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Hypersensitivity to Acetaldehyde-Protein Adducts

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SUMMARY

Acetaldehyde, the first product in the metabolism of ethanol, is known to condense with plasma proteins, forming stable adducts. We have previously shown that these adducts can be recognized as foreign by the immune system. In the present study the existence of type I hypersensitivity-mediating antibodies against these adducts was investigated in humans and in animals. Immunization of mice with acetaldehyde-protein condensates, followed by adoptive transfer of splenocytes, led to the production of IgE anti-acetaldehyde adducts. A monoclonal IgE antibody was obtained by the hybridization technique. This antibody recognized acetaldehyde adducts, independently of the carrier protein used, indicating that the acetaldehyde moiety behaves as a hapten. The affinity of the antibody for the acetaldehyde adduct of polylysine was 7 orders of magnitude higher than that for polylysine. Passive immunization by intradermal or intravenous administration of this monoclonal antibody to rats rendered the animals hypersensitive to acetaldehyde-protein conjugates, as shown by marked anaphylaxis. A study was conducted to determine the existence of naturally occurring hypersensitivity reactions to alcohol in >1000 non-Oriental individuals. A prevalence of severe hypersensitivity reactions of 0.46% was found. The reactions were severe enough to deter these individuals from consuming all types of alcoholic beverages. Individuals presenting such reactions had significantly elevated levels of circulating anti-acetaldehyde-protein IgE antibodies.

Acetaldehyde, the first product in the metabolism of ethanol, has been shown to form adducts with a number of proteins, including albumin (1), hemoglobin (2), tubulin (3, 4), and various other proteins (5, 6). Acute and chronic administration of alcohol to rats results in the formation of acetaldehyde adducts with hepatic proteins (7–9), and reports also indicate the existence of acetaldehyde adducts in plasma (10, 11) and red cells of both alcoholics (11) and healthy volunteers given ethanol acutely (12).

Studies from our laboratory (13), and from other groups (14–18), have shown that the molecular structures formed upon the acetaldehyde-protein condensation are recognized as foreign by the immune system. Immunization of animals with acetaldehyde-protein adducts leads to the formation of polyclonal antibodies that recognize acetaldehyde-containing epitopes (13–18). It has also been shown that animals fed alcohol chronically also develop circulating antibodies against acetaldehyde adducts (13, 19). Humans naturally exhibit basal antibody titers against acetaldehyde adducts (18, 20), likely due to the varying amounts of alcohol consumed during the life of an individual. Some alcoholics present significantly higher levels of anti-acetaldehyde adduct antibodies (18, 20), which have been proposed to play a role in the perpetuation of alcoholic-induced liver injury (20). Studies have also shown the existence in alcoholics of antibodies that specifically react with hepatocytes of alcohol-pretreated animals (21, 22).

Because different immunoglobulin classes can mediate the immune response to a common epitope, the possibility exists that allergy-mediating antibodies, such as anti-acetaldehyde adduct IgE, might be formed in some individuals. IgEs are known to bind to receptors on mast cells and basophils and, upon antigen recognition, stimulate the release of histamine and other mediators responsible for a number of allergic/anaphylactic reactions (23). These types of reactions are known to occur for drugs that form metabolite-protein adducts, such as halothane and penicillin (24). For the latter, drug hypersensitivity reactions have been reported to occur in 1.8% of the population (25).

The existence of allergic and general hypersensitivity reactions to ethanol has been implied in a number of case reports describing such reactions (26–29). However, the prevalence of such reactions and the possible mechanisms have not been established. The objective of this study was to determine whether polyclonal and monoclonal IgE anti-acetaldehyde adducts could be induced in animals by immunization and whether transfer of such antibodies could result in anaphylactic reactions against acetaldehyde adducts in the recipients. In addition, we have estimated the prevalence of hypersensitivity

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ABBREVIATIONS: ELISA, enzyme-linked immunosorbent assay; RAST, radioallergosorbent test.

reactions to alcohol in the human population, and we have demonstrated the existence of the IgE anti-acetaldehyde adducts in such individuals.

Materials and Methods

Animal studies. Female C57BL/6J × BALB/c mice were immunized with acetaldehyde-keyhole limpet hemocyanin conjugate reduced with sodium cyanoborohydride, prepared as described previously (13). Two micrograms of the conjugate in 4 mg of aluminum hydroxide gel were administered intraperitoneally, in a volume of 0.5 ml of saline (30). Seven days after the first injection, a second injection of acetaldehyde-keyhole limpet hemocyanine, as described above, was administered. At day 8, mice were subjected to 250-R whole-body X-irradiation (31). On day 15, spleen cells of animals were adoptively transferred into naive C57BL/65 × BALB/c female mice that had been irradiated at 700 R. Another booster injection was given 30–60 min after cell transfer (30). Blood was collected 14–21 days after the adoptive transfer, and titers were determined by the passive cutaneous anaphylaxis method (32). Essentially, for this procedure 0.1 ml of mouse serum, at different dilutions, was injected intradermally into male Sprague Dawley rats (400–500 g) anesthetized with pentobarbital. Forty-eight hours after the passive immunization, 1 mg of protein-acetaldehyde adduct in 1 ml of saline containing 0.5% Evans Blue was administered intravenously. Extravasation of Evans Blue that follows local vasodilation resulting from mast cell degranulation yields a blue spot in the skin. The relative intensity of mast cell degranulation was measured by determining the area of the spot 1 hr after the injection of the acetaldehyde-protein adduct, or the respective control protein.

