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ETHANOL-MEDIATED REGULATION OF TRANSCRIPTION FACTORS IN IMMUNOCOMPETENT CELLS

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1. ABSTRACT

The immunomodulatory effects of acute and chronic alcohol use are characterized by impaired antigen-specific immune activation and by increased susceptibility to infections due to alterations in innate immune responses and inflammatory mediator production. The central feature of cellular responses to inflammatory and stress signals is the activation of the nuclear regulatory kappa B/Rel family of transcriptional factors via various surface receptor systems in immunocompetent cells. Activation of NF-kappa B, however, is regulated at multiple levels including I-kappa B degradation, nuclear translocation, and by interaction of NF-kappa B/Rel with other transcription factors. Data from our and other laboratories demonstrate that acute alcohol treatment inhibits activation and nuclear binding of the p65/p50 NF-kappa B functional heterodimer in human monocytes, a mechanism likely contributing to inhibition of pro-inflammatory cytokine production. Here we show that acute alcohol-mediated inhibition of NF-kappa B activation in various monocytic cells including human monocytes and murine macrophages. Inhibition of NF-kappa B activation by alcohol in monocytic cells was independent of I-kappa B alpha degradation. These acute-alcohol-induced changes in monocytic cells were different compared to T lymphocytes, both in Jurkat CD4 cells and peripheral human T cells, acute alcohol had a biphasic effect on TNF-alpha-induced NF-kappa B activation via an I-kappa B alpha-dependent mechanism. Inhibition of NF-kappa B activation by acute alcohol in LPS-activated human monocytes was associated with an increase in nuclear glucocorticoid receptor (GR) levels and reduced GR binding to the glucocorticoid response element (GRE). Together these findings support the hypothesis that in the presence of alcohol, nuclear interaction of NF-kappa B (p65) with glucocorticoid receptor and/or other transcription factors may contribute to the reduced NF-kappa B activation. In contrast to the inhibitory effects of acute alcohol on NF-kappa B activation in monocytic cells, chronic alcohol use and alcoholic hepatitis result in an augmentation of NF-kappa B activation and pro-inflammatory cytokine induction. These results suggest that

the complex interactions of the NF-kappa B/Rel and related transcription factors including GR and heat-shock responses determine the level of activation of the immunocompetent cells in response to the challenge of acute and chronic alcohol use at the single cell level.

2. INTRODUCTION: ALCOHOL, IMMUNITY, AND TRANSCRIPTIONAL REGULATION

Alcohol, the most commonly used and abused substance in human history, has diverse effects on human health and organ systems including the immune system and results in immunomodulatory effects. Alcohol consumption is associated with abnormalities of both the innate and adaptive immune systems leading to increased susceptibility to infections, decreased delayed-type hypersensitivity responses, and increased incidence of certain cancers (1, 2). The most profound effects of both acute and chronic alcohol use are seen on cells and functions of the innate immune system. The fist line of defense against infectious microorganisms relies on innate immunity mediated by monocytes, tissue macrophages (including Kupffer cells), dendritic cells and neutrophil leukocytes (3, 4). The innate immune system relies on germ-line decoded pattern recognition receptors (PRRs) to recognize pathogen-derived substances, phagocytose and kill pathogens, and express a vast array of antimicrobial effector molecules that attack microorganisms at many different levels (4, 5). In addition, monocytes and dendritic cells have a pivotal role as antigen presenting cells and provide the antigenic signal derived from the microorganisms to initiate adaptive immune responses and T cell activation. However, the efficiency of antigen presenting function of monocytes is reduced by both acute and chronic alcohol use (6-9).

The inhibitory effects of acute alcohol on innate immune functions are even more striking. Acutely, alcohol has been shown to significantly suppress the production of NF-kappa B-regulated pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha), and interleukin-1-beta (IL-1-beta) in alveolar macrophages, Kupffer cells, and human monocytes (8, 10-14). This is associated with suppressed induction of the inducible nitric oxide synthase, another gene regulated by NF-kappa B, and required for adequate host defense against intracellular pathogens such as Listeria monocytogenes and Mycobacterium tuberculosis (15, 16). The induction of these pro-inflammatory cytokines is critical for antimicrobial host defense involving coordinated upregulation of adhesion molecules on vascular endothelial cells and the subsequent recruitment of neutrophil leukocytes, a process suppressed by acute alcohol (17).

In contrast to acute alcohol, chronic alcohol use in humans is associated with increased serum levels of pro-inflammatory cytokines (18-20). In patients with alcoholic hepatitis, elevated production of pro-inflammatory cytokines was found in circulating monocytes (21). Additional in vitro studies in monocytic cells also showed increased TNF-alpha production after chronic alcohol treatment (22). TNF-alpha is an inflammatory cytokine that mediates many systemic manifestations and, at least in part, the tissue injury in various inflammatory disorders including alcoholic liver disease (18, 23). TNF-alpha is considered as the primary pro-inflammatory cytokine induced both in response to infections to initiate sufficient host defense and during chronic inflammatory processes often leading to tissue injury. Thus, alteration of TNF-alpha induction by alcohol during these processes has a major impact on cellular immune responses and inflammation.

