Biochemical Pharmacology, Vol. 52, pp. 1499–1505, 1996.
Copyright © 1996 Elsevier Science Inc.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00524-2

Impairment of the Asialoglycoprotein Receptor by Ethanol Oxidation

Dahn L. Clemens, Christine M. Halgard, Jack R. Cole, Rodney M. Miles, Michael F. Sorrell and Dean J. Tuma*

Liver Study Unit, Department of Veterans Affairs Medical Center and Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68105, U.S.A.

ABSTRACT. It is well established that ethanol exposure impairs the process of receptor-mediated endocytosis in hepatic cells, although the molecular mechanism(s) and the physiological consequence(s) of this impairment are unclear. Because addressing these mechanistic questions is difficult *in vivo*, we have developed a recombinant cell line of hepatic origin capable of metabolizing ethanol. In this study, we have used these recombinant cells, designated HAD cells, to investigate the ethanol-induced impairment to the receptor-mediated endocytosis of the hepatic asialoglycoprotein receptor. Comparing the binding of the ligand asialoorosomucoid in both the parental Hep G2 cells and the recombinant HAD cells, maintained in the presence and absence of ethanol, revealed decreased ligand binding in the HAD cells. This impairment was accentuated by prolonging the ethanol exposure, reaching approximately 40% in both surface and total receptor populations by 7 days. Addition of the alcohol dehydrogenase inhibitor pyrazole to the ethanol-containing medium abolished this impairment, indicating that the decreased binding was a result of the alcohol dehydrogenase-mediated oxidation of ethanol. Furthermore, using antibody specific to the asialoglycoprotein receptor, it was demonstrated that the ethanol-induced impairment in ligand binding was a consequence of decreased ligand binding and not a result of diminished receptor numbers. These results indicated that ethanol oxidation was required for the ethanol-induced impairment in ligand binding, and that the reduced ligand binding was a result of a decrease in the ability of the ligand to bind to the receptor. BIOCHEM PHARMACOL 52;10:1499–1505, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. ethanol oxidation; receptor-mediated endocytosis; alcohol dehydrogenase

Studies employing chronic ethanol administration to animals have been instrumental in characterizing a variety of ethanol-induced hepatic impairments. Unfortunately, many times the use of *in vivo* models has made studies designed to investigate the mechanisms of these impairments difficult or impossible to perform. The difficulty of performing *in vivo* mechanistic studies has been exacerbated by the fact that isolated hepatocytes lose their differentiated phenotype in culture [1]. Concomitant with this phenotypic loss is a corresponding loss in liver-specific functions [2, 3]. Among the liver-specific functions that are rapidly lost are the ability to express alcohol dehydrogenase and to oxidize ethanol efficiently [4]. Thus, development of an *in vitro* hepatic cell culture system capable of efficiently metabolizing ethanol would be an extremely useful model system for studying ethanol-induced hepatic changes and defining the mechanisms of alcohol-induced liver cell injury. We have developed such a cell culture system [5]. Using the differentiated hepatoblastoma cell line Hep G2, which metabolizes very little ethanol, we have established a recombinant hepatic cell line that efficiently oxidizes ethanol and produces acetaldehyde. These recombinant cells, termed HAD cells, were established by stably transfecting Hep G2 cells with an eukaryotic expression vector designed to express alcohol dehydrogenase. HAD cells have been shown to metabolize ethanol efficiently and to produce micromolar amounts of acetaldehyde [5]. Thus, through the use of these two cell lines, it is possible to investigate the direct effects of ethanol metabolism on cellular functions.

In this study, we have employed both the parental Hep G2 and the recombinant HAD 73.1 cell lines to investigate the ethanol-induced impairment in receptor-mediated endocytosis. It is well established that chronic ethanol administration to rats dramatically impairs the receptor-mediated endocytosis processes of various receptors, including the epidermal growth factor receptor [6] and the liver-specific asialoglycoprotein receptor [7, 8]. Marked decreases in specific ligand binding to these receptors as well as alterations in other steps of the endocytosis process have been identified [6–8]. Although impairments in receptor-mediated endocytosis of these receptors are well established in hepatocytes isolated from ethanol-fed animals, the mechanism(s) of these impairments has yet to be elucidated. Furthermore,

* Corresponding author: Dahn L. Clemens, Ph.D., VAMC, 4101 Woolworth Ave., Omaha, NE 68105. Tel. (402) 346-8800, Ext. 3738; FAX (402) 449-0604; E-mail: DLCLEMEN@UNMCVM.UNMC.EDU.

Received 21 March 1996; accepted 28 May 1996.

the direct involvement of ethanol metabolism in these impairments has yet to be demonstrated.