Monoclonal antibodies from animals showing high passive cutaneous anaphylaxis titers (dilution greater than 1/1000) were obtained by generating hybridomas utilising immune spleen cells from these animals and NS-1 myeloma cells as the fusion partner (33). Clones were screened by the ELISA method (33), with goat anti-mouse IgE/Fc (Immunovision Laboratories, Springdale, AR) as a secondary antibody. The latter was subsequently recognized by a mouse anti-goat IgG (H+L) conjugated to horseradish peroxidase (Jackson Immuno-Research Labs, West Grove PA). Color was developed with *ortho*-phenylenediamine (Zymed Labs, San Francisco, CA). Positive clones reacting strongly with human plasma protein-acetaldehyde adducts, but not with control plasma proteins, were further tested *in vivo* by the passive cutaneous anaphylaxis reaction, as described above. One stable IgE-producing clone (2–1–3) was allowed to grow in the intraperitoneal cavity of BALB/c × C57BL/6J mice that had been pretreated with Pristane (30). The ascitic fluid was subsequently collected and used for the passive systemic anaphylaxis studies. Ascites fluid (0.1–0.5 ml) was administered intravenously to male Sprague Dawley rats (240–260 g). The same amount of ascites from an IgE-generating clone of non-anti-acetaldehyde adduct specificity (clone 1–4–2) was used as control. Forty-eight hours after antibody administration, two cannulae were implanted under ether anesthesia, one in the femoral artery for blood pressure measurements and one into the jugular vein for the administration of proteins and drugs. The cannulae were then exteriorized and blood pressure measurements were performed by means of a Narco-physiological pressure transducer (Narco Houston, TX), in the nonanesthetized state.

In experiments designed to address the nature of the groups reacting with monoclonal antibody 2–1–3, poly-L-lysine (M_r 102,000–109,000; Sigma Chemical Co., St. Louis, MO) and human serum albumin were dialyzed at a concentration of 1.8 mg/ml for 24 hr at 22° in separate flasks containing either 1 liter of 10 mM acetaldehyde + 10 mM sodium cyanoborohydride or 10 mM sodium cyanoborohydride (control). After that time the dialysis bags were removed and were exhaustively dialyzed against phosphate-buffered saline. The resulting adducts and their respective controls were adsorbed on ELISA plates at a concentration of 3 μg/ml. After blocking, the plates were incubated for 2 hr with different concentrations of monoclonal antibody 2–1–3 (ascites), and the assay was continued to the peroxidase step, using 3,3′,5,5′-tetramethylbenzidine (Kirkegaard and Perry, Gaithersberg, MD) as peroxidase substrate.

Human studies. Individuals selected at random from the Metropolitan Toronto region were contacted by phone (7–9 p.m.). The interviewer indicated his University of Toronto affiliation and explained our interest in conducting research on possible unpleasant reactions to small amounts of alcoholic beverages. Individuals with such symptoms were asked additional information on the reactions and on the type of beverages involved, the amount that elicited the reactions, and whether they had Oriental ancestry (and so might have presented adverse reactions due to the lack of low-K_m aldehyde dehydrogenase activity) (44). Individuals who described hypersensitivity reactions to all alcoholic beverages and who had no Oriental ancestry were asked about the possible family history of such reactions and were further asked about their drinking habits and about the existence of other types of hypersensitivity reactions, including allergies to common allergens and prescription drugs. One thousand questionnaires were completed in the initial phase. This sample is referred to as the "survey group." In a second phase 287 additional individuals were given the same questionnaire, to determine the percentage who refused to provide enough information to complete the questionnaire after learning about its nature. It was assumed that these individuals did not have hypersensitivity reactions to alcohol. In this group 30.3% of individuals surveyed did not complete the questionnaire (200 questionnaires were completed after 287 calls). Thus, to calculate the prevalence of hypersensitivity reactions the initial 1000 completed questionnaires were assumed to have been derived from 1435 individuals. Confidence intervals (95% limits) were calculated for a binomial distribution.

In order to obtain actual clinical samples from individuals hypersensitive to alcohol, a newspaper advertisement was placed requesting volunteers who would present reactions of the type encountered and would donate a blood sample. Sixty calls were received after a 1-day advertisement in a Sunday paper. Of these, 12 Caucasians fulfilled the criteria of hypersensitivity reactions to all types of alcoholic beverages. This sample is referred to as the "clinical group." Five of the 12 subjects in the clinical group were virtual abstainers at the time of the study. Controls were University personnel who were social drinkers and who did not experience hypersensitivity reactions to alcoholic beverages.

Measurement of human IgE anti-acetaldehyde-protein adducts was performed by a RAST method (34). Essentially, acetaldehyde-conjugated human plasma proteins (10 mg/ml) or control human plasma proteins (14) were allowed to adsorb for 2 hr onto 10-mm-diameter nitrocellulose discs (Transblot; Bio-Rad, Richmond, CA). Excess available sites were blocked with reconstituted Carnation skim milk. Human plasma from hypersensitive and control individuals diluted in phosphate-buffered saline-Tween 20, was allowed to react with the discs for 2 hr at room temperature. After washing, retained IgE was determined after an 18-hr incubation with ^{125}I-labeled anti-human IgE (5000 cpm, 12.5 μCi/μg; Pharmacia RAST test). To calculate the specific binding to acetaldehyde adducts, counts retained by the discs on which control protein was adsorbed were subtracted from the total counts of disks containing the acetaldehyde conjugates.

Total IgE was determined with a Pharmacia (Uppsala, Sweden) IgE radioimmunoassay kit. Determination of the IgG, IgM, and IgA anti-acetaldehyde antibodies was made by the ELISA method (33). Human serum albumin- or human transferrin-acetaldehyde conjugates were absorbed onto ELISA plates (Corning, Corning, NY), and gelatin was used to block unbound sites. Goat anti-human IgG, IgM, or IgA labeled with peroxidase was added, and color was developed with 3,3′,5,5′-tetramethylbenzidine as peroxidase substrate. Absorbance was read on an ELISA reader at 450 nm (Titertek Multiscan; Flow Instruments, McLean, VA).