Alcohol exerts its immunomodulatory effects by interacting with intracellular signal transduction pathways of the immunocompetent cells. The production of TNF-alpha and pro-inflammatory cytokines is regulated at various levels of the signaling cascade including surface receptor expression and receptor-linked signaling events that lead to activation of proteins in the nuclear factor kappa B/Rel pathway. NF-kappa B/Rel binding site is common to the promoter region of various pro-inflammatory genes including TNF-alpha, IL-1, IL-6, IL-8 and MCP-1 (24-28). In addition to NF-kappa B activation, regulation of these pro-inflammatory genes is influenced by interaction of NF-kappa B with other nuclear regulatory factors including the glucocorticoid receptor (29, 30). Subsequent cellular events contributing to alcohol-induced changes in the production of the pro-inflammatory cytokines, particularly that of TNF-alpha, involve post-transcriptional and post-translational modulation. Recent studies demonstrating stabilization of TNF-alpha mRNA in Kupffer cells of chronically alcohol-fed rats involved activation of the p38 mitogen-activated kinase pathway leading to increased TNF-alpha production (31). Post-translational regulation of TNF-alpha production was shown by acute alcohol involving inhibition of TNF-alpha processing and TNF-alpha-converting enzyme (TACE) in two monocytic cell lines, Mono Mac 6 and DRM (32).

This communication will discuss the effects of acute and chronic alcohol use on activation of transcription factors in immunocompetent cells relevant to the immunomodulatory effects of alcohol. Alcohol-mediated modulation of the activation of the Rel/nuclear factor kappa B (NF-kappa B) proteins and their potential interactions with transcription factors including GR and heat shock response elements is in the focus. Experimental data presented will reveal intracellular mechanisms closely relevant to the biological effects of acute and chronic alcohol use on immune cells.

3. MATERIALS AND METHODS

3.1. Monocyte and T lymphocyte isolation and cell lines

Healthy individuals, ages 18 to 60, females and males, donated 120 ml peripheral blood/experiment. Blood was obtained by venipuncture and anticoagulated by heparin. Blood donors had no alcohol at least 48 hours prior to blood donation. Furthermore, donors had no previous alcohol abuse history and consumed less than 6 drinks/week.

Human peripheral blood monocytes were isolated by Ficoll-Hypaque density centrifugation and selective adherence as described before (33-35). Informed consent was obtained and the study was approved by the Institutional Committee for Protection of Human Subject in Research. After overnight rest, monocytes were stimulated

with LPS (0.1-1 µg/ml, E. Coli), dexamethasone (1µM) , in the presence or absence of 25 mM ethanol. This in vitro ethanol concentration approximates 100 mg/dl blood alcohol levels in humans achieved after moderate acute alcohol intake. Cell viability was not affected by ethanol or LPS treatment. T lymphocytes were isolated from mononuclear cells by rosetting with sheep-red blood cells as described (6, 8). RAW 264.7 murine macrophage cell line was maintained and stimulated in RPMI medium supplemented with 10% FBS and Jurkat, human CD4 T lymphocyte, cell line in RPMI with 10% FBS and Hepes buffer (25mM). Monocytes were stimulated for various lengths of time as indicated for each experiment and then nuclear and cytoplasmic extracts were prepared as described (34). Protein concentration was determined in the cytoplasmic and nuclear extracts, respectively, using the Biorad Protein Assay method.

3.2. Western blots and EMSA

Western blots were performed as described before with equal amounts of protein (10µg/sample) loaded for each stimulation group. Proteins were separated on SDS-polyacrylamide gel and electroblotted onto nitro-cellulose membranes. Non-specific binding was blocked by incubation of the membranes in Tris-buffered saline (TBS)/1% nonfat dried milk/0.1% Tween-20 for 2 hours at room temperature. Immunoreactive protein was detected by incubating the blot with GR antibody at 4C overnight (GR antibody sc-1003). After washing the blots, in TBS/0.1% Tween-20, the filters were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Amersham, Inc.). Blots were then developed by the use of an enhancer chemiluminescence assay reagents from Amersham.