To further investigate the ethanol-induced impairments in receptor-mediated endocytosis, we have employed these two hepatic cell lines. The purpose of this study was to determine if ethanol metabolism is required for the impaired binding of asialoorosomucoid to the asialoglycoprotein receptor and, if so, if this impairment is due to a diminution of the number of receptors or to altered binding properties of the receptor.

MATERIALS AND METHODS

Cells and Culture Conditions

HAD 73.1 [5] and Hep G2 cells [9] were cultured in DMEM† containing high glucose supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 10% fetal bovine serum and 50 μg/mL gentamicin (complete DMEM). Recombinant HAD cells were cultured in complete DMEM containing 200 μg/mL hygromycin B. All cells were maintained at 37° in a humidified environment containing 5% CO_2. During ethanol exposure studies, the growth medium was supplemented with 25 mM ethanol and 25 mM HEPES (pH 7.3), and the cells were cultured in tightly sealed flasks to minimize evaporation of ethanol. To ensure that sufficient concentrations of ethanol were present in the culture system, the growth medium was changed every 2–3 days. By culturing the cells in this manner, the ethanol concentration was always maintained above 12.6 mM. In some experiments, alcohol dehydrogenase was inhibited by inclusion of 1 mM pyrazole [10, 11] to the growth medium.

Binding Assays

Human orosomucoid was desialylated with *Clostridium perfringens* neuraminidase as previously described [7, 12]. ^{125}I-Asialoorosomucoid and ^{125}I-protein G were prepared by the procedure described by Weigel and Oka [13]. Surface ligand binding assays were performed essentially as described by Weigel [14], and receptor binding was determined by the specific binding of ^{125}I-asialoorosomucoid at 0–4°. Briefly, cells were seeded onto 12.5 cm^2 tissue culture flasks (Falcon, Franklin Lakes, NJ) and incubated overnight in growth medium. The following morning, the growth medium was removed and replaced with new medium, in some cases containing 25 mM ethanol. The cells were incubated for various periods of time prior to assay. Following ethanol exposure, the medium was removed from the flasks, and the cells were washed three times in DMEM. The washed cells were incubated with 2.0 μg/mL of ^{125}I-asialoorosomucoid at 0–4° for 4 hr with gentle shaking. Following this incubation, the ^{125}I-asialoorosomucoid solution was removed, and the cell sheet was washed three times with DMEM. The cells were removed from the surface of the flask by incubation in 1 mL of PBS containing 10 mM EDTA (PBS/EDTA) for 10 min. The PBS/EDTA solution containing the cells was collected, and each flask was washed with an additional 1 mL of PBS/EDTA; these two samples were pooled. These cell suspensions contained surface bound ^{125}I-asialoorosomucoid. At least one flask from each experimental group was incubated with a 100-fold excess of unlabeled asialoorosomucoid. The counts from these flasks were deemed to be from nonspecific binding. Specific binding was determined by subtracting the counts detected in nonspecific binding flasks from those detected in experimental flasks. Total asialoorosomucoid binding (surface plus intracellular binding) was determined by assaying the cells as described above in the presence of 0.075% digitonin to permeabilize the cells [8].

DNA content of the cultures was determined by the method described by Cesarone *et al.* [15] using 33258 Hoechst. Fluorescence at 356 nm excitation and 458 nm emission was measured on a Perkin-Elmer LS-SB luminescence spectrometer (Norwalk, CT). Binding of asialoorosomucoid to the asialoglycoprotein receptor was expressed as femtomoles ^{125}I-asialoorosomucoid bound per million cells, using a value of 10.38 μg DNA/10^6 cells.

Antibody Binding Assay

Antisera specific to the human asialoglycoprotein receptor was used to estimate the relative number of surface asialoglycoprotein receptors. Briefly, the medium was removed from the flasks, and the cells were washed as above and incubated with 1 mL of rabbit polyclonal antiserum raised against the purified human asialoglycoprotein complex (a gift from Dr. Richard Stockert) [16] for 1 hr at 0–4°. Following this incubation, the flasks were again washed three times with DMEM and then incubated with 1 mL of 2.0 μg/mL of ^{125}I-protein G for 2 hr. Bound ^{125}I-protein G was determined, and the binding expressed as described above. Nonspecific binding was accounted for by incubating the cells in the presence of non-immune rabbit serum and ^{125}I-protein G as above. The counts from these flasks were deemed to be from nonspecific binding. As with ligand binding, specific antibody binding was determined by subtracting counts detected in the nonspecific flasks from those detected in experimental flasks.