Results

Fig. 1 shows the immunoreactivity of polyclonal sera of mice after immunization with hemocyanin-acetaldehyde conjugate.

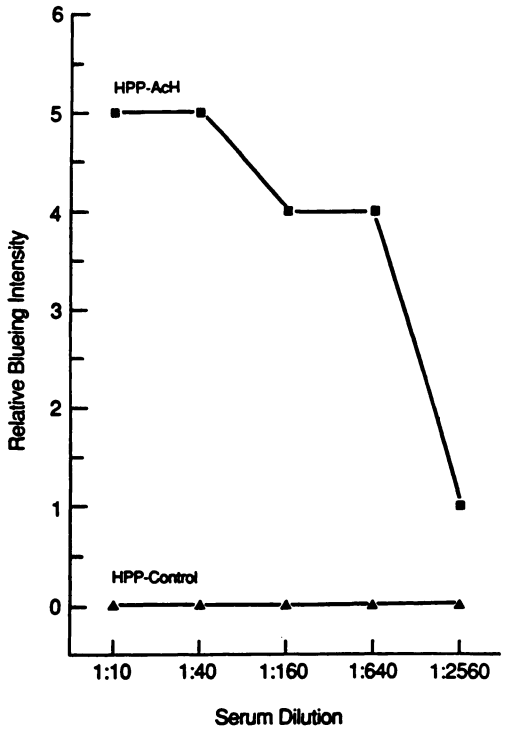

Fig. 1. Passive cutaneous anaphylaxis in rats after intravenous injection of the acetaldehyde conjugate of human plasma protein (*HPP-AcH*) or control human plasma protein (*HPP-Control*). Rats were injected intradermally with sera of mice immunized with keyhole limpet hemocyanin-acetaldehyde conjugates, at the dilutions indicated. Highly conjugated human plasma protein-acetaldehyde and keyhole limpet hemocyanin-acetaldehyde, prepared as described previously (13) were administered (1 mg) 48 hr after passive immunization.

Rats injected intradermally with mouse immune sera showed cutaneous anaphylaxis after the intravenous injection of acetaldehyde-conjugated human plasma proteins. No effects were observed when control unconjugated proteins were injected. Sera of mice immunized with control (unconjugated) keyhole limpet hemocyanin did not present reactions against conjugated or control human serum albumin.

Fig. 2 presents the immunoreactivity of monoclonal IgE 2–1–3 antibody, as assessed by the ELISA method. The antibody reacted with human, rat, and bovine serum albumin-acetaldehyde conjugates, prepared at 1 mM and 100 μM acetaldehyde (Fig. 2, A–C). Strong reactivity was also observed against human hemoglobin (Fig. 2D) adducts at 1 mM and 100 μM acetaldehyde, indicating that the acetaldehyde residue in the adducts is recognized independently of the protein carrier.

In order to determine the chemical structures recognized by monoclonal antibody 2–1–3, ELISAs were carried out in which the immunoreactivity of 2–1–3 was compared against both polylysine conjugated with acetaldehyde and control polylysine. The immunoreactivity was further compared against conjugated human serum albumin adducts prepared under the same conditions. Data in Fig. 3 indicate that monoclonal antibody 2–1–3 had a 10^7-fold higher affinity for the polylysine-acetaldehyde conjugate than for polylysine control.

Monoclonal IgE 2–1–3 was further tested *in vivo* to assess whether anaphylaxis-type reactions could be obtained against human albumin-acetaldehyde adducts. Rats administered acetaldehyde-modified human serum albumin intravenously after the intradermal injection of monoclonal IgE 2–1–3 showed cutaneous anaphylaxis. As shown in Fig. 4, the passive cutaneous anaphylaxis test detected adducts formed at acetaldehyde concentrations as low as 20 μM.

Fig. 5 shows the ability of monoclonal antibody 2–1–3 to generate systemic anaphylaxis upon human serum albumin-acetaldehyde adduct administration. Animals passively immunized systemically with the monoclonal IgE showed a marked reduction in blood pressure, of the order of 50%, after the intravenous injection of human serum albumin-acetaldehyde adduct (conjugated at 20 μM acetaldehyde). Administration of an antihistaminic drug, diphenhydramine, completely reversed this effect. When a highly conjugated human serum albumin was used the effect of the diphenhydramine was only partial. No effect on blood pressure was seen after the administration of control human serum albumin. No anaphylactic reactions were observed when control monoclonal IgE (1–4–2) was used (data not shown).

In order to determine the prevalence of reported hypersensitivity reactions in the general population, 1000 individuals (without Oriental ancestry) were initially contacted. Six individuals, five females and one male, reported strong physical reactions to all types of alcohol-containing beverages (Table 1). Five of the six subjects in the survey group reported hypersensitivity to several allergens, including pollens and prescription drugs. In the replicate-survey group two of 200 individuals reported these severe hypersensitivity reactions. The estimated prevalence of these hypersensitivity reactions in the population is 0.46%. The 95% confidence interval is 0.14–0.78%.