A consensus double-stranded NF-kappa-B oligonucleotide (5'AGTTGAGGGGACTTTCCCAGGC3'), GRE oligonucleotide (5'TCGACTGTACAGGATGTTCTAGCTACT3') and a HSE oligonucleotide (5'GCCTCGAATGTTCGCGAAGTT3") were used for EMSA. End-labeling was accomplished by treatment with T4 polynucleotide kinase in the presence of gamma-32P-ATP (Dupont-NEN, Boston, MA). Labeled oligonucleotides were purified on a polyacrylamide copolymer column (Bio-Rad). Five micrograms of nuclear protein were added to a binding reaction mixture containing 50 mM Tris-HCl (pH 7.5), 5mM $MgCl_2$, 2.5 mM EDTA, 2.5 mM DTT, 250 mM NaCl, 20% glycerol, 20µg/ml of bovine serum albumin, 2µg of poly (dI-dC) and 30,000cpm of ^{32}P labeled NF-kappa B oligonucleotide. Samples were incubated at room temperature for 30 min. All reactions were run on a 5% polyacrylamide gel and the dried gel was exposed to an x-ray film at –80°C overnight. For the cold competition reaction a 20-fold excess of specific unlabeled double-stranded probe was added to the reaction mixture before adding the labeled oligonucleotide as described (34).

4. RESULTS AND DISCUSSION

4.1. Regulation of nuclear factor-kappa B activation by alcohol

Nuclear factor kappa B (NF-kappa B) denotes homo- or heterodimeric transcriptional factors of the Rel family of proteins including RelA (p65), RelB, and cRel, which contain transactivation domains, and p50 and p52, expressed as the precursor proteins p105 and p100, respectively (24, 25). These proteins require post-translational processing and do not contain transactivation domains. NF-kappa B is thought to play a pivotal role in immune and inflammatory responses and in stress-mediated cellular responses through the regulation of genes encoding pro-inflammatory cytokines, chemokines, adhesion molecules, growth factors, and inducible enzymes such as cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS) (24-28). NF-kappa B is kept inactive in the cytoplasm of resting cells due to its association with an inhibitory kappa B (I-kappa B) protein (24, 25). Three forms of I-kappa B have been identified in immune cells, including I-kappa B alpha, I-kappa B beta, and I-kappa B epsilon (36-38). The consensus pathway for NF-kappa B activation in response to bacterial or pro-inflammatory cytokine stimuli involve the activation of the I-kappa B kinase (IKK) that consists of three subunits alpha, beta, and gamma and leads to phosphorylation of the I-kappa Bs typically followed by I-kappa B ubiquitination and degradation via the proteasome pathway (5, 24, 39, 40). This is associated with unmasking of the nuclear localization sequences on the NF-kappa B complex and its nuclear translocation and DNA binding. NF-kappa B binding site is common to the promoter region of all of those inflammation-related genes that appear to be affected by alcohol.

Previous studies showed that acute alcohol administration in various animal models resulted in inhibition of production of the pro-inflammatory cytokine, TNF-alpha, in the serum and in alveolar macrophages (10). The attenuated pro-inflammatory cytokine activation has been proposed as a mechanism contributing to increased susceptibility to infections after acute alcohol treatment (41, 42). Similar down-regulation of TNF-alpha, IL-1-beta and IL-8 production was seen after acute alcohol consumption in human volunteers and in isolated human blood monocytes after acute alcohol treatment (33, 35). Many of these previously reported changes in inflammatory cytokine production have subsequently been linked to inhibition of NF-kappa B activation by acute alcohol use.

In recent years, several laboratories, including ours, investigated modulation of the NF-kappa B activation by acute or chronic alcohol in various cell types of the immune system. In human blood monocytes, acute alcohol treatment inhibited NF-kappa B activation by LPS, one of the most potent inducers of NF-kappa B activation (34). This reduction in NF-kappa B binding in the alcohol treated monocytes was mostly due to a decrease in the p65/p50 heterodimer binding that was also reflected by the reduced nuclear levels of p65 (RelA) in alcohol-exposed cells (34). The biologically active NF-kappa B dimer is the p65/p50 heterodimer and the p65 protein, but not the p50, has a DNA transactivation domain. The homodimer of p50/p50 that is also seen in monocytes after LPS or alcohol stimulation has a DNA binding site but no transactivation potential. Thus, p50/p50 homodimers can act as functional inhibitors of NF-kappa B-mediated DNA transactivation (43). Interestingly, the level of the p50/p50 NF-kappa B