Immunoblotting

Total receptor content was determined by immunoblot analysis essentially as previously described [17]. Briefly, cells were lysed in 0.1% SDS and protein concentrations were determined using the BCA Protein Assay Reagent (Pierce Chemical Co., Rockford, IL). Fifteen micrograms of total cellular protein was separated by SDS–PAGE on 10% gels. Proteins were transferred by electroblotting to nitrocellulose membranes, and blots were blocked in PBS containing 5% nonfat dry milk and 0.01% Tween 20. The blots were reacted with a 1:1000 dilution of the rabbit antiserum de-

† *Abbreviation:* DMEM, Dulbecco's Modified Eagles's Medium.

scribed above. Following this incubation, the blots were washed with PBS containing 0.1% Tween 20 prior to incubation with an alkaline phosphatase conjugated goat anti-rabbit antiserum (ZYMED, South San Francisco, CA). The blots were washed as described above, and the reacting proteins visualized by the addition of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT). The blots were quantified by densitometric analysis using a Hewlett Packard ScanJet 4c/T (Sunnyvale, CA) and Sigma Gel software (Sigma, St. Louis, MO).

RESULTS

It is well documented that the binding of asialoorosomucoid to the hepatic asialoglycoprotein receptor is impaired in hepatocytes isolated from animals that have been fed ethanol [7, 8]. We were interested in determining if this impairment was due to the presence of ethanol or if the metabolism of ethanol was required for this dysfunction.

To test this, we first compared the surface binding of ^{125}I-asialoorosomucoid in Hep G2 cells (cells that metabolize ethanol very poorly) and HAD 73.1 cells (cells that metabolize ethanol efficiently) cultured in the presence or the absence of 25 mM ethanol. The data presented in Fig. 1 indicate that while there was no difference in the ability of the Hep G2 cells cultured in the presence or absence of ethanol to bind asialoorosomucoid, there was a dramatic reduction in the binding of ^{125}I-asialoorosomucoid in the HAD 73.1 cells cultured in the presence of ethanol. This impaired surface binding was evident after 2 days of ethanol exposure and became more pronounced with increased time of exposure to ethanol. These results indicated that ethanol oxidation was required for this impairment.

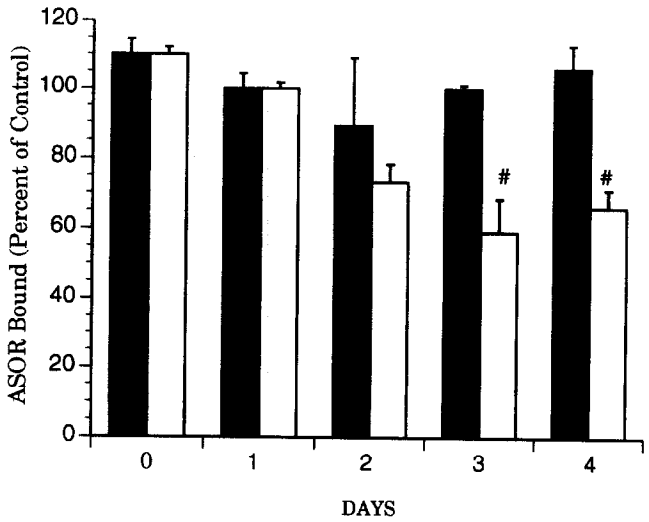

FIG. 1. Time course of asialoorosomucoid binding to HAD 73.1 and Hep G2 cells in the presence and absence of ethanol. Hep G2 (■) and HAD 73.1 (□) cells were cultured in either the presence or the absence of 25 mM ethanol and then assayed at the indicated times for the ability of ^{125}I-asialoorosomucoid to bind to the surface asialoglycoprotein receptors. Using the cells cultured in the absence of ethanol as controls, impairments in ligand binding were determined. The absolute values for ^{125}I-asialoorosomucoid binding to cells maintained in the absence of ethanol at time zero were 48.8 and 43.7 fmole/10^6 cells for Hep G2 and HAD 73.1 cells respectively. The data are expressed as the means ± SEM of four determinations. Key: (#) significant difference between the Hep G2 cultures and the HAD 73.1 cell cultures, $P < 0.05$.

To further implicate alcohol dehydrogenase-mediated oxidation of ethanol in the observed impaired binding of the asialoglycoprotein receptor, HAD 73.1 cells were cultured in medium containing 25 mM ethanol either in the presence or the absence of the alcohol dehydrogenase inhibitor pyrazole. The results depicted in Fig. 2 show that addition of pyrazole to the ethanol-containing growth medium abolished the binding impairment observed in HAD 73.1 cells. The abolition of this impairment was observed at both time points tested, 4 days (Fig. 2A) and 7 days (Fig. 2B) of ethanol exposure. These results further supported the conclusion that alcohol dehydrogenase-mediated metabolism of ethanol was required for the ethanol-induced impairment in ligand binding to the asialoglycoprotein receptor observed in HAD 73.1 cells.