Fig. 6 shows the titers of specific IgE against acetaldehyde-conjugated human plasma proteins from 12 Caucasians (clinical group) reporting hypersensitivity reactions to all types of alcoholic beverages. Immunoreactivity against the plasma protein-acetaldehyde adducts was significantly higher ($p < 0.02$) in the alcohol-hypersensitive clinical group than in controls. There was no relationship in the hypersensitive group between total IgE and anti-acetaldehyde adduct IgE immunoreactivity ($r = 0.103$, not significant). In the hypersensitive clinical group, five individuals reported experiencing reactions that were unpleasant enough to fully deter them from consuming alcoholic beverages. In this group one 34-year-old female presented 12 times higher anti-acetaldehyde adduct IgE levels than the mean control value. She reported cutaneous inflammation, nausea, vomiting, and fainting after consuming minute amounts of alcohol. One female (BJ) in this group, who did not present elevated IgE anti-acetaldehyde adduct titers, displayed elevated IgM and IgG titers to human serum albumin-acetaldehyde and human transferrin-acetaldehyde conjugates (Fig. 7).

Discussion

The present studies extend the observations on the immunological abnormalities associated with ethanol metabolism (35) into the study of reaginic antibodies. Data obtained reveal that anaphylaxis against acetaldehyde-protein adducts can be induced in animals by passive immunization with monoclonal antibodies of the IgE type. These antibodies recognized acetaldehyde adducts formed at low concentrations of acetaldehyde, which have been reported to occur in the blood after ethanol ingestion (36). The monoclonal antibody was also shown to recognize polylysine-acetaldehyde conjugates, prepared under reducing conditions, with an affinity 7 orders of magnitude higher than that for control polylysine.

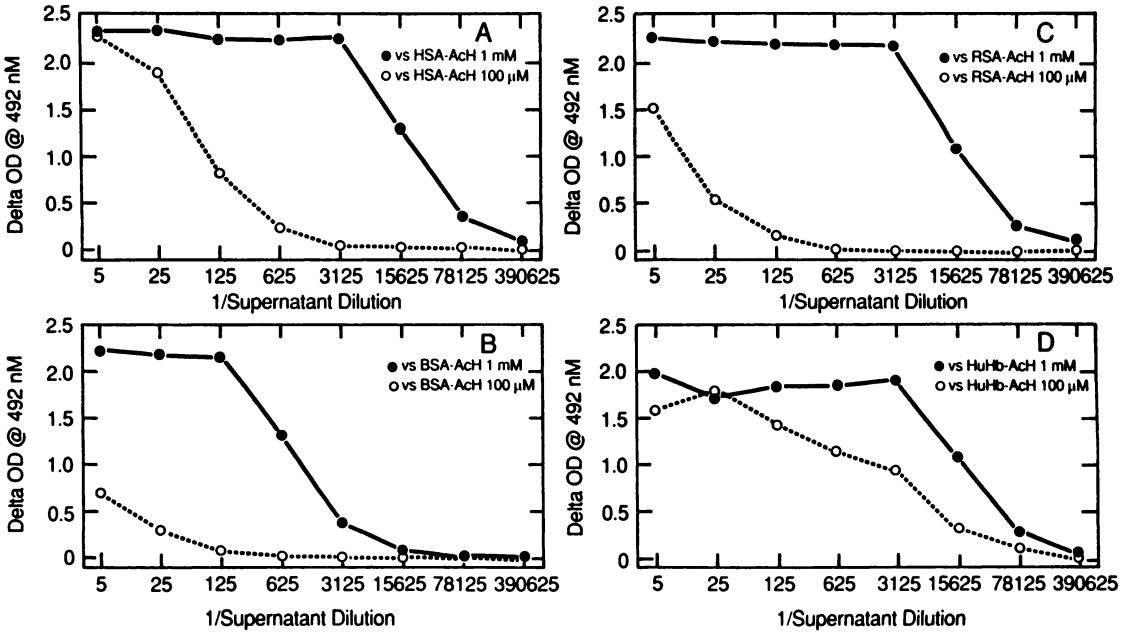

Fig. 2. Immunoreactivity of monoclonal IgE 2–1–3 against acetaldehyde-conjugated proteins, assessed by the ELISA method. *HSA*, human serum albumin; *RSA*, rat serum albumin; *BSA*, bovine serum albumin; *HuHb*, human hemoglobin; *AcH*, acetaldehyde; Acetaldehyde-protein conjugates were prepared at the concentration of acetaldehyde indicated. Absorbance was measured at 492 nm. Background values for unconjugated protein have been subtracted.

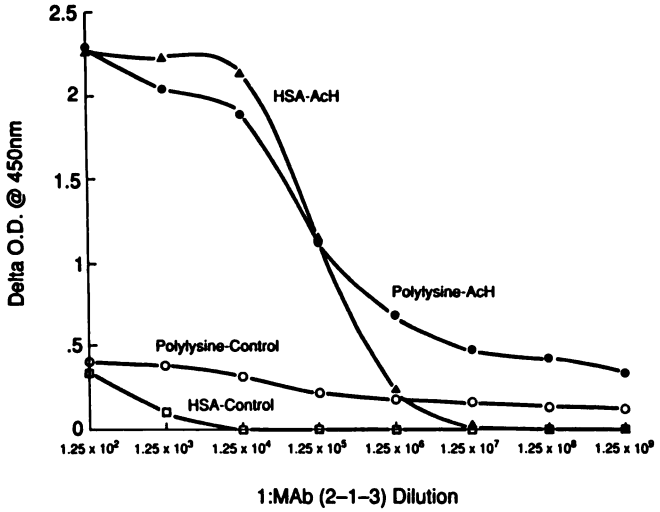

Fig. 3. Immunoreactivity of monoclonal IgE 2–1–3 against highly conjugated polylysine and human serum albumin-acetaldehyde adducts. *HSA*, human serum albumin; *AcH*, acetaldehyde.

