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Effects of ethanol consumption and withdrawal on B cell subpopulations in murine bone marrow

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SUMMARY

We designed studies to examine the effects of ethanol consumption and withdrawal on the numbers of pre-B and B cells in murine bone marrow. Flow cytometric analysis of B220 and surface IgM expression on bone marrow cells revealed that consumption of ethanol by mice for 7 days led to a significant reduction in pre-B cells. The number of mature B cells in the bone marrow of these animals, however, did not differ from that of control mice. In contrast, examination of bone marrow obtained from mice at various times after withdrawal from ethanol showed significantly fewer numbers of mature B cells and an even greater loss of pre-B cells. This effect was seen for relatively long periods after withdrawal. These study findings are interpreted to suggest that ethanol consumption results in changes in the pre-B cell population in murine bone marrow. It also appears that withdrawal from ethanol results in more profound changes in the mature B cell population of the bone marrow than those that occur during ethanol consumption.

Keywords ethanol bone marrow B cells pre-B cells

INTRODUCTION

Consumption of ethanol (ETOH) is associated with many adverse effects on immune function. Accordingly, alcoholic human beings have a higher incidence of certain diseases, such as tumours (particularly of the head, neck, and gastrointestinal tract), bacterial pneumonias, infections with opportunistic pathogens [1,2], and autoimmune disorders [3], than their non-alcoholic cohorts. This increased incidence is presumably a result of alcohol-induced immune dysfunction. Although the effects of ETOH on the general health of human beings are multifactorial, and include nutritional effects, it is clear from animal models that ETOH consumption in a controlled environment is associated with many changes in the immune system. This is not to imply that other factors like smoking, nutrition and aspiration are not important, but it is clear that effects are noted in the absence of these confounding variables. To date, many aspects of cellular and humoral immune responses and non-specific host defence mechanisms, such as phagocytosis and chemotaxis, are inhibited by ETOH [4–10]. With the use of a murine model, we have shown that ETOH consumption results in diminished T cell function, such as reduced proliferative responses to T cell mitogens and alloantigens, as well as a decreased antibody production to T cell-dependent antigens [11]. These changes in T cell function are associated with impaired immunity to bacterial infection [12]. It is interesting that B cell function, as determined by measuring proliferative responses to B cell mitogens and antibody responses to T cell-independent antigens, seems to be unaffected by ETOH [11,13].

Work by Jerrells & Saad has shown that consumption of ETOH is also associated with a significant loss of lymphocytes from lymphoid tissues such as spleen and thymus. In the thymus, immature thymocytes are depleted predominantly, but mature cells are also depleted [14]. Although both T and B cells are lost from the spleen after 7 days of ETOH consumption, B cells are depleted to the greater extent [14]. The cellularity of the spleen returns to normal levels by day 7 after animals are fed laboratory chow diet, rather than ETOH diet [11]. The defect in antibody production to T cell-dependent antigens associated with ETOH consumption [11] could be due to many factors, but in general might be associated with a defect in T helper cell function, a loss of a special B cell population, or both. The lack of an effect on T cell-independent B cell responses [11] in spite of marked B cell loss would support this notion. The present study is an attempt to define further the effects of ETOH on B cell production.

In light of these observations, we hypothesize that the ETOH-induced effects on B cell numbers in the periphery are in part a result of the effects of ETOH on B cell haematopoiesis. Recently, investigators have shown that a number of B cell-

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associated antigens, such as B220, cytoplasmic IgM (cIgM), and surface IgM (sIgM), can define specific stages of B cell development in the murine bone marrow [15–19]. With the use of these markers and terminal deoxynucleotidyl transferase (TdT), at least five phenotypically distinct stages have been ascertained: (i) early pro-B cells, TdT^+ $B220^-$; (ii) intermediate pro-B cells, TdT^+ $B220^{lo}$; (iii) late pro-B cells, TdT^- $B220^{lo}$; (iv) pre-B cells, TdT^- $B220^{lo}$ $cIgM^+$; and (v) B lymphocytes, $B220^{hi}$ $cIgM^-$ $sIgM^+$ [15,16]. In this study, we examined alterations in the B cell lineage markers B220 and sIgM on bone marrow cells isolated from mice that had consumed ETOH and were then withdrawn from ETOH. These markers allowed us to discern intermediate-to-late pro-B and pre-B cell populations ($B220^{lo}$ or $B220^+$ $sIgM^-$; collectively referred to here as pre-B cells) and mature B cell populations ($B220^{hi}$ or $B220^+$ $sIgM^+$) in these animals. The results of these studies suggest that ETOH consumption and withdrawal are associated with a selective depletion of pre-B and mature B cells (respectively) from the bone marrow.

