Effects of Alcohol Consumption on the Allergen-Specific Immune Response in Mice

Allan Linneberg, Martin Roursgaard, Lars-Georg Hersoug, and Søren Thor Larsen

Background: There is evidence that chronic alcohol consumption impairs the T-helper 1 (Th1) lymphocyte-regulated cell-mediated immune response possibly favoring a Th2 deviation of the immune response. Moreover, a few epidemiological studies have linked alcohol consumption to allergen-specific IgE sensitization.

Objective: To investigate the effects of alcohol consumption on the allergen-specific immune response in mice.

Methods: BALB/cJBomTac mice were immunized intraperitoneally with ovalbumin (OVA) using a low dose sensitization protocol. Throughout the experiment, mice were kept on isocaloric liquid diets containing 0 to 6.2% ethanol. Evaluation of immunomodulatory effects of ethanol was based on measurements of total serum IgE, as well as OVA-specific IgE, IgG1, and IgG2a. Furthermore, levels of OVA-induced interleukin (IL)-4 and interferon-γ were determined in ex vivo splenocyte cultures.

Results: Alcohol intake decreased the level of OVA-specific IgG2a in a dose-dependent manner, whereas high levels of alcohol markedly increased the level of total IgE, but not OVA-specific IgE. Th1 suppression was supported by the cytokine profile.

Conclusions: Alcohol consumption induced a marked decrease in markers of the Th1-type allergen-specific immune response and an increase in total serum IgE. In this model, there was no effect of alcohol on OVA-specific IgE. Studies using other routes of immunization may be warranted.

Key Words: Alcohol Drinking, Allergens, Allergy, Ethanol, IgE.

ALCOHOL CONSUMPTION IS a frequent and increasing lifestyle habit in many countries and an established risk factor for liver disease and different cancers such as cancers of the breast and oro-pharynx (Boffetta and Hashibe, 2006). Furthermore, there is evidence of a nonlinear relationship (J- or U-shaped relationship) of alcohol consumption to the risk of cardiovascular disease and all cause mortality (Gronbaek, 2006). It is further well-documented that chronic alcohol consumption impairs the T-helper 1(Th1) lymphocyte-regulated cell-mediated immune response increasing the susceptibility to viral and bacterial infections such as infections of the lower respiratory tract (e.g., pneumococcal pneumonia and tuberculosis) (Cook, 1998). There is relatively strong evidence to support a causal link between alcohol consumption and increased levels of total serum IgE, as reviewed by Gonzalez-Quintela et al. (2004). Thus, alcohol consumption may be associated with changes in the cytokine profile consistent with a relative T-helper 2 (Th2) lymphocyte deviation of the immune response. Despite these immune-modulating effects of alcohol, little is known about its potential effects on immune-mediated diseases such as allergic diseases. In humans, alcohol consumption has been linked to IgE sensitization against inhalant and food allergens, but at present the evidence is not clear (Assing et al., 2007; Bakos et al., 2006; Gonzalez-Quintela et al., 2003; Linneberg et al., 2001, 2003, 2006; Vidal et al., 2002). This might be partly because of problems inherent in observational epidemiological studies such as confounding by factors associated with both alcohol exposure and health outcomes as well as inaccurate assessment of alcohol exposure. These problems may be overcome by experimental animal studies.

To our knowledge there is no previously published experimental animal study on the effects of alcohol consumption on the risk of allergen-specific IgE sensitization. In a mouse model, we aimed to investigate the hypothesis that alcohol consumption induces a Th2-dominated allergen-specific immune response and increases the risk of allergen-specific IgE sensitization.