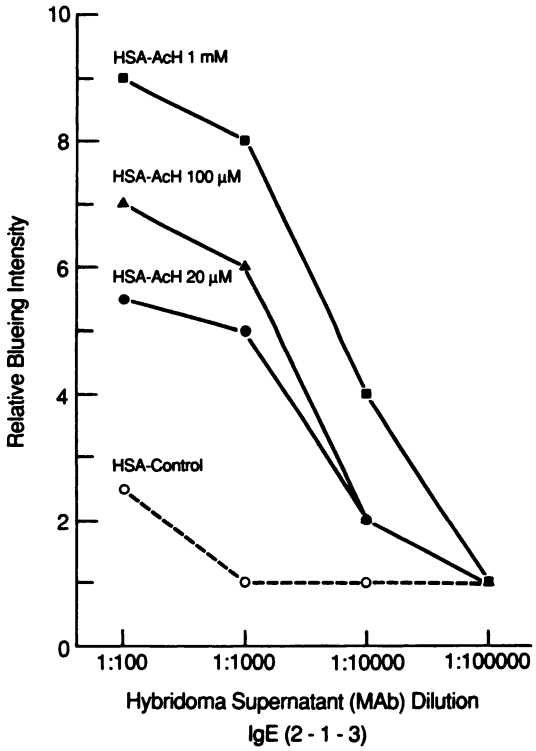

Fig. 4. Passive cutaneous anaphylaxis in rats after administration of monoclonal IgE 2–1–3. Animals were injected intradermally with monoclonal IgE 2–1–3, at the dilutions indicated. Forty-eight hours later, the animals were injected intravenously with 1 mg of unmodified human serum albumin (*HSA-Control*) or acetaldehyde-conjugated human serum albumin (*HSA-AcH*) prepared at the concentrations indicated.

Anti-acetaldehyde-adduct IgE was also found in humans presenting hypersensitivity reactions against alcohol. Although earlier case reports have implied the existence of allergic reactions to ethanol (26–29), the mechanism of the reactions has remained obscure. Allergic reactions and specific IgE antibodies have also been reported to occur in patients exposed to environmental formaldehyde (37), another reactive aldehyde known to bind to proteins. Antibodies against formaldehyde-protein adducts have also been reported in hemodialysis patients treated with formaldehyde-sterilized dialyzers and in nursing personnel presenting formaldehyde-induced asthma (38).

It is of interest to note that an important proportion of patients with Hodgkins disease, known to have marked elevations in circulating IgE (39), present severe hypersensitivity reactions after ingesting minute amounts of alcohol, including pain, itching, flushing, and nausea (40). Irradiation therapy, which reduces IgE levels, reduces or abolishes the hypersensitivity to alcohol (40).

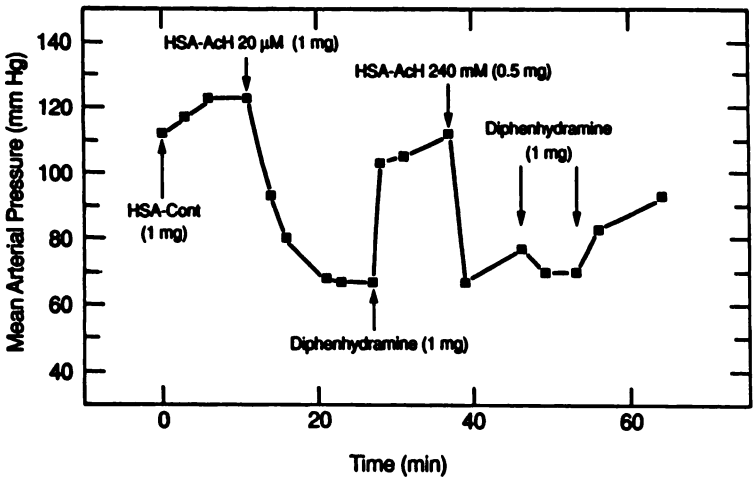

Fig. 5. Systolic blood pressure after administration of acetaldehyde-conjugated human serum albumin. Rats were passively immunized by intravenous administration of ascites fluid containing monoclonal IgE 2–1–3 (0.2 ml/animal). Forty-eight hours after the administration of antibody 2–1–3, animals were administered human serum albumin control (*HSA-Control*) or human serum albumin-acetaldehyde (*HSA-AcH*), intraveneously.

TABLE 1

Reported symptomatology in individuals with severe hypersensitivity reactions to alcoholic beverages, from a random sample of 1000 individuals

No.	Age	Sex	Symptoms	Other allergies	Minimum amount of alcohol
	years				
1.	37	F	Skin blotching, rash, itching of lips, abdominal pain (family history: mother)	Yes	Few sips, <1 drink[a]
2.	34	M	Skin blotching, watering eyes, perspiration	Yes	1 drink
3.	45	F	Generalized rash, abdominal pain	Yes	1 drink
4.	50	F	Generalized swelling, disorientation, hyperactivity (family history: father, grandson)	Yes	1 sip
5.	38	F	Abdominal pain, headaches for 48 hr, dizziness	Yes	<1 drink
6.	44	F	Face and body heat, shortness of breath	No	<2 drinks

[a] One drink is defined as 1 glass of wine, 1 beer, or 1 standard serving of liquor.