MATERIALS AND METHODS

Mice and ETOH administration

Male C57Bl/6 mice (5–6 weeks of age) were obtained from Harlan Sprague-Dawley (Houston, TX) and maintained in the animal facility for 1–2 weeks to acclimatize. Individual mice were fed either a Lieber–DeCarli liquid diet containing 7% (v/v) ETOH; an identical liquid diet, with the exception that the diet was made isocaloric with dextrose and maltose in a pair-feeding protocol; or laboratory chow and water. The animals receiving the control liquid diet were fed with the use of a pair-feeding protocol as previously described [11]. Animals fed the ETOH-containing diet or laboratory chow and water were fed *ad libitum*. In all experiments described in this study, animals were provided liquid diets for 7 days and either studied at this time, or the liquid diet was discontinued and animals were provided a diet of laboratory chow and water *ad libitum*.

Antibodies

Rat anti-mouse B220 antibody was obtained from PharMingen (San Diego, CA). Streptavidin–PE and goat anti-mouse IgM–FITC were obtained from Southern Biotechnology Associates, Inc. (Birmingham, AL). Antibodies were used at a final dilution of 1:100. Streptavidin–PE was used at a final dilution of 1:50.

Bone marrow isolation and staining for flow cytometry

Bone marrow cells from animals fed ETOH diet, control diet, or laboratory chow were obtained by lavage (two femurs per mouse) with calcium- and magnesium-free Hanks' balanced salt solution (HBSS; Biofluids, Rockville, MD). Bone marrow cells were washed three times in HBSS, and erythrocytes were removed by hypotonic lysis in haemolytic Geys solution as described elsewhere [20]. Bone marrow cells were stained with the appropriate antibodies in 96-well, round-bottomed plates (10^6 cells/well) for 20 min at 4°C. Cells were fixed in 1% paraformaldehyde for 10 min, washed, and then resuspended in PBS to remove excess paraformaldehyde. Cells were analysed with the use of a Coulter Profile II flow cytometer/cell sorter (Coulter Cytometry, Hialeah, FL) provided by the University Core Facility for Flow Cytometry at Louisiana State University Medical Center (Shreveport, LA). Debris, dead cells, and erythrocytes were excluded from the analysis on the basis of forward-angle light scatter gating. The spectral overlap between FITC and PE was compensated for electronically on the basis of single fluorochrome-stained samples. The percentage of cells stained with the appropriate antibody was determined by analysing at least 10^4 cells from each sample.

Statistical analysis

Differences between groups were determined with the use of a one-way analysis of variance.

RESULTS

We examined the effects of ETOH consumption and withdrawal on cells in the B cell lineage in murine bone marrow in three separate experiments. Throughout these experiments, the number of bone marrow cells recoverable from the femurs of ETOH-treated mice was approximately 50% of that recoverable from control animals. The mean number of cells isolated from pair-fed animals was $8{\cdot}1 \pm 1 \times 10^7$, compared with $4{\cdot}3 \pm 0{\cdot}5 \times 10^7$ cells recovered from ETOH-fed mice.

With the data obtained from the flow cytometric studies and total bone marrow counts we determined the numbers of dual-positive ($B220^+$ $sIgM^+$) B cells in the bone marrow of each animal (two femurs each) from three separate experiments. Mean numbers of each population of cells were