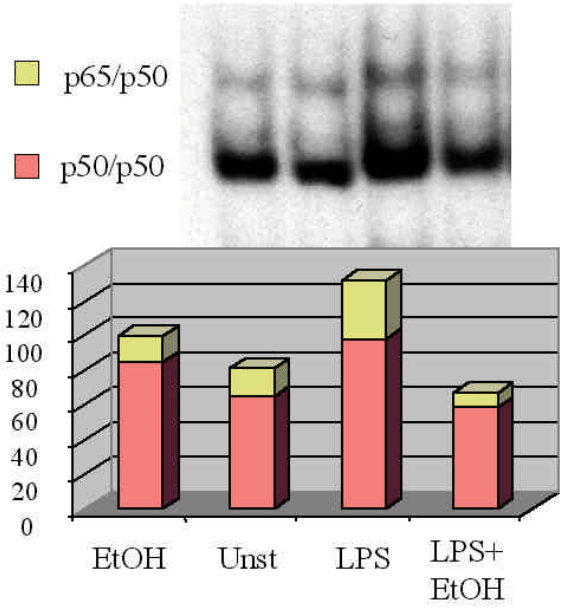

Figure 1. Acute alcohol inhibits NF-kappa-B activation in monocytes and differently regulates DNA binding of the p65/p50 and p50/p50 NF-kappa-B/Rel complexes. Adherence-isolated peripheral blood monocytes were unstimulated or activated with LPS (0.1 µg/ml), ethanol (25 mM), or their combination for one hour. Equal protein amounts of nuclear extracts were evaluated in EMSA (top panel). Densitometric values of the p65/p50 and p50/p50 complexes are shown (bottom panel). The specificity of p65 and p50 complexes was determined in supershift experiments (data not shown). Data is one representative of eight blood donors with similar results.

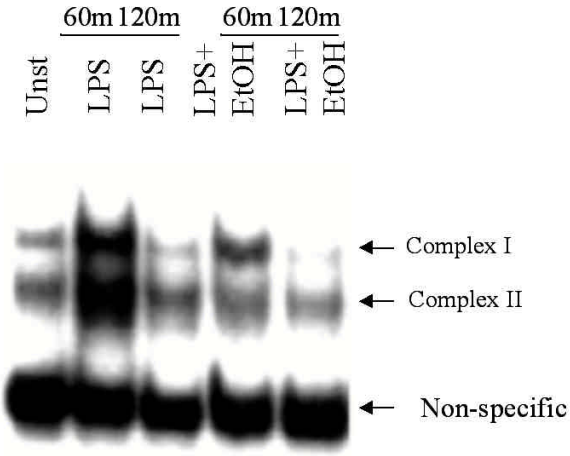

Figure 2. Acute alcohol inhibits NF-kappa-B activation in murine macrophages. RAW 264.7 murine macrophages were stimulated with 1µg/ml LPS with or without 25mM ethanol for 60 and 120 minutes and nuclear extracts subjected to electrophoretic mobility shift assays as described in the Methods. Complex I represents the p65/p50 heterodimer and Complex II represents the p50/p50 homodimer.

homodimer was increased in monocytes treated only with acute alcohol while LPS induced activation of the p65/p50 heterodimer (44). In alcohol plus LPS challenged monocytes, the reduction in the absolute level of NF-kappa B binding was associated with a proportional increase of the p50/p50 homodimer at the expense of the activating p65/p50 heterodimer (Figure 1). Thus, acute alcohol appears to inhibit NF-kappa B activation, and likely NF-kappa B-mediated gene activation, first, by reducing the absolute levels of NF-kappa B activation and second, by resulting in a proportional increase in nuclear binding of the transcriptionally inactive p50/p50 homodimer.

NF-kappa B activation is largely dependent on degradation of I-kappa Bs in most cell types, although increasing evidence suggest various, I-kappa B-independent mechanisms for activation of NF-kappa B (45). We reported that acute alcohol-induced inhibition of NF-kappa B in human monocytes occurred in the presence of I-kappa B alpha degradation (34) suggesting that I-kappa B alpha-independent mechanisms may be involved. These may include regulation via I-kappa B epsilon, a prominent I-kappa B type in monocytes mediating rapid and transient NF-kappa B activation. (37, 38). I-kappa B beta involvement is less likely as I-kappa B beta degradation, unlike degradation of I-kappa B alpha or I-kappa B epsilon, is prolonged after LPS stimulation and mediates longer-lasting NF-kappa B activation (24, 46). Thus, I-kappa B beta is less likely to have a major role in NF-kappa B regulation by acute alcohol treatment. NF-kappa B activation and nuclear binding can also be regulated at the nuclear level via interaction of NF-kappa B with other nuclear proteins including the glucocorticoid receptor.