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MATERIALS AND METHODS

Animals, Diets, and Experimental Design

Inbred female BALB/cJBomTac mice aged 5 to 6 weeks were purchased from Taconic M&B, Ry, Denmark, and were housed in polypropylene cages (380 mm× 220 mm× 150 mm) with pinewood sawdust bedding (Lignocel S8, Brogaarden, Denmark). The cages, housing up to 11 mice per cage, were furnished with bedding materials, gnaw sticks, and cardboard tubes. The photo period was from 6 AM to 6 PM, and the temperature and mean relative humidity in the animal room were 19 to 22°C and 43 ± 8% (SD), respectively. Cages were sanitized twice weekly. Food was available ad libitum. Body weights were monitored throughout the experiment as a general health indicator. Treatment of the animals followed procedures approved by The Animal Experiment Inspectorate, Denmark.

Mice were fed with AIN-93 G purified ethanol liquid rodent diet (710301 AIN-93 G from Dyets Inc, Bethlehem, PA). Mice received diets are shown in Table 1. In the control diet, alcohol was replaced by maltose-dextrin according to manufacturer's instructions to keep all diet mixtures isocalorical. Fresh diets were prepared every day. Mice in the high continuous alcohol (HCA) group (Table 1) received the highest alcohol concentration that did not compromise the well-being of the animals. The low continuous alcohol group (LCA) was exposed to an alcohol concentration that was approximately half of the HCA group (Table 1). The high pulsing alcohol exposure group (HPA) was included to mimic the exposure pattern that is relevant for many humans.

All mice were kept on control diet the first 5 days of a 21-day acclimatization period. Hereafter, alcohol was gradually increased in the diet of the exposure groups as shown in Fig. 1.

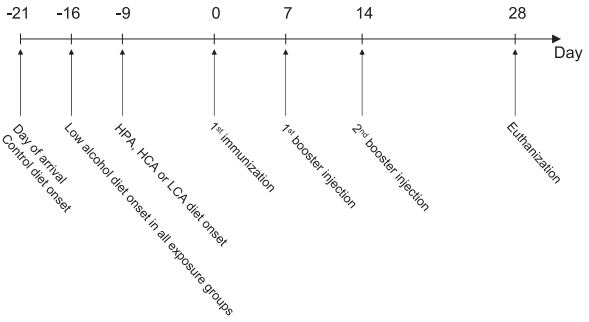

Fig. 1. Time line for the experiment. For full information on treatments, see Materials and Methods and Table 1.

Immunization Protocol

Mice were immunized by intraperitoneal injection of 1 μg ovalbumin (OVA) in 100 μl saline on day 0 (Fig. 1). An adjuvant control group received 1 μg OVA adsorbed to 270 μg Al $(OH)_3$ in 100 μl saline. Mice were boosted with 0.1 μg OVA on day 7 and 14. The mice were euthanized on day 28 where also blood samples and spleens were collected.

Spleen Cell Assay and Measurements of Cytokine Production

On termination of the experiment on day 28, the spleen was excised from the animals. The spleens were disintegrated using a 70-μm nylon cell strainer (BD Falcon Biosciences, Bedford, MA). Cells were cultured in 24-well plates (Nunc, Roskilde, Denmark), 1.0-ml cell suspension per well (2.7×10^6 cells/ml) in RPMI 1640 medium (Cambrex, Vervier, Belgium) supplemented with Nutridoma (1%), gentamycin (50 μg/ml), and monothioglycerol (13.5 μl/l). Cells were either unstimulated or stimulated with 1 mg/ml of lipopolysaccharide-purified OVA. After 5 days, supernatants were harvested and analyzed for cytokines.

Concentrations of the Th2-related interleukin (IL)-4 and the Th1-related cytokine interferon (IFN) γ were measured by the Gyrolab Bioaffy System (Gyros AB, Uppsala, Sweden) according to the manufacturer's instructions. The detection limits were 200 and 6 pg/ml for IL-4 and IFNγ, respectively.

Antibody Production

At the end of the experiment, on day 28, mice were anesthetized and blood samples were collected by heart puncture. Sera were assayed for content of total IgE as well as OVA-specific IgE, IgG1, and IgG2a. In mice, IgG2a is a Th1-dependent antibody, whereas both IgE and IgG1 are Th2-dependent. Total IgE was measured by ELISA kit (Catalog no. 6370, Alpha diagnostics Inc, San Antonio, TX) according to manufacturers' instruction whereas OVA-specific antibodies were measured by ELISA methods as described previously (Larsen et al., 2003).