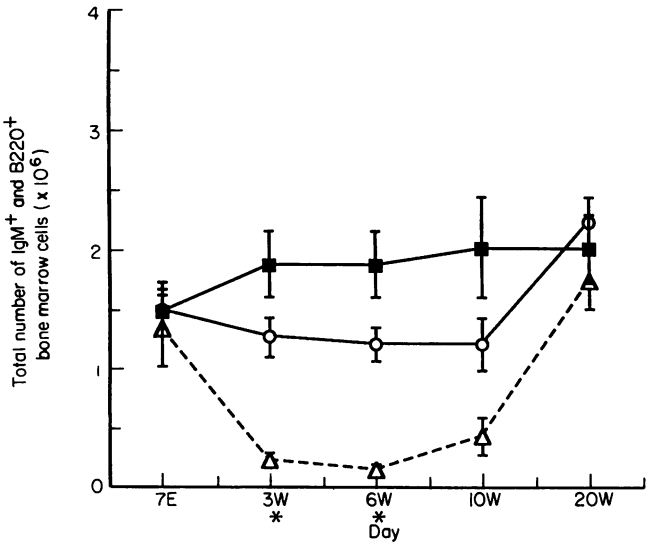

Fig. 1. Analysis of numbers of dual-positive ($B220^+$ $sIgM^+$) bone marrow cells in mice fed ethanol (ETOH) diet (△), control diet (○), or laboratory chow (■). Mice were maintained on the appropriate diet for 7 days and either killed (7E) or placed back on laboratory chow and water (withdrawal) for 3, 6, 10, or 20 days (3W, 6W, 10W, or 20W, respectively) before analysis of bone marrow. The data from three separate experiments are presented as the mean ± s.e.m. * Times when the numbers of dual-positive cells obtained from ETOH diet-fed mice were statistically different ($P < 0{\cdot}05$) from those of control diet- and laboratory chow-fed mice, as determined by a one-way analysis of variance. No statistically significant differences were observed between laboratory chow- and control diet-fed mice at any time.

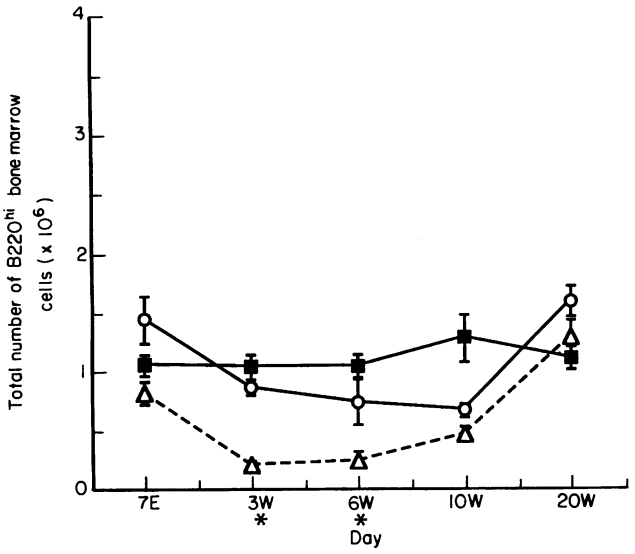

Fig. 2. Analysis of numbers of $B220^{hi}$ bone marrow cells in mice fed ethanol (ETOH) diet (△), control diet (○), or laboratory chow (■) as described in the legend for Fig. 1. The data from three separate experiments are presented as the mean ± s.e.m. *Times when the numbers of $B220^{hi}$ bone marrow cells obtained from ETOH diet-fed mice were statistically different ($P < 0.05$) from those of control diet- and laboratory chow-fed mice. No statistically significant differences were observed between laboratory chow- and control diet-fed mice at any time.

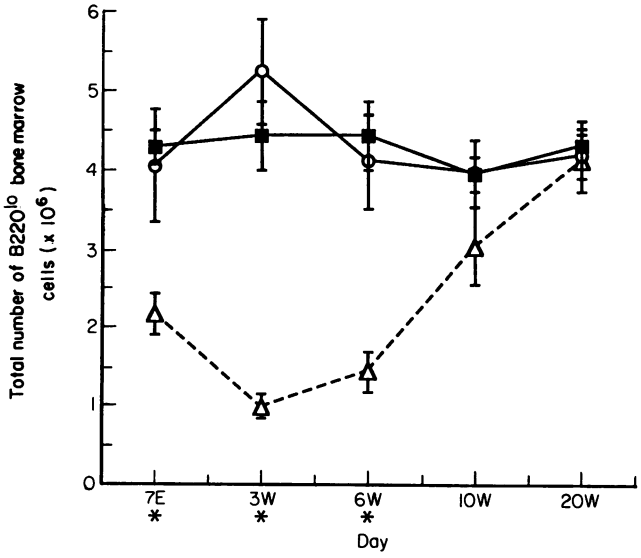

Fig. 3. Analysis of numbers of $B220^{lo}$ bone marrow cells in mice fed ethanol (ETOH) diet (△), control diet (○), or laboratory chow (■) as described in the legend for Fig. 1. The data from three separate experiments are presented as the mean ± s.e.m. *Times when the numbers of $B220^{lo}$ bone marrow cells obtained from ETOH diet-fed mice were statistically different ($P < 0.05$) from those of control diet- and laboratory chow-fed mice. No statistically significant differences were observed between control diet- or laboratory chow-fed mice at any time.