We were interested in determining if the binding impairment observed in HAD 73.1 cells cultured in the presence of ethanol was a result of a decrease in the binding properties of the receptor, or if this effect was due to fewer receptors located on the surface of the cells. To differentiate between these two possibilities, we performed assays designed to determine the relative number of the asialoglycoprotein receptors on the surface of the cells, and the ability of these receptors to bind ligand. To estimate the relative number of asialoglycoprotein receptors on the cell surface, cells cultured in the presence or the absence of ethanol were incubated with antiserum specific to the asialoglycoprotein receptor. To ensure that there was a demonstrable impairment in the binding of the ligand, sister flasks were assayed for the ability of the cells to bind ^{125}I-asialoorosomucoid. The results of these experiments show that although the ability of the cells cultured in the presence of ethanol exhibited a reduction in their ability to bind ligand, there was very little difference in the ability of the antiserum to recognize the receptor (Fig. 3). These results were observed following both 3 days (Fig. 3A) and 7 days (Fig. 3B) of ethanol exposure. In addition, Hep G2 cells were cultured in the presence and absence of ethanol for 7 days and assayed for the ability to bind asialoglycoprotein receptor antiserum and ligand as described above. No significant difference was found in either the binding of antibody or ligand between these two culture conditions (data not shown). Thus, these data indicated that the ethanol-induced impairment in ligand binding observed in HAD 73.1 cells cultured in the presence of ethanol was due to a decrease in the binding of the ligand to the receptor and not due to a depletion of receptor numbers on the surface of the cells.

To determine if the ethanol-induced impairment of asi-

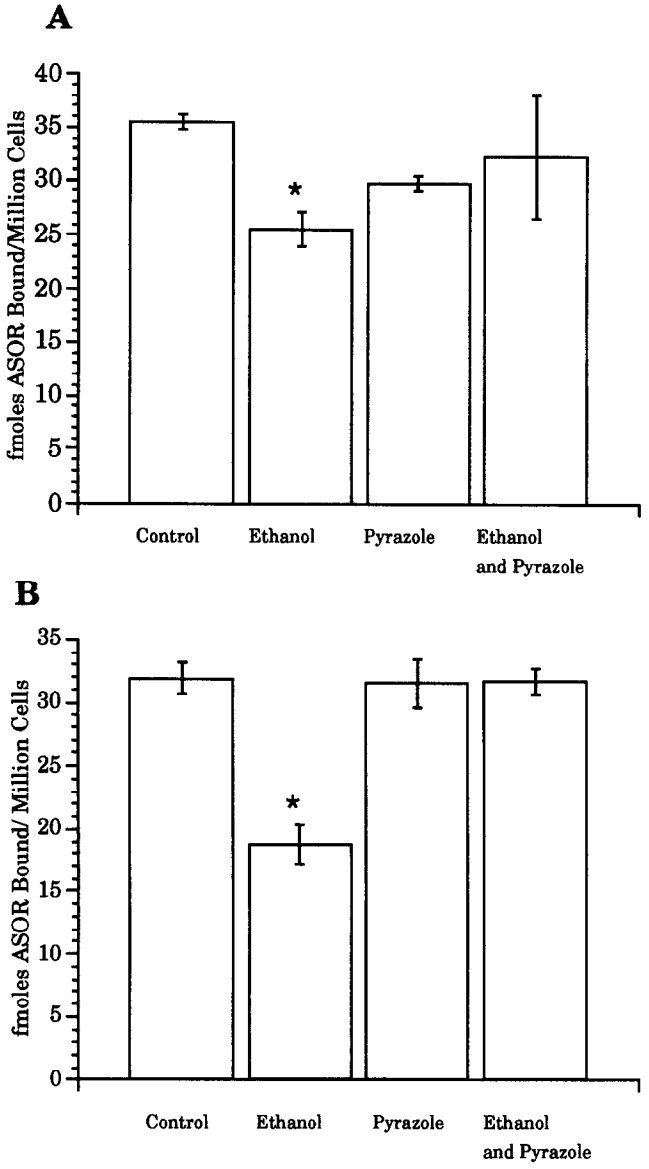

FIG. 2. Asialoorocomucoid binding to HAD 73.1 cells in the presence and absence of pyrazole. HAD 73.1 cells were exposed to 25 mM ethanol for either 4 days (A) or 7 days (B) in the presence or absence of the alcohol dehydrogenase inhibitor pyrazole and then assayed for the ability of ^{125}I-asialoorosomucoid to bind to the surface asialoglycoprotein receptors. The data are expressed as the means ± SEM of four determinations. Key: (*) significant difference between the HAD 73.1 cell cultures maintained in the presence of ethanol and controls maintained in its absence, $P < 0.01$.