Inhibition of NF-kappa B activation by acute alcohol treatment was also reported in LPS-activated rat Kupffer cells, the resident macrophages of the liver (13). In this study, inhibition of NF-kappa B by alcohol (100mM) was concomitant to reduced secretion of TNF-alpha. Reduced levels of mRNA for TNF-alpha in this study further suggested transcriptional regulation of TNF by ethanol (13). In our experiments, acute alcohol treatment reduced LPS-mediated activation of NF-kappa B in the murine macrophage, RAW 264.7 cells. Substantial reduction in the binding of both p65/p50 heterodimer and the p50/p50 homodimer was seen in alcohol plus LPS treated cells compared to the LPS activated macrophages at 60 and 120 minutes (Figure 2). These studies collectively suggest that acute alcohol treatment inhibits NF-kappa B activation in cells of the monocyte/macrophage lineage. Considering the pivotal role of these cells in innate immunity and in inflammation, inhibition of NF-kappa B activation by acute alcohol has a major clinical importance in attenuation of the initial signaling steps of inflammatory responses. Inhibition of NF-kappa B activation by ethanol was not limited to monocyte stimulation with LPS, but also occurred when monocytes were activated with staphylococcal enterotoxin B (SEB), IL-1 or TNF-alpha (35) (Szabo unpublished data). These inducers act via various surface receptors that include CD14 and Toll-like receptor 4 (TLR4) for LPS, HLA-Class II for SEB, IL-1R for IL-1, and TNFR1 for the cell-activating effects of TNF-alpha (5, 47-51). Thus, universal inhibition of NF-kappa B by acute alcohol suggest a target for the effects of alcohol

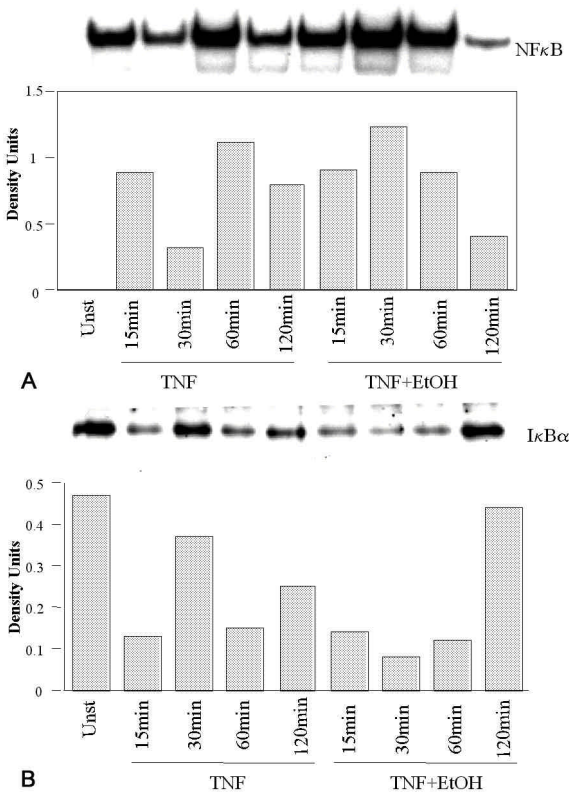

Figure 3. A: Acute ethanol increases TNF-induced NF-kappa-B binding in Jurkat T cells. Jurkat T cells were stimulated with 10ng/ml TNF-alpha in the presence or absence of 25mM ethanol at the indicated times. Nuclear extracts were prepared and subjected to gel shift analysis as described in the methods. One representative experiment out of a total of four is shown here. B: Effect of acute alcohol on TNF-induced I-kappa-B-alpha degradation in Jurkat T cells. Jurkat T cells were stimulated with 10ng/ml TNF-alpha in the presence or absence of 25mM ethanol at the indicated times. Cytoplasmic extracts (10μg/group) were subjected to Western blotting using the anti-I-kappa-B-alpha antibody (sc-371) from Santa Cruz Biotechnology. One representative experiment out of a total of four is shown here.

that is downstream, yet common, to these receptors in NF-kappa B activation.

It has been long recognized that although both cause immunosuppression, acute and chronic alcohol use have diverse effects on the production of pro-inflammatory cytokines, particularly, that of TNF-alpha (22, 33, 52). In patients with alcoholic hepatitis, peripheral blood monocytes have been proposed as one of the cellular sources of the increased levels of pro-inflammatory cytokines (18-20, 52). A recent study suggested that the intracellular signaling mechanisms for the increased pro-inflammatory cytokine production in these monocytes involves increased activation of NF-kappa B (21) suggesting that acute and chronic alcohol use may differently affect NF-kappa B activation. Thus, differential NF-kappa B activation by acute and chronic alcohol use in monocytic cells may be a key in their opposing effects on pro-inflammatory cytokine induction. A recent study also showed increased TNF-alpha induction after chronic alcohol feeding in LPS-stimulated rat Kupffer cells (53). Chronic alcohol feeding increased LPS-stimulated extracellular receptor-activated kinases 1/2 (ERK1/2). Activation of ERK1/2 was required for maximal increases in TNF-alpha mRNA associated with and increased binding of the early growth response-1 (EGR-1), and not NF-kappa B, to the TNF promoter. There was no change in AP-1 binding (53). These data suggested that enhanced activation of ERK 1/2 and Egr may also contribute to increased TNF-alpha production after chronic alcohol feeding in Kupffer cells.

