Schwarz: Crystal structure of the complex of the variable domain of antibody D1.3 and turkey egg white lysozyme: a novel conformational change in antibody CDR-L3 selects for antigen. *J Mol Biol* 257, 889-894 (1996)
89. Braun K.P., R.B. Pearce, & C.M. Peterson: Acetaldehyde-serum protein adducts inhibit interleukin-2 secretion in concanavalin A-stimulated murine splenocytes: a potential common pathway for ethanol-induced immunomodulation. *Alcohol Clin Exp Res* 19, 345-349 (1995)
90. Kirichenko A., L. Li, M.T. Morandi, & A. Holian: 4-hydroxy-2-nonenal-protein adducts and apoptosis in murine lung cells after acute ozone exposure. *Toxicol Appl Pharmacol* 141, 416-424 (1996)
91. Li L., R.F. Hamilton, A. Kirichenko Jr., & A.Holian: 4-Hydroxynonenal-induced cell death in murine alveolar macrophages. *Toxicol Appl Pharmacol* 139, 135-143 (1996)
92. Han C.Y. & Y.K. Pak: Oxidation-dependent effects of oxidized LDL: proliferation or cell death. *Exp Mol Med* 31, 165-173 (1999)
93. Li W., X.M. Yuan, & U.T. Brunk: OxLDL-induced macrophage cytotoxicity is mediated by lysosomal rupture and modified by intralysosomal redox-active iron. *Free Radic Res* 29, 389-398 (1998)
94. Willis M.S., L.W. Klassen, D.J. Tuma, & G.M. Thiele: Malondialdehyde-Acetaldehyde (MAA) adduct formation on protein induces cell death in a non-necrotic manner. *Hepatology* 28, 433A-433A (1998)
95. Haberland M.E., R.R. Rasmussen, C.L. Olch, & A.M. Fogelman: Two distinct receptors account for recognition of maleyl-albumin in human monocytes during differentiation in vitro. *J Clin Invest* 77, 681-689 (1986)
96. Mukhopadhyay A., B. Mukhopadhyay, R.K. Srivastava, & S.K. Basu: Scavenger-receptor-mediated delivery of daunomycin elicits selective toxicity towards neoplastic cells of macrophage lineage. *Biochem J* 284, 237-241 (1992)
97. Wickramasinghe S.N., R. Hasan, & Z. Khalpey: Differences in the serum levels of acetaldehyde and cytotoxic acetaldehyde-albumin complexes after the consumption of red and white wine: in vitro effects of flavonoids, vitamin E, and other dietary antioxidants on cytotoxic complexes. *Alcohol Clin Exp Res* 20, 799-803 (1996)
98. Wickramasinghe S.N. & R. Hasan: In vivo effects of vitamin C on the cytotoxicity of post-ethanol serum. *Biochem Pharmacol* 48, 621-624 (1994)
99. Wickramasinghe S.N. & R. Hasan: In vitro effects of vitamin C, thioctic acid and dihydrolipoic acid on the cytotoxicity of post-ethanol serum. *Biochem Pharmacol* 43, 407-411 (1992)
100. Niemela O. & Y. Israel: Hemoglobin-acetaldehyde adducts in human alcohol abusers. *Lab Invest* 67, 246-252 (1992)
101. Brecher A.S., K. Hellman, & M.H. Basista: A perspective on acetaldehyde concentrations and toxicity in man and animals. *Alcohol* 14, 493-496 (1997)
102. Maher J.J., C. Tzagarakis, & A.Gimenez: Malondialdehyde stimulates collagen production by hepatic

lipocytes only upon activation in primary culture. *Alcohol Alcohol* 29, 605-610 (1994)
103. Kamimura S., K. Gaal, R.S. Britton, B.R. Bacon, G. Triadafilopoulos, & H. Tsukamoto: Increased 4-hydroxynonenal levels in experimental alcoholic liver disease: association of lipid peroxidation with liver fibrogenesis. *Hepatology* 16, 448-453 (1992)
104. Comment C.E., B.L. Blaylock, D.R. Germolec, P.L. Pollock, Y. Kouchi, & H.W. Brown: Thymocyte injury after in vitro chemical exposure: potential mechanisms for thymic atrophy. *J Pharmacol Exp Ther* 262, 1267-1273 (1992)
105. Zhou Y.F., E. Guetta, Z.X. Yu, T. Finkel, & S.E. Epstein: Human cytomegalovirus increases modified low density lipoprotein uptake and scavenger receptor mRNA expression in vascular smooth muscle cells. *J Clin Invest* 98, 2129-2138 (1996)
106. Cook R.T.: Alcohol abuse, alcoholism, and damage to the immune system--a review. *Alcohol Clin Exp Res* 22, 1927-1942 (1998)
107. Laskin C.A., E. Vidins, L.M. Blendis, & C.A. Soloninka: Autoantibodies in alcoholic liver disease. *Am J Med* 89, 129-133 (1990)
108. Wehr H., M. Rodo, C.S. Lieber, & E. Baraona: Acetaldehyde adducts and autoantibodies against VLDL and LDL in alcoholics. *J Lipid Res* 34, 1237-1244 (1993)
109. Willis M.S., G.M. Thiele, D.J. Tuma, & L.W. Klassen: T cell proliferative responses to malondialdehyde-acetaldehyde haptenated protein are scavenger receptor mediated. *Int Immunopharmacol* 3, 1381-1399 (2003)
110. Muller K., S.J. Hardwick, C.E. Marchant, N.S. Law, G. Waeg, & H. Esterbauer: Cytotoxic and chemotactic potencies of several aldehydic components of oxidised low density lipoprotein for human monocyte-macrophages. *FEBS Lett* 388, 165-168 (1996)
111. Takase S., M. Yasuhara, A. Takada, & Y. Ueshima: Changes in blood acetaldehyde levels after ethanol administration in alcoholics. *Alcohol* 7, 37-41 (1990)
112. Steinbrecher U.P., M. Fisher, J.L. Witztum, & L.K. Curtiss: Immunogenicity of homologous low density lipoprotein after methylation, ethylation, acetylation, or carbamylation: generation of antibodies specific for derivatized lysine. *J Lipid Res* 25, 1109-1116 (1984)
113. Thiele G.M., D.J. Tuma, & L.W. Klassen: Alcohol, Anesthetics, and Analgesics in Autoimmune Reactivity. In: Contemporary Immunology: Autoimmune Reactions. Eds. Paul S, Humana Press Inc., NJ (1998)

Key Words: Acetaldehyde, Malondialdehyde, Oxidized Low Density Lipoprotein, MAA, Autoimmunity, Scavenger Receptors, Alcoholic Liver Disease, Hepatitis, Haptens, Self Tolerance, 4-Hydroxynonenal, Apoptosis, Ethanol, Necrosis, Heat Shock Proteins, Immune Suppression, Review

Send correspondence to: Dr. Geoffrey M. Thiele, Ph.D, Omaha VA Medical Center, Research Service 151, Rm 325, 4101 Woolworth Avenue, Omaha, NE 68105, Tel: 402-346-8800 Ext. 3550, Fax: 402-449-0604, E-mail: GTHIELE@unmc.edu