determined and compared for statistically significant differences by using a one-way analysis of variance. Figure 1 shows the total numbers of $B220^+$ $sIgM^+$ (mature) B cells in animals fed either ETOH diet, control diet, or laboratory chow at the indicated times. There were no statistically significant differences ($P > 0.05$) in the total numbers of $B220^+$ $sIgM^+$ cells in the bone marrow of these animals after 7 days of diet feeding ($n = 13$, 9, and 6 for animals fed ETOH diet, control diet, or laboratory chow, respectively). Examination of bone marrow cells after 3 and 5 days of ETOH consumption also showed no change in the percentage or number of $B220^+$ $sIgM^+$ cells (data not shown). At day 3 after withdrawal (from ETOH and control diets), however, there was a statistically significant difference ($P < 0.001$) in the total number of $B220^+$ $sIgM^+$ bone marrow cells isolated from mice that consumed ETOH (mean, 2.31×10^5 cells per two femurs; $n = 7$), compared with findings in mice that consumed control diet or laboratory chow (1.2 and 1.8×10^6 cells per two femurs, respectively; $n = 8$ and 6). This represents a range in loss of dual-positive mature B cells from the bone marrow of ETOH-consuming mice of 82–88%.

The effects of ETOH consumption and withdrawal were still evident at day 6 after withdrawal ($P = 0.0001$), at which time mice fed ETOH diet had approximately 1.48×10^5 $B220^+$ $sIgM^+$ B cells, compared with 1.2 and 1.8×10^6 $B220^+$ $sIgM^+$ B cells in mice fed control diet and laboratory chow, respectively ($n = 5$, 5, and 6, respectively). This represents a decrease of 88–92% of the bone marrow-associated $B220^+$ $sIgM^+$ B cells in the mice that consumed ETOH and underwent withdrawal for 6 days, relative to control diet-treated animals.

At day 10 after withdrawal, the level of $B220^+$ $sIgM^+$ B cells in mice that had consumed ETOH ($n = 7$) was statistically different from that observed in laboratory chow-fed mice ($n = 5$; $P = 0.0025$). No statistically significant differences were observed between control diet- ($n = 8$) and laboratory chow-fed mice, nor between ETOH diet- and control diet-fed mice ($P > 0.05$).

By day 20 after withdrawal, the numbers of dual-positive B cells in the ETOH diet-fed animals ($n = 5$) returned to those observed in the control diet- ($n = 6$) and laboratory chow-fed mice ($n = 6$).

Figure 2 illustrates the total number of $B220^{hi}$ (mature) B cells isolated from mice fed either ETOH diet, control diet, or laboratory chow at the indicated times. There were no statistically significant differences in the number of $B220^{hi}$ B cells isolated from the bone marrow of mice in the three diet groups at day 7 of ETOH consumption ($P > 0.05$). As with the $B220^+$ $sIgM^+$ B cells, however, there was a statistically significant loss of $B220^{hi}$ B cells from the bone marrow of ETOH-consuming mice at day 3 after withdrawal ($P < 0.001$). The numbers of $B220^{hi}$ B cells in ETOH-consuming mice (mean, 2.09×10^5 per mouse) were lower (range 76–80%) than those in control diet- or laboratory chow-fed mice (mean, 8.65×10^5 and 1.04×10^6 per mouse, respectively) at this time. The effects of ETOH consumption and withdrawal were still evident at day 6 after withdrawal, when the number of $B220^{hi}$ B cells in ETOH-consuming mice (mean, 2.48×10^5 per mouse) was lower (range 67–77%) than that obtained from mice fed control diet (mean, 7.46×10^5 per mouse) or laboratory chow (mean, 1.04×10^6) ($P = 0.001$). By day 10 after withdrawal, the numbers of $B220^{hi}$ B cells in ETOH-consuming mice were significantly different from those in mice fed laboratory chow