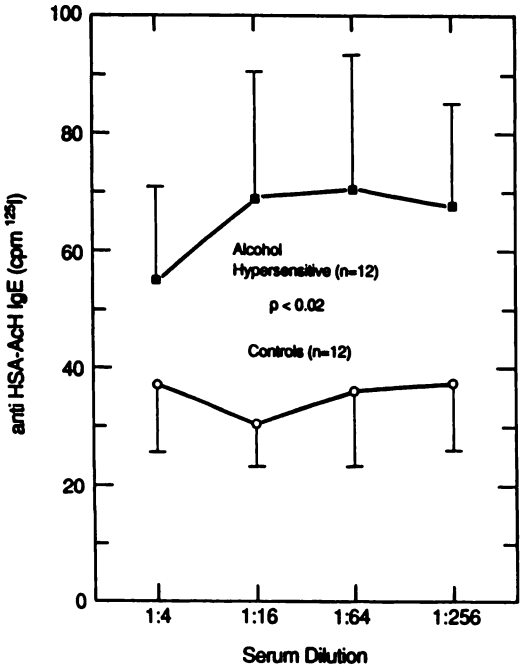

Fig. 6. Anti-acetaldehyde-human serum albumin IgE immunoreactivity in 12 subjects presenting hypersensitivity reactions to alcohol. Values represent IgE immunoreactivity, determined by the RAST method, against acetaldehyde-conjugated human serum albumin (*HSA-AcH*) minus the immunoreactivity against control, unmodified, human serum albumin.

Sensitization to alcohol-derived haptens may also occur through cell-mediated processes. Stotts and Ely (41) were able to induce allergic dermatititis in response to ethanol after a 3-week cutaneous application of ethanol. The reaction was observed in six of 93 individuals, in some of whom allergic reactivity was observed 18 months after sensitization. Acetaldehyde, but not acetate, could replace alcohol in eliciting the allergic reactions. In one of the volunteers, cutaneous testing with acetaldehyde led to accidental sensitization to both acetaldehyde and ethanol (41).

A non-IgE-mediated form of urticaria also exists that involves the liberation of histamine from basophils and mast cells via the complement pathway, in which anaphylatoxins are released. This pathway is mediated by antigen-antibody reactions involving IgM and some IgG subtypes (42). In our clinical group, one individual presenting severe hypersensitivity reactions to alcohol showed elevated IgM and IgG titers against acetaldehyde adducts.

Hypersensitivity reactions to alcohol, as reported here, would be expected either in individuals presenting high titers of anti-acetaldehyde adduct antibodies, including IgEs, or in individuals with low or intermediate antibody titers in whom the levels of acetaldehyde are markedly increased after alcohol consumption. The latter condition (43), accompanied by the formation of high levels of acetaldehyde-protein adducts (13), is known to occur in Oriental individuals in whom a point mutation renders mitochondrial acetaldehyde dehydrogenase inactive (44). Such a mutation, existent in 40–50% of the Oriental population, is associated with facial flushing, itching, and nausea upon alcohol consumption (44). Individuals presenting such a mutation have virtual protection against alcoholism (43, 45, 46). Similar reactions occur in individuals receiving disulfiram, an antialcohol drug that inhibits aldehyde dehydrogenase (47). It is of note that both the disulfiram-alcohol cutaneous reaction and the Oriental flushing are blocked by antihistaminics (48, 49). It is not known, however, whether these effects are due to a direct action of acetaldehyde on mast cells or whether immune

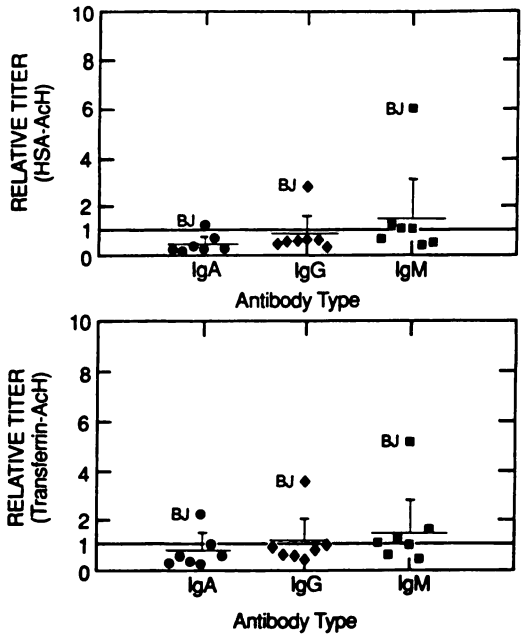

Fig. 7. Relative anti-acetaldehyde-protein IgG, IgM, and IgA titers in five subjects with severe hypersensitivity to alcoholic beverages. Each value represents the relative titer of a single hypersensitive individual, compared with the mean titer for controls. A value of 1.0 represents no difference in titer between the hypersensitive individual and the mean for the control group. One individual (BJ) who did not show elevated IgE anti-acetaldehyde-protein titers showed significant elevations (>2 SD over control) in IgG and IgM titers. Titers represent the immunoreactivity of the serum against the protein-acetaldehyde conjugates minus the reactivity against the control protein. *HSA*, human serum albumin; *AcH*, acetaldehyde.

mechanisms mediate these reactions. Our studies suggest that anti-acetaldehyde-protein adduct antibodies might be involved in the Oriental flushing reaction. Further study of such effects may lead to therapeutic applications for alcohol abuse patients.

It should be noted that, in our studies, we cannot discard the possibility of a mutation in the high-K_m cytosolic aldehyde dehydrogenase, such as the one described by Yoshida *et al.* (50) in Caucasians or other, as yet unreported, mutations. In such a case, a reduction in acetaldehyde dehydrogenase activity would potentiate the effect of high antiacetaldehyde antibody levels found in these subjects.

In conclusion, we have shown that antibodies of the IgE type can recognize acetaldehyde-protein adducts. Administration of acetaldehyde protein adducts to animals passively immunized with specific IgEs led to the production of anaphylactic reactions. About 0.46% (0.14–0.78%; 95% confidence interval) of the population presents severe hypersensitivity reactions against all types of alcoholic beverages, which deter them from consuming alcohol. These individuals present significantly elevated IgE titers against acetaldehyde-protein adducts. If our data are extrapolated, >1,000,000 individuals could present this type of reaction in North America alone.