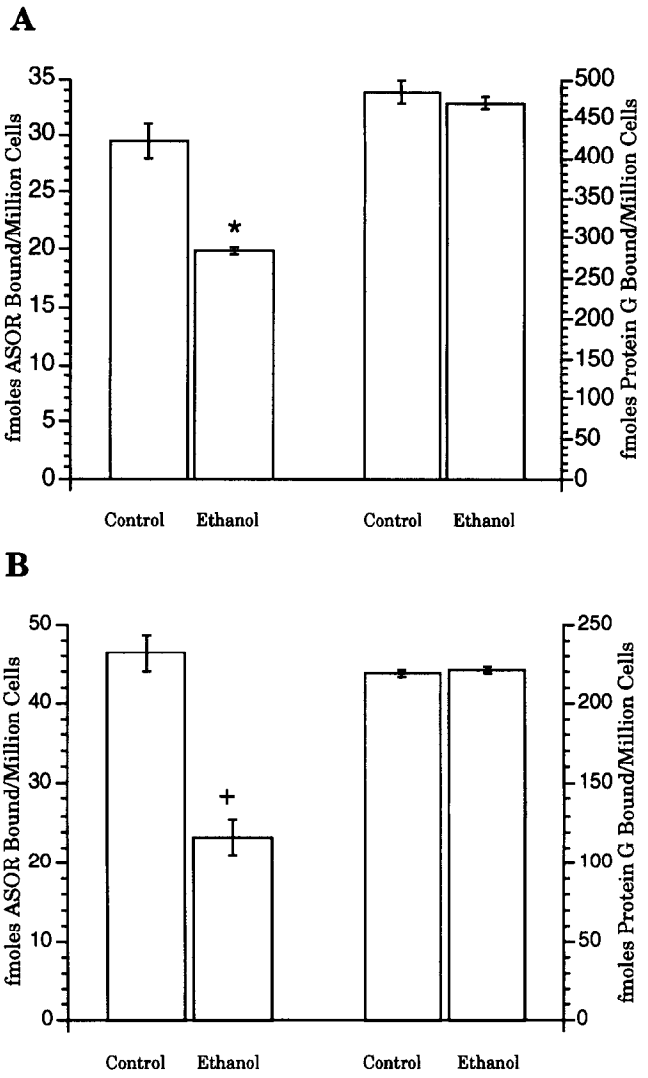

FIG. 3. Comparison of ligand binding to the asialoglycoprotein receptor and binding of anti-asialoglycoprotein receptor serum following ethanol exposure. HAD 73.1 cells were exposed to 25 mM ethanol for either 3 days (A) or 7 days (B). One set of flasks was assayed for the ability of ^{125}I-asialoorosomucoid to bind to the surface asialoglycoprotein receptors, and another set of flasks was assayed for the presence of asialoglycoprotein receptors on the surface of the cells using anti-asialoglycoprotein receptor serum followed by ^{125}I-protein G. The data are expressed as the means ± SEM of five or six determinations. Key: (*) significant difference between the HAD 73.1 cell cultures maintained in the presence of ethanol and controls maintained in its absence, $P < 0.01$; and (+) significant difference between the HAD 73.1 cell cultures maintained in the presence of ethanol and controls maintained in its absence, $P < 0.005$.

aloorosomucoid binding to the asialoglycoprotein receptor was solely a surface effect, or if the total cellular receptor population was also affected, we also assayed ligand binding to the total receptor population. To determine this, HAD 73.1 cells were cultured either in the presence or the absence of 25 mM ethanol for 7 days, and binding assays were performed. Following the ethanol exposure, one half of the flasks were assayed in the presence of 0.075% digitonin to permeabilize the cells. The results shown in Fig. 4 indicate that the impairment in asialoglycoprotein receptor binding was virtually identical in both the surface and total receptor populations, being impaired approximately 40%. These results indicated that the total cellular population of receptor was being similarly affected by exposure to ethanol.

To ensure that the decrease in ligand binding was not simply due to a decrease in the total cellular population, immunoblots were performed, and representative samples are shown in Fig. 5. Densitometric analysis of the asialo-