Statistics

Antibody and cytokine levels in serum and cell culture supernatant, respectively were analyzed by nonparametric statistics. Initially, groups were compared using the Kruskall–Wallis test. Only if a statistically significant difference in response between groups was apparent, the individual alcohol groups (i.e., LCA, HPA, HCA) were further compared pair-wise with the OVA control group by the Mann–Whitney's *U*-test. A *p*-value of less than 0.05 was considered statistically significant. Calculations were performed using the Minitab Statistical Software, Release 14 XTRA (Minitab Inc., State College, PA).

RESULTS

Body Weight

The body weights of mice from different groups are shown in Fig. 2. The weight gain of mice in the HCA group was lower than that of mice in the control group. Thus on day 28, mice in the HCA group on average had increased their body weight by 3% (SD 14%) since the day of arrival (day 14).

Table 1. Groups Included in the Study

Group	Diet	Immunization
Control (n = 10)	Alcohol-free control diet	ovalbumin (OVA)
Adjuvant control (n = 10)	Alcohol-free control diet	OVA + A1$(OH)_3$
Low continuous alcohol intake (LCA) (n = 10)	Low (2.7% vol/vol) alcohol intake 7 d/wk	OVA
High pulsing alcohol intake (HPA) (n = 10)	High (6.2% vol/vol) alcohol 3 d/wk consecutively Alcohol-free control diet 4 d/wk consecutively	OVA
High continuous alcohol intake (HCA) (n = 11)	High (4.8% vol/vol) alcohol 7 d/wk	OVA

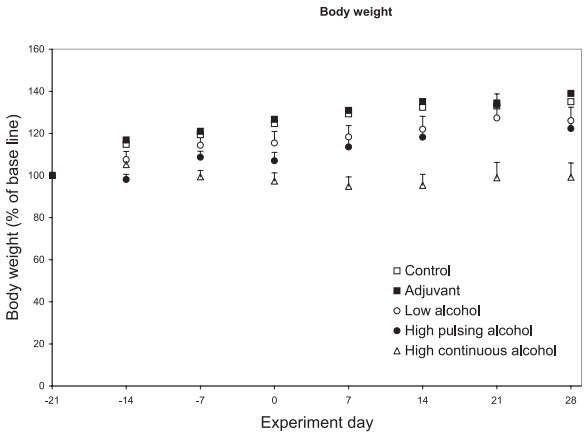

Fig. 2. The body weight of the mice was followed from day of arrival (day -21 = base line body weight) until termination of the experiment (day 28). Values are means of 10–11 animals with upper 95% confidence limit.

This weight gain was significantly lower than that of mice in the control group, which gained 35% (SD 8%), compared with day of arrival. On the day of arrival the mean weight of the animals was 18.4 g with a standard deviation of 1.3 g.

Antibodies

Alcohol intake was significantly associated with the production of total serum IgE antibodies ($p = 0.018$, Kruskal–Wallis). When the alcohol groups were compared pair-wise with the control, it was observed that this was because of a significantly increased level of total IgE in the HCA group (Table 2). The median level of IgE increased from 357 ng/ml in the control group to 2062 ng/ml in the HCA group, i.e., by a factor of 5.8. The median level of total IgE in the HPA and LCA was increased 2.6- and 3.4-fold, respectively, but none of these groups reached statistical significance in the Mann–Whitney *U*-test.

Furthermore, alcohol intake clearly affected the production of OVA-specific IgG2a antibodies. Mice in the HCA group had a dramatically reduced IgG2a level, corresponding to 98% reduction of the control mice level ($p = 0.004$), whereas the HPA treatment led to a 90% reduction from control level ($p = 0.04$). LCA treatment did not significantly reduce the IgG2a production, albeit the production was reduced by almost 70% (Table 2).