References

1. Tuma, D. J., T. M. Donohue, V. A. Medina, and M. F. Sorrell. Acetaldehyde adducts with proteins: binding of [^{14}C]-acetaldehyde to serum albumin. *Arch. Biochem. Biophys.* **220**:239–246 (1983).
2. Stevens, V. J., W. F. Fantl, C. B. Newman, R. V. Sims, A. Cerami, and C. M. Peterson. Acetaldehyde adducts with hemoglobin. *J. Clin. Invest.* **67**:364–369 (1981).
3. Jennett, R. B., M. F. Sorrell, A. Saffari-Ward, J. L. Ockner, and D. J. Tuma. Preferential covalent binding of acetaldehyde to the α-chain of purified rat liver tubulin. *Hepatology* **9**:57–62 (1989).
4. Smith, S. L., R. B. Jennett, M. F. Sorrell, and D. J. Tuma. Acetaldehyde substoichiometrically inhibits bovine neurotubulin polymerization. *J. Clin. Invest.* **84**:337–341 (1989).
5. Nomura, F., and C. S. Lieber. Binding of acetaldehyde to rat liver microsomes: enhancement after chronic alcohol consumption. *Biochem. Biophys. Res. Commun.* **100**:131–137 (1981).
6. Jukkola, A., and O. Niemelä. Covalent binding of acetaldehyde to type III collagen. *Biochem. Biophys. Res. Commun.* **159**:163–169 (1989).
7. Barry, R. E., A. S. Williams, and J. D. McGivan. The detection of acetaldehyde/liver plasma membrane protein adduct formed *in vivo* by alcohol feeding. *Liver* **7**:364–368 (1987).
8. Lin, R. C., S. R. Smith, and L. Lumeng. Detection of a protein-acetaldehyde adduct in the liver of rats fed alcohol chronically. *J. Clin. Invest.* **81**:615–619 (1988).
9. Zhong, X. Detection of acetaldehyde protein adducts in the liver of rats treated with ethanol. M.Sc. thesis, University of Toronto (1989).
10. Peterson, C. M., L. Jovanovic-Peterson, and F. Schmid-Formby. Rapid association of acetaldehyde with hemoglobin in human volunteers after low dose ethanol. *Alcohol* **5**:371–374 (1988).
11. Lin, R. C., L. Lumeng, S. Shahidi, T. Kelly, and D. C. Pound. Protein-acetaldehyde adducts in serum of alcoholic patients. *Alcohol. Clin. Exp. Res.* **14**:438–443 (1990).
12. Niemelä, O., Y. Israel, Y. Mizoi, T. Fukunaga, and C. J. P. Eriksson. Hemoglobin-acetaldehyde adducts in human volunteers following acute ethanol ingestion. *Alcohol. Clin. Exp. Res.* **14**:838–841 (1990).
13. Israel, Y., E. Hurwitz, O. Niemelä, and R. Arnon. Monoclonal and polyclonal antibodies against acetaldehyde-containing epitopes in acetaldehyde-protein adducts. *Proc. Natl. Acad. Sci. USA* **83**:7923–7927 (1986).
14. Fleisher, J. H., C. C. Lung, G. C. Meinke, and J. L. Pinnas. Acetaldehyde-albumin adduct formation: possible relevance to an immunologic mechanism in alcoholism. *Alcohol Alcohol.* **23**:133–141 (1988).
15. Klassen, L. W., D. S. Xu, and D. J. Tuma. Immune responses to acetaldehyde adducts, in *Alcohol, Immunomodulation and AIDS* (D. Seminara, R. Watson, and A. Pawlowski, eds.). Alan R. Liss Inc., New York, 333–340 (1990).
16. Trudell, J. R., C. M. Ardies, and W. R. Anderson. Cross-reactivity of antibodies raised against acetaldehyde adducts of protein with acetaldehyde adducts of phosphatidylethanolamine: possible role in alcoholic cirrhosis. *Mol. Pharmacol.* **38**:587–593 (1990).
17. Steinbrecher, U. P., M. Fisher, J. L. Witztum, and L. K. Curtis. 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).
18. Hoerner, M., U. J. Behrens, T. Worner, and C. S. Lieber. Humoral immune response to acetaldehyde adducts in alcoholic patients. *Res. Commun. Chem. Pathol. Pharmacol.* **54**:3–12 (1986).
19. Worrall, S., J. DeJersey, B. C. Shanley, and P. A. Wilce. Ethanol induces the production of antibodies to acetaldehyde-modified epitopes in rats. *Alcohol Alcohol.* **24**:217–223 (1989).
20. Niemelä, O., F. Klajner, H. Orrego, E. Vidins, L. Blendis, and Y. Israel. Antibodies against acetaldehyde-modified protein epitopes in human alcoholics. *Hepatology* **7**:1210–1214 (1987).
21. MacSween, R. N. M., R. S. Anthony, and M. Farquharson. Antibodies to alcohol-altered hepatocytes in patients with alcoholic liver disease. *Lancet* **2**:803–804 (1981).
22. Neuberger, J., I. R. Crossley, J. B. Saunders, M. Davis, B. Portmann, A. L. Eddleston, and R. Williams. Antibodies to alcohol altered liver cell determinants in patients with alcoholic liver disease. *Gut* **25**:300–304 (1984).
23. Ishizaka, K., and T. Ishizaka. Immunology of IgE-mediated hypersensitivity, in *Allergy: Principles and Practice*, Ed. 2 (E. Middleton, C. E. Reed, and E. F. Ellis, eds.), Vol. 1. Mosby, St. Louis, 43–74 (1983).
24. Pohl, L. R., H. Satoh, D. D. Christ, and J. G. Kenna. The immunological and metabolic basis of drug hypersensitivities. *Annu. Rev. Pharmacol.* **28**:367–387 (1988).
25. Anderson, J. A., and N. F. Adkinson. Allergic reactions to drugs and biological agents. *J. Am. Med. Assoc.* **258**:2891–2899 (1987).
26. Hicks, R. Ethanol, a possible allergen. *Ann. Allergy* **26**:641–643 (1968).
27. Ormerod, A. D., and P. J. A. Holt. Acute urticaria. *Br. J. Dermatol.* **108**:723–724 (1983).
28. Ting, S., D. O. Rauls, P. Ashbaugh, and L. E. Mansfield. Ethanol induced urticaria: a case report. *Ann. Allergy* **60**:527–530 (1988).
29. Kelso, J. M., M. V. Keating, D. L. Squillance, E. J. O'Connell, J. W. Yunginger, and M. I. Sachs. Anaphylactoid reaction to ethanol. *Ann. Allergy* **62**:452–454 (1990).
30. Eshhar, Z., M. Ofarim, and T. Waks. Generation of hybridomas secreting murine reaginic antibodies of anti-DNP specificity. *J. Immunol.* **124**:775–780 (1980).
31. Tung, A. S. Production, purification and characterization of antigen-specific murine monoclonal antibodies of IgE class. *Methods Enzymol.* **92**:47–66 (1983).
32. Ovary, Z. Passive cutaneous anaphylaxis. *Handb. Exp. Immunol.* **1**:33.1–33.9 (1986).
33. Eshhar, Z. Monoclonal antibodies, in *Hybridoma Technology in the Bio-*