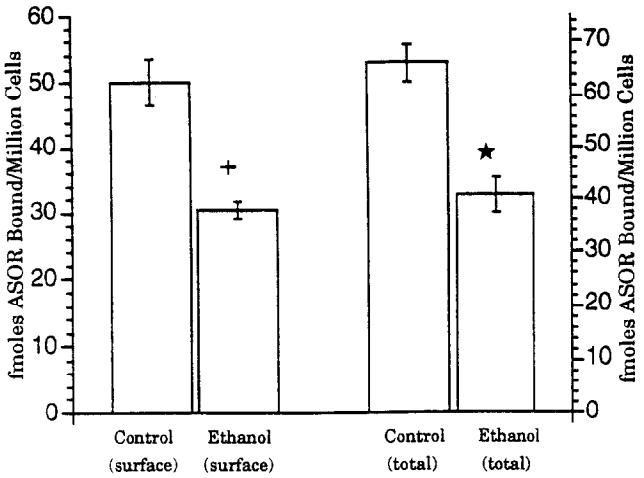

FIG. 4. Comparison of the effects of ethanol exposure on the surface and total binding of asialoorosomucoid to HAD 73.1 cells. HAD 73.1 cells were exposed to 25 mM ethanol for 7 days and assayed for the ability of ^{125}I-asialoorosomucoid to bind to the surface and total asialoglycoprotein receptors. The data are expressed as the means ± SEM of five or six determinations. Key: (+) significant difference between the HAD 73.1 cell cultures maintained in the presence of ethanol and controls maintained in its absence, $P < 0.005$; and (★) significant difference between the HAD 73.1 cell cultures maintained in the presence of ethanol and controls maintained in its absence, $P < 0.001$.

glycoprotein receptor bands from cells cultured in the presence or absence of ethanol confirmed that there was no difference in the asialoglycoprotein receptor content between the control and the ethanol-exposed cells.

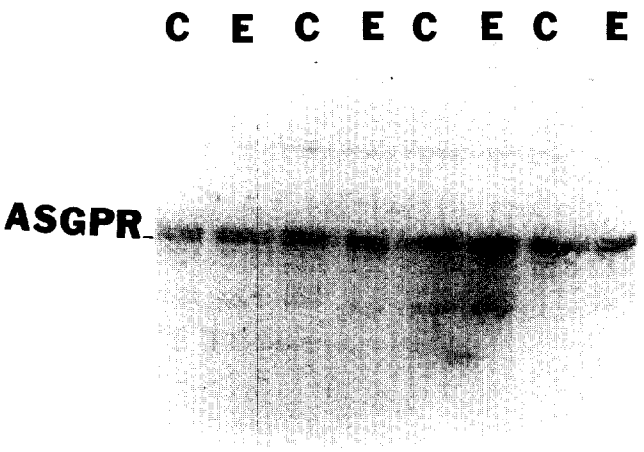

FIG. 5. Immunoblot analysis of the asialoglycoprotein receptor (ASGPR) in HAD 73.1 cells maintained in the presence or the absence of ethanol. HAD 73.1 cells were maintained in the presence of 25 mM ethanol for 7 days. Cell lysates were prepared, and proteins were separated by SDS–PAGE. Proteins were blotted onto nitrocellulose and probed with anti-asialoglycoprotein receptor serum. Shown are representative samples from four different flasks of cells maintained in the absence of ethanol (C) and from four different flasks of cells maintained in the presence of 25 mM ethanol (E).

DISCUSSION

Receptor-mediated endocytosis is responsible for the uptake and degradation of many biologically important molecules including hormones, growth factors, and cytokines [18, 19]. Receptor-mediated endocytosis is also involved in regulating the expression of surface receptors and signal transduction [20, 21]. Thus, alterations in hepatic receptor-mediated endocytosis could have broad biological consequences, being detrimental to the liver. Numerous studies have shown that chronic ethanol exposure to animals affects many hepatic processes. Unfortunately, use of animal models makes mechanistic studies extremely difficult. In an attempt to create a system more amenable to mechanistic studies, we have established a recombinant cell line that efficiently metabolizes ethanol and produces acetaldehyde [5]. We have termed these cells HAD 73.1 cells. HAD 73.1 cells are a recombinant cell line constructed by stably transfecting the differentiated hepatoblastoma cell line, Hep G2, with an eukaryotic expression vector designed to express alcohol dehydrogenase [5]. Thus, the only known differences between these two cells lines are the ability (a) to express alcohol dehydrogenase, and (b) to efficiently metabolize ethanol. Use of this model system eliminates many of the variables associated with animal feeding models, thus simplifying many types of studies.

It is well established that ethanol feeding to animals impairs the processes of hepatic receptor-mediated endocytosis. Therefore, to validate the use of the HAD/Hep G2 culture system we have investigated the effects of alcohol dehydrogenase-mediated oxidation of ethanol on the process of receptor-mediated endocytosis. Initially, we observed that when the recombinant HAD 73.1 cells were cultured in the presence of ethanol, binding of the ligand, asialoorosomucoid, to the asialoglycoprotein receptor was reduced in comparison to either HAD 73.1 cells cultured in the absence of ethanol or Hep G2 cells cultured either in the presence or the absence of ethanol. These results indicated that the metabolism of ethanol was required for the impairment in ligand binding. To further test this, HAD 73.1 cells were cultured in the presence of ethanol either in the absence or the presence of pyrazole, an alcohol dehydrogenase inhibitor. The only group of cells that showed an impaired ability to bind the ligand were the HAD 73.1 cells cultured in the medium containing ethanol but lacking pyrazole. Thus, by inhibiting alcohol dehydrogenase-mediated oxidation of ethanol, we were able to abolish the deleterious effect of ethanol. These data taken together provide very strong evidence that the oxidation of ethanol, and not the presence of ethanol itself, mediated this dysfunction.