Regulation of NF-kappa B by acute alcohol has been investigated in other cell types of the immune system, including T lymphocytes. Dong et al reported enhanced TNF-alpha inducible NF-kappa B activation in CD4+ Jurkat T lymphocytes and this NF-kappa B activation was associated with increased transcription of HIV-1-LTR suggesting a role for alcohol in HIV infection (54). Potentiation of TNF-alpha-inducible NF-kappa B by alcohol involved enhanced NF-kappa B proteolysis and was inhibited by the proteasome inhibitor, PDTC suggesting that NF-kappa B activation in T cells is mediated via I-kappa B alpha degradation (54). In the Jurkat cells, potentiation of NF-kappa B by acute alcohol treatment did not require de novo protein synthesis. In our experiments, acute alcohol also increased the TNF-alpha-induced NF-kappa B activation in Jurkat cells at a physiologically relevant, 25 mM dose (Figure 3a). The augmentation of TNF-alpha-induced NF-kappa B by alcohol was rapid and transient seen at 15-60 minutes after stimulation. However, at a later timepoint, at 120 minutes, NF-kappa B binding was reduced in the alcohol plus TNF-alpha stimulated cells compared to TNF only induced cells. The biological relevance of the biphasic regulation of NF-kappa B by acute alcohol needs further investigation, however, increased NF-kappa B in T cells is considered as one of the protective mechanisms against apoptosis (25). In the Jurkat T cells, IKB-alpha levels showed changes reciprocal to the alcohol-induced activation of NF-kappa B binding suggesting that the alcohol-induced changes in NF-kappa B activation were I-kappa B alpha-dependent (Figure 3b). I-kappa B alpha-dependent regulation of NF-kappa B by alcohol in T cells is different for monocytes, where we found reduced NF-kappa B binding along with ongoing degradation of I-kappa B alpha in alcohol plus LPS-treated monocytes. Similar to Jurkat cells, in T lymphocytes from healthy individuals, alcohol augmented TNF-alpha-induced NF-kappa B activation. Augmentation of TNF-alpha-inducible NF-kappa B was seen in the presence of 25 mM alcohol in the TNF-alpha-activated peripheral T cells compared to TNF-alpha stimulation alone (Figure 4). These results suggest that acute alcohol treatment has distinct effects on NF-kappa B activation in T lymphocytes compared to monocytes that may contribute to the anti-inflammatory effects of acute alcohol use (34).

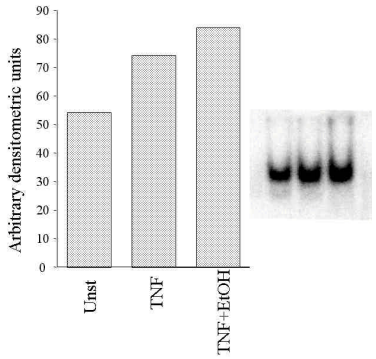

Figure 4. Acute alcohol increases the TNF-induced NF-kappa-B binding in peripheral T cells. T cells were isolated by rosetting Ficoll-Hypaque-isolated PBMCs with neuraminidase treated sheep red blood cells and stimulated with 10ng/ml TNF-alpha in the presence or absence of 25mM ethanol for 2 hrs. Nuclear extracts were prepared and subjected to gel shift analysis using the [^{32}P]NF-kappa-B oligo as described in the methods. One representative experiment out of a total of four is shown.

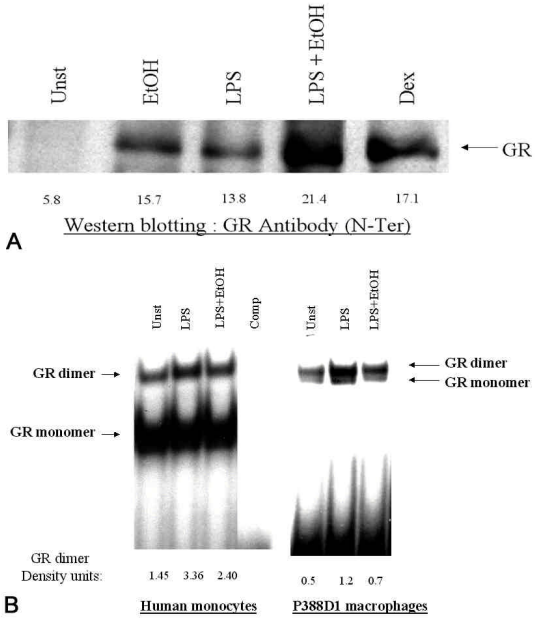

Figure 5. A: Alcohol augments nuclear glucocorticoid receptor levels in peripheral blood monocytes. Adherence-isolated monocytes were stimulated with 1µg/ml LPS with or without 25mM alcohol and 1µM Dexamethasone (Dex) for 1 h. Nuclear extracts (10µg/group) were subjected to immunoblotting using the anti-GR antibody (Santa Cruz Biotechnology) as described in the Methods. Data are representative of four experiments showing similar results. B: Alcohol affects DNA binding to GRE oligonucleotide in peripheral blood monocytes. Nuclear extracts from adherence-isolated monocytes stimulated with either 1 µg/ml LPS in the presence or absence of 25 mM alcohol for 1 h were subjected to gel shift analysis using the [^{32}P]GRE oligonucleotide probe as described in the Methods. Band I, homodimer of GR (functional); Band II, monomer of GR (non-functional).