Alcohol intake did not lead to statistically significant differences in the levels of OVA-specific IgE or IgG1.

Cytokine Production

Alcohol consumption had a clear effect on the IFNγ response, leading to suppressed IFNγ production in spleen cultures from mice exposed to low, high or alternating alcohol intake (Fig. 3*A*). No differences were seen on the IL-4 productions, albeit IL-4 tended to be higher in the adjuvant control group when compared pair-wise with the control group.

Table 2. Antibody Production

		Ovalbumin-specific antibodies		
Group	Total IgE (ng/ml)	IgE(AU)	IgGl (AU)	IgG2a(AU)
Control	357 (2(52–1205)	156 (101–307)	621 (210–1628)	1793 (169–2913)
Adjuvant control	766 (339–1619)	333 (203–1110)	561 (355–1185)	636 (153–3500)
Low continuous alcohol intake (LCA)	1221 (821–1405)	116 (73–131)	741 (353–1538)	533 (271–1637)
High pulsing alcohol intake (HPA)	910 (298–1363)	97 (66–156)	1315 (275–1864)	199 (39–473)*
High continuous alcohol intake (HCA)	2062 (1253–4800)*	157 (39–257)	268 (88–552)	42 (0–173)**

AU, arbitrary units.
Statistically significantly difference when compared to control group is indicated by *$p = 0.05$ or **$p = 0.01$.
Values are medians with 25 and 75 percentiles in brackets.

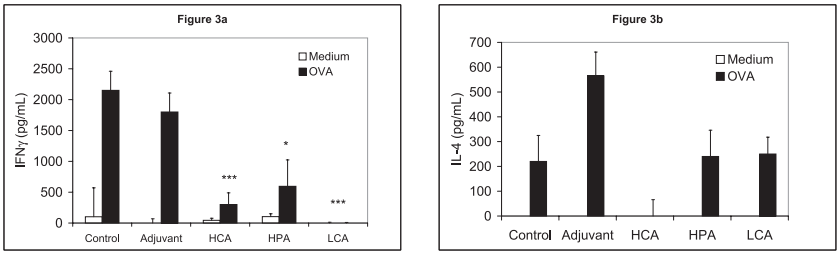

Fig. 3. Concentrations of IFNγ; (**A**) and IL-4 (**B**) in spleen cell culture supernatant. Spleen cell cultures ($n = 10$–11) were incubated 5 days with either medium or medium with 1 mg/mL LPS-free OVA. For the medium without OVA, levels of IL-4 were below the detection limit for all groups. LCA: Low continuous alcohol intake. HPA: High pulsing alcohol intake. HCA: High continuous alcohol intake. Values are means ± SEM. Levels of significance are *$p < 0.05$ and ***$p < 0.001$. P-values represent test for comparison with Control group. Refer to Materials and Methods for full information.

Furthermore, a very low level of IL-4 was seen in the HCA group (Fig. 3*B*).

DISCUSSION

Alcohol consumption had a marked effect on the immune response as reflected by a significantly increased production of total IgE and a significantly reduced OVA-specific IgG2a production. Th1 suppression was supported by the ex vivo cytokine production in spleen cell cultures, showing that alcohol intake markedly suppressed the Th1-dependent cytokine IFNγ in response to OVA. The above effects exceeded those of the adjuvant.

The strong effect of alcohol on IgE production appears to be independent of allergen exposure as alcohol increased the level of total IgE but not the level of OVA-specific IgE. In line with this interpretation, Starkenburg et al. (2001) found similar effects on total serum IgE in alcohol-fed mice that were not immunized with allergen. The results are consistent with data in humans showing that total serum IgE is increased in alcoholics (Dominguez-Santalla et al., 2001; Gonzalez-Quintela et al., 1995; Gonzalez-Quintela et al., 1999; Hallgren and Lundin, 1983; Smith et al., 1980; Vidal et al., 1994) and moderate to heavy drinkers in other patient populations (Criqui et al., 1990; Vidal et al., 2002) and in general populations (Gonzalez-Quintela et al., 2003; Linneberg et al., 2003). This effect appears to be independent of other factors, e.g., gender and smoking that may influence levels of total serum IgE (Barbee et al., 1987; Burrows et al., 1982; Criqui et al., 1990; Jarvis et al., 1999; Omenaas et al., 1994; Wuthrich et al., 1996). The present animal experimental study supports that alcohol itself or one of its metabolites is the substance responsible for the increased levels of total serum IgE associated with alcohol consumption in humans.