By using antiserum specific to the asialoglycoprotein receptor in binding assays and immunoblots, we also demonstrated that the ethanol-induced impairment in ligand binding was due to a decrease in the ability of the ligand to bind to the receptor, and not to diminished receptor content. This was the case for both surface and total cellular

receptor populations. It has been reported recently that the asialoglycoprotein receptor of hepatocytes isolated from rats fed ethanol for 4–7 days showed a similar decrease in the ability to bind ligand on the surface of the cell [8]. Thus, these results indicate that the HAD cell system may be a suitable *in vitro* model for the study of ethanol-induced hepatic cellular dysfunction.

The fact that inhibition of alcohol dehydrogenase-mediated oxidation of ethanol in HAD cells abolished the ethanol-induced impairment in ligand binding strongly suggests that a by-product or consequence of ethanol oxidation is responsible for this impairment. Two of the more dramatic consequences of alcohol dehydrogenase-mediated oxidation of ethanol by hepatocytes, which have been proposed as possible mediators of ethanol-induced hepatic toxicity, are the shift in redox potential and the production of the highly reactive compound acetaldehyde. We have shown that ethanol oxidation by HAD cells is limited by alcohol dehydrogenase activity; thus, the redox change observed in hepatocytes is attenuated in the HAD cell system [5]. Conversely, HAD cells actively metabolizing ethanol produce substantial quantities of acetaldehyde, reaching levels of over 50 μM [5]. Acetaldehyde has been shown to bind to and inactivate proteins [22–25]. Additionally, acetaldehyde has been strongly implicated as a mediator of defects in hepatic protein trafficking [26]. Thus, it is possible that acetaldehyde is also involved in the impairment of ligand binding to the asialoglycoprotein receptor observed in this study.

In summary, we have used a recombinant cell line of hepatic origin capable of alcohol dehydrogenase-mediated oxidation of ethanol to investigate the ethanol-induced impairment of hepatic receptor-mediated endocytosis. Use of these cells has allowed us to demonstrate that the binding of asialoorosomucoid to the asialoglycoprotein receptor is impaired in cells that metabolize ethanol (HAD 73.1 cells) and not in those unable to metabolize ethanol (Hep G2 cells). Furthermore, in all the circumstances analyzed, impairment of the binding of asialoorosomucoid to the asialoglycoprotein receptor appeared to be dependent on the oxidation of ethanol, thus providing very strong evidence for a requirement of ethanol metabolism in this dysfunction. It is hoped that further studies using this recombinant cell culture system will yield further information regarding the underlying mechanism(s) responsible for this impairment.

This work was supported by the Omaha Veterans Administration Alcohol Research Center and a grant to D.L.C. from the University of Nebraska Medical Center. We would also like to thank Richard Stockert for the gift of antisera specific to the human asialoglycoprotein receptor and Carol Casey for valuable discussion.