4.2. Glucocorticoid response element and its modulation by alcohol

One of the most studied nuclear receptors of the superfamily of nuclear receptors is the glucocorticoid receptor (GR), which plays an important role in the modulation of inflammatory responses via repression of NF-kappa B activity (29, 30). Alcohol has been shown to exert indirect immunosuppressive effects by affecting glucocorticoid responses (55, 56). Upon cellular activation, the cytoplasmic glucocorticoid receptor (GR) binds to its ligand and translocates to the nucleus to exert its transactivating and transrepressive effects. The GR represses the *de novo* transcription of inflammatory cytokine genes, including TNF-alpha, IL-1-beta, IL-6 as well as GM-CSF and IFN-gamma? (57, 58). Transrepression of target genes by GR involves either its interaction with transcription factors such as NF-kappa B and AP-1 or up-regulation of I-kappa B synthesis resulting in inhibition of NF-kappa B regulated target genes (59-61). Various mechanisms have been described for GR-mediated inhibition of NF-kappa B including interaction of GR with p65 (61), protein kinase A (PKA)-dependent cross-repression of NF-kappa B by GR (62), and GR-mediated repression of NF-kappa B-driven genes by disturbance in the interaction of p65 with the basal transcription machinery (63).

We investigated regulation of glucocorticoid receptor activation and GR nuclear binding to the glucocorticoid response element (GRE) in human monocytes after acute alcohol treatment. The nuclear levels of GR were increased by alcohol or LPS-treatment in monocytes but to a lesser extent than the increase induced by the GR-ligand, dexamethasone (positive control). However, the combination of alcohol plus LPS treatment resulted in higher nuclear GR levels than ethanol or LPS treatments alone (Figure 5A). Although high levels of GR protein existed in the nucleus of LPS plus ethanol treated monocytes, binding of the GR to the GRE was not increased in these cells. Electromobility gel shift assays revealed that the increase in GR DNA binding after LPS stimulation was prevented by simultaneous alcohol administration (Figure 5B). Reduced GRE binding was found both in human monocytes and in P388D1 mouse macrophages in alcohol exposed and LPS stimulated cells (Figure 5B). Changes in the GR homodimer (higher molecular weight band) represent the biologically functional GR complex (64, 65). Taken together, in acute alcohol-exposed monocytes, LPS induction resulted in increased nuclear GR levels that appeared to be in a non-GRE-bound form. Based on these observations, we propose a model in which excess nuclear GR levels in alcohol exposed and LPS-activated monocytes may become available to interact with other nuclear proteins, including p65 of the NF-kappa B/Rel family to prevent NF-kappa B activation (Figure 6) (29, 30). Increasing evidence suggest that GR modulates inflammatory gene expression both positively (transactivation) and negatively (transrepression). It has been proposed that the transactivation function of the GR could contribute to the anti-inflammatory actions of GR activation by enhancing expression of anti-inflammatory cytokines including IL-1

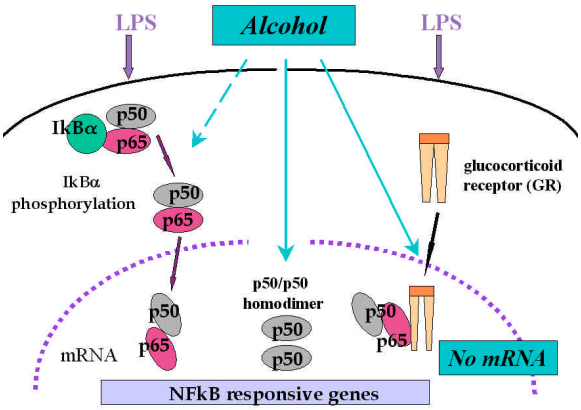

Figure 6. Proposed model of GR regulation and interaction of p65-NF-kappa-B and GR by acute alcohol treatment. Solid arrows indicate stimulatory and the dotted arrow inhibitory effects.