Sciences and Medicine, Ed. 2 (T. A. Springer, ed.). Plenum Press, New York, 3–34 (1985).
34. Gleich, G. J., J. W. Yunginger, and J. D. Stobo. Laboratory methods for studies of allergy, in *Allergy* (E. Middleton, C. E. Reed, and E. F. Ellis, eds.), Vol. 1. Mosby, St. Louis, MO, 271–293 (1983).
35. Israel, Y., H. Orrego, and O. Niemelä. Immune responses to alcohol metabolites: pathogenic and diagnostic implications. *Semin. Liver Dis.* **8**:81–87 (1988).
36. Nuutinen, H., K. O. Lindros, and M. Salaspuro. Determinants of blood acetaldehyde level during ethanol oxidation in chronic alcoholics. *Alcohol. Clin. Exp. Res.* **7**:163–168 (1983).
37. Wilhelmsson, B., and M. Holmstrom. Positive formaldehyde-RAST after prolonged formaldehyde exposure by inhalation. *Lancet* **2**:164 (1987).
38. Dolovich, J., S. Evans, V. Baurmeister, H. Schulze, M. Ali, and A. Shimizu. Antibody responses to hemodialysis-related antigens in chronic hemodialysis patients. *Artif. Organs* **11**:93–96 (1987).
39. Romagnani, S., P. L. R. Ferrini, and M. Ricci. The immune derangement in Hodgkin's Disease. *Semin. Hematol.* **22**:41–55 (1985).
40. Brewin, T. B. Alcohol intolerance in neoplastic disease. *Br. Med. J.* **2**:437–441 (1966).
41. Stotts, J., and W. J. Ely. Induction of human skin sensitization to ethanol. *J. Invest. Dermatol.* **69**:219–222 (1977).
42. Kaplan, A. P. Urticaria and angioedema, in *Allergy: Principles and Practice*, Ed. 2 (E. Middleton, C. E. Reed, and E. F. Ellis, eds.), Vol. 2. Mosby, St. Louis, MO, 1341–1360 (1983).
43. Harada, S., D. P. Agarwal, H. W. Goedde, S. Tagaki, and B. Ishikawa. Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. *Lancet* **2**:982 (1982).
44. Yoshida, A., I.-Y. Huang, and M. Ikawa. Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. *Proc. Natl. Acad. Sci. USA* **81**:258–261 (1984).
45. Mizoi, Y., Y. Tatsuno, J. Adachi, M. Kogame, T. Fukunaga, S. Fujiwara, S. Hishida, and I. Ojiri. Alcohol sensitivity related to polymorphism of alcohol-metabolizing enzymes in Japanese. *Pharmacol. Biochem. Behav.* **18** (Suppl. 1):127–133 (1983).
46. Goedde, H. W., S. Harada, and D. P. Agarwal. Racial differences in alcohol sensitivity: a new hypothesis. *Hum. Genet.* **51**:331–334 (1979).
47. Peachey, J. E., and C. Naranjo. The use of disulfiram and other alcohol-sensitizing drugs in the treatment of alcoholism, in *Research Advances in Alcohol and Drug Problems* (R. Smart, F. B. Glaser, Y. Israel, H. Kalant, R. E. Popham, and W. Schmidt, eds.), Vol. 7. Plenum Press, New York, 397–431 (1983).
48. Kissin, B., and M. M. Gross. Drug therapy in alcoholism. *Curr. Psychiatry* **10**:135–144 (1970).
49. Miller, N. S., D. W. Goodwin, F. C. Jones, W. F. Gabrielli, M. D. Pardo, M. M. Auand, and T. B. Hale. Antihistamine blockade of alcohol-induced flushing in Orientals. *J. Stud. Alcohol* **49**:16–20 (1988).
50. Yoshida, A., V. Dave, R. J. Ward, and T. J. Peters. Cytosolic aldehyde dehydrogenase (ALDH 1) variants found in alcohol flushers. *Ann. Hum. Genet.* **53**:1–7 (1989).

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