References

1. Schuetz EG, Li D, Omiecinski CJ, Muller-Eberhard U, Kleinman KH, Elswick B and Guzelain PS, Regulation of gene expression in adult rat hepatocytes cultured on a basement membrane matrix. *J Cell Physiol* **134:** 309–323, 1988.
2. Clayton DF and Darnell JE Jr, Changes in liver-specific compared to common gene transcription during primary culture of mouse hepatocytes. *Mol Cell Biol* **3:** 1552–1561, 1983.
3. Ben-Ze'ev A, Robinson GS, Bucher NLR and Farmer SR, Cell–cell and cell–matrix interactions differentially regulate the expression of hepatic cytoskeletal genes in primary cultures of rat hepatocytes. *Proc Natl Acad Sci USA* **85:** 2161–2165, 1988.
4. Crabb DW and Li TK, Expression of alcohol dehydrogenase in primary monolayer cultures of rat hepatocytes. *Biochem Biophys Res Commun* **128:** 12–17, 1985.
5. Clemens DL, Halgard CM, Miles RM, Sorrell MF and Tuma DJ, Establishment of a recombinant hepatic cell line stably expressing alcohol dehydrogenase. *Arch Biochem Biophys* **321:** 311–318, 1995.
6. Dalke DD, Sorrell MF, Casey CA and Tuma DJ, Chronic ethanol administration impairs receptor-mediated endocytosis of epidermal growth factor by rat hepatocytes. *Hepatology* **12:** 1085–1091, 1990.
7. Casey CA, Kragskow SL, Sorrell MF and Tuma DJ, Chronic ethanol administration impairs the binding and endocytosis of asialo-orosomucoid in isolated hepatocytes. *J Biol Chem* **262:** 2704–2710, 1987.
8. Tworek BL, Tuma DJ and Casey CA, Decreased binding of asialoglycoproteins to hepatocytes from ethanol-fed rats: Consequence of both impaired synthesis and inactivation of the asialoglycoprotein receptor. *J Biol Chem* **271:** 2531–2538, 1996.
9. Knowles BB, Howe CC and Aden DP, Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis surface antigens. *Science* **209:** 497–499, 1980.
10. Theorell H and Yonetani T, Liver alcohol dehydrogenase–DPN–pyrazole complex: A model of a ternary intermediate in the enzyme reaction. *Biochem Z* **338:** 537–553, 1963.
11. Grunnet N, Quistorff B and Thieden HID, Rate-limiting factors in ethanol oxidation by isolated rat-liver parenchymal cells. Effect of ethanol concentration, fructose, pyruvate and pyrazole. *Eur J Biochem* **40:** 275–282, 1973.
12. Oka JA and Weigel PH, Recycling of the asialoglycoprotein receptor in isolated rat hepatocytes. *J Biol Chem* **258:** 10253–10262, 1983.
13. Weigel PH and Oka JA, Endocytosis and degradation mediated by asialoglycoprotein receptor in isolated hepatocytes. *J Biol Chem* **257:** 1201–1207, 1982.
14. Weigel PH, Characterization of the asialoglycoprotein receptor on isolated rat hepatocytes. *J Biol Chem* **253:** 6111–6120, 1980.
15. Cesarone CF, Bolognesi C and Santi L, Improved microfluorometric DNA determination in biological material using 33258 Hoechst. *Anal Biochem* **100:** 188–197, 1979.
16. Paietta E, Stockert RJ, McManus M, Thompson D, Schmidt S and Wiernik PH, Hodgkin's cell lectin, a lymphocyte adhesion molecule and mitogen. *J Immunol* **143:** 2850–2857, 1989.
17. Clemens DL, Wolfinbarger JB, Mori S, Berry BD, Hayes SF and Bloom ME, Expression of Aleutian mink disease parvovirus capsid proteins by a recombinant vaccinia virus: Self-assembly of capsid proteins into particles. *J Virol* **66:** 3077–3085, 1992.
18. Pastan IH and Willingham MC, Receptor-mediated endocytosis of hormones in cultured cells. *Annu Rev Physiol* **43:** 239–250, 1981.
19. Burwen SJ and Jones AL, Intracellular pathway of immunoglobulin A and epidermal growth factor. In: *Intracellular Trafficking of Proteins* (Eds. Steer CJ and Hanover JA), pp. 248–278. Cambridge University Press, Cambridge, 1991.
20. Felder S, Miller K, Moehren G, Ullrich A, Schlessinger J and Hopkins CR, Kinase activity controls the sorting of the epi-

dermal growth factor receptor within the multivesicular body. *Cell* **61:** 623–634, 1990.
21. Schlessinger J, Ullrich A, Honegger AM and Moolenaar WH, Signal transduction by epidermal growth factor. *Cold Spring Harb Symp Quant Biol* **LIII:** 515–519, 1988.
22. Jennett RB, Sorrell MF, Johnson EL and Tuma DJ, Covalent binding of acetaldehyde to tubulin: Evidence for preferential binding to the α-chain. *Arch Biochem Biophys* **256:** 10–18, 1987.
23. Jennett RB, Sorrell MF, Saffari-Fard A, Ochner JL and Tuma DJ, Preferential covalent binding of acetaldehyde to the α-chain of purified rat liver tubulin. *Hepatology* **9:** 57–62, 1989.
24. Smith SL, Jennett RB, Sorrell MF and Tuma DJ, Acetaldehyde substoichiometrically inhibits bovine neurotubulin polymerization. *J Clin Invest* **84:** 337–341, 1989.
25. Mauch TJ, Donohue TM, Zetterman RK, Sorrell MF and Tuma DJ, Covalent binding of acetaldehyde selectively inhibits the catalytic activity of lysine-dependent enzymes. *Hepatology* **6:** 263–269, 1986.
26. Volentine GD, Ogden KA, Kortje DK, Tuma DJ and Sorrell MF, Role of acetaldehyde in the ethanol-induced impairment of hepatic glycoprotein secretion in the rat *in vivo*. *Hepatology* **7:** 490–495, 1987.