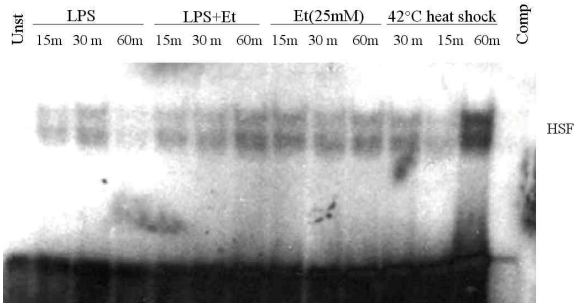

Figure 7. Acute ethanol increases heat shock element binding in monocytes. Adherence-isolated monocytes were stimulated with 1µg/ml LPS with or without 25mM alcohol and heat-shocked by exposure to 42 C for the indicated time periods. Nuclear extracts were prepared and subjected to EMSA using the [^{32}P] HSE oligonucleotide as described in the Methods. A 20-fold excess of the unlabeled HSE oligo was included as a cold competitor (Comp). Results from one experiment are shown from four experiments performed.

receptor antagonist (which inhibits binding of IL-1 to its receptor), and IL-10 (an anti-inflammatory cytokine) (66). Elevated IL-10 has been shown as a mediator of the anti-inflammatory effects of acute alcohol and of the immunosuppression after chronic alcohol use (6, 7, 67). However, activation of the IL-10 gene by GR requires higher GR concentrations than the transrepressive actions thought to be necessary for the direct anti-inflammatory actions of the GR (66). Thus, our results support the contention that the transrepressive actions of the GR are relevant to anti-inflammatory functions of the GR induced by acute alcohol use.

4.3. Effects of acute alcohol treatment on heat shock promoter element (HSE) activation

Alcohol treatment at the cellular level can be considered as a danger signal and it is associated with activation of signaling mechanisms that are induced in response to cellular stress/shock. In addition to the NF-kappa B and Jak/STAT pathways, heat shock response (HSR) and activation of heat shock factors, proteins that bind to the heat shock promoter element (HSEs), are fundamental in cellular responses. Thus, activation of heat shock response (HSR) during alcohol use may interact with other cellular activation pathways. In multiple cell models, prior induction of the HSR inhibits subsequent activation of NF-kappa B (68). It is now well established that prior induction of the HSR has an inhibitory effect on NF-kappa B activation which accounts, for the large part, for the mechanisms by which the HSR inhibits cellular inflammatory responses (69). The mechanism by which the HSR inhibits activation of NF-kappa B involves inhibition of I-kappa B alpha degradation and stabilization of the NF-kappa B I-kappa B alpha complex (69). Further regulation involved inhibition of IKK activation by HSR and augmentation of intracellular phosphatase activity in macrophages (69, 70).

Considering that production of heat shock protein genes can be regulated by activation of the heat shock promoter element, we investigated HSE activation in human monocytes. There was a moderate increase in HSE binding upon LPS stimulation in human monocytes with maximal induction at 30 minutes (Figure 7). Alcohol treatment alone (25mM) induced an early HRE activation at 15 minutes that was still increased at 60 minutes after stimulation (Figure 7). The kinetics of the alcohol-induced HSE was similar to heat shock-induced HSE activation, however, as expected, the extent of activation was much greater with heat shock. The combination of alcohol plus LPS resulted in maximal activation of HSE at 60 minutes and was not substantially greater than HRE activation induced by LPS or ethanol alone. These results suggest that after acute alcohol exposure of immune cells various signaling pathways are induced, including HRE activation that in concert with other nuclear regulatory factors determine the final cellular response to alcohol and other cell activators.

5. CONCLUSIONS

The complex biological effects of acute and chronic alcohol use are the net-result of activation of various elements of signaling pathways at the cellular level. Here, we reviewed the interactions between acute and chronic alcohol use and some of the most prominent cell activation pathways of immunocompetent cells that contribute to the immunomodulatory effects of alcohol use. Results from the reviewed literature and from our laboratory collectively demonstrate that one of the major targets for the effects of acute and chronic alcohol in modulation of cellular signaling is the NF-kappa B/Rel pathway. Interestingly, NF-kappa B activation is regulated differently depending on the cell types and on acute or chronic alcohol use. Data also suggest that regulation of NF-kappa B by acute or chronic alcohol use involves interactions with other transcription factors, including the glucocorticoid receptor and elements of the heat-shock response. Thus, further investigation of these interactions may offer explanation for the diverse effects of acute and

chronic alcohol in different cell types. The biological significance of inhibition of NF-kappa B activation by acute alcohol use in monocytic cells appears to be linked to decreased production of pro-inflammatory cytokines and impaired elimination of certain types of infections. In contrast, overproduction of inflammatory mediators in patients with alcoholic hepatitis is associated with hyper-elevated NF-kappa B activation. Further investigation of the NF-kappa B and related transcription factors in immune cells will be instrumental for better understanding of the molecular mechanisms of the immunosuppressive effects of alcohol.

6. ACKNOWLEDGEMENT

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