Recently, Heinz and Waltenbaugh (2007) proposed a unifying hypothesis for the underlying mechanisms of alcohol's effect on immune function. They suggest that ethanol modifies dendritic cell function and their interaction with naïve CD4+ T cells. By down-regulating IL-12 and up-regulating IL-10 dendritic cells induce T cell differentiation away from Th1 and toward Th2 effector function (Heinz and Waltenbaugh, 2007).

The present results were obtained in female mice. In humans, there is evidence to suggest that females as compared to males may be more susceptible to some of the deleterious effects of alcohol (Ward and Coutelle, 2003). These differential effects are believed to be because of gender differences in the pharmacokinetics of ethanol, e.g., because of higher body fat/water ratio or reduced liver mass/body weight ratio in females, or lower first-pass metabolism in females. Therefore, it would be relevant to include male mice in future studies.

In contrast to the clear effect of alcohol on total serum IgE, there was no significant effect on OVA-specific IgE. To the best of our knowledge, this is the first published experimental data on this issue. It may be speculated that the route of allergen administration is important to the effects of alcohol ingestion on immune tolerance. For example, alcohol induced gastritis appears to be able to prevent oral tolerance induction in mice (Andrade et al., 2006) suggesting that other routes of allergen exposure may be important.

Although the specific role of the IgG2a response in the induction of immune tolerance is not clear, it is interesting that in the present mouse model, alcohol markedly decreased the OVA-specific IgG2a response thereby potentially skewing the Th1/Th2 balance towards Th2. Interestingly, in a murine model, a high OVA-specific IgG2a response was associated with a low level of OVA-specific IgE, while a low OVA-specific IgG2a response was associated with OVA-specific IgE sensitization (Sakai et al., 1999). Nevertheless, in the present study there was no increase in the OVA-specific IgE response.

It has been reported that alcoholics admitted to hospital with alcohol abstinence syndrome have increased levels of some Th2-related cytokines compared with healthy controls (Gonzalez-Quintela et al., 1999). Experimental studies in animals have shown that alcohol impairs the Th1 lymphocyte-regulated cell-mediated immune response thereby possibly favoring a Th2 deviation of the immune response (Latif et al., 2002; Schodde et al., 1996; Starkenburg et al., 2001; Waltenbaugh et al., 1998; Waltenbaugh and Peterson, 1997). The finding of a marked impairment of the OVA-induced IFNγ response further supports that alcohol induces a strong suppression of the Th1 response. Although the observed decreased Th1 response and the increase in total serum IgE might suggest a Th2 skewing of the immune response, the lack of a OVA-induced IL-4 response (that even appeared to be decreased in the HCA group) does not support this notion. Further experiments are needed to explore this either involving an in vivo challenge protocol or in vitro experiments investigating the numbers of Th2 cells using intracellular flow cytometry.

In summary, alcohol consumption induced a marked decrease in markers of the Th1-type immune response (decreased OVA-specific IgG2a and IFNγ) and an increase in total serum IgE. In this animal model using the intraperitoneal route of immunization, there was no effect of alcohol on OVA-specific IgE or OVA-induced IL-4 response. However, we speculate that a potential effect of alcohol on the specific IgE immune response would be dependent on the route of immunization. In line with this, ethanol-induced gastritis appears to be able to prevent oral tolerance induction in mice (Andrade et al., 2006). Experimental studies using other routes of immunization would be of particular interest.

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