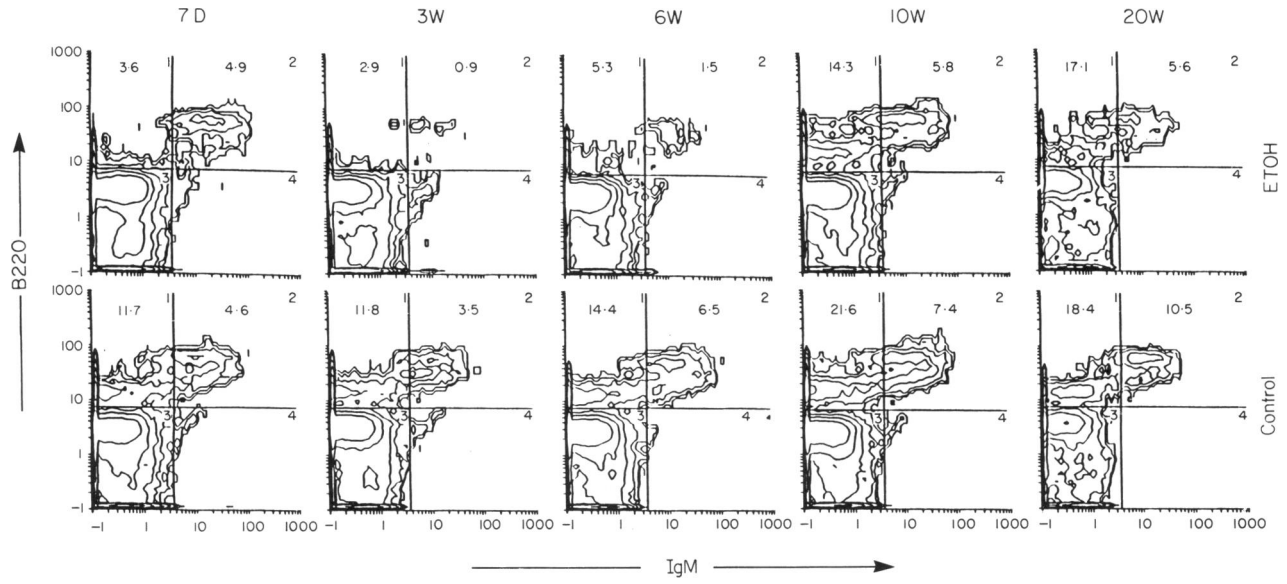

Fig. 4. Two-colour flow cytometric evaluation of B220 and sIgM expression on bone marrow cells of individual mice fed ethanol (ETOH) or control diet. Animals were treated as described in the legend for Fig. 1. The percentage of $B220^+$ $sIgM^-$ or $B220^+$ $sIgM^+$ cells is listed in the appropriate quadrants. A summary of these data from all mice analysed appears in Fig. 1.

($P < 0{\cdot}001$), but not from those in mice fed control diet. At day 20 after withdrawal, the number of $B220^{hi}$ B cells in ETOH-consuming mice was not significantly different from that for control diet- or laboratory chow-fed animals.

Figure 3 illustrates the effects of ETOH consumption and withdrawal on the number of $B220^{lo}$ pre-B cells in the bone marrow of mice at the indicated times. After 7 days of receiving either ETOH or control diet, the ETOH-consuming mice showed a 47–50% reduction ($P = 0{\cdot}001$) in the number of bone marrow-derived pre-B cells ($B220^{lo}$) ($2{\cdot}1 \times 10^6$ per mouse), compared with the findings in animals consuming control diet or laboratory chow ($4{\cdot}05 \times 10^6$ and $4{\cdot}28 \times 10^6$, respectively). A progressive loss of $B220^+$ $sIgM^-$ pre-B cells (as determined by dual parameter flow cytometry) was first noted after 5 days of ETOH consumption (data not shown). The loss of $B220^{lo}$ pre-B cells from the bone marrow of ETOH-consuming mice was even more pronounced 3 days after withdrawal ($P < 0{\cdot}001$). A comparison of the total number of $B220^{lo}$ cells isolated from ETOH-consuming mice ($9{\cdot}98 \times 10^5$ per mouse) with that obtained from mice consuming laboratory chow and control diet (mean, $4{\cdot}44 \times 10^6$ and $5{\cdot}24 \times 10^6$, respectively) revealed a 78–81% reduction in the bone marrow pre-B cells of mice that had consumed ETOH.

At day 6 after withdrawal, the number of pre-B cells remained significantly lower in ETOH-consuming mice, compared with that in mice consuming control diet or laboratory chow ($P = 0{\cdot}0001$). No statistically significant differences were observed among the three diet groups at days 10 and 20 after withdrawal.

Figures 4 and 5 show typical dual parameter (B220, sIgM) and single parameter (B220) flow cytometric profiles (respectively) and the percentage of each cell population obtained with the use of bone marrow cells isolated from animals fed control diet and those fed ETOH diet at the indicated times. These figures demonstrate that the changes in pre-B and B cell populations summarized in Figs 1–3 are the same whether we define these populations by co-expression of two cell surface markers (B220, sIgM, Fig. 4) or by the relative distribution of a single cell surface marker ($B220^{lo}$ *versus* $B220^{hi}$, Fig. 5). More importantly, these profiles demonstrate that the changes in the bone marrow pre-B and B cell populations are not accompanied by a change in fluorescence intensity, but rather a reduction in the number of positive events.

DISCUSSION

These studies were designed to examine the effects of ETOH consumption and withdrawal on B cell populations in murine bone marrow. The results support the suggestion that ETOH consumption in a mouse model leads to a selective depletion of pre-B cells ($B220^+$ $sIgM^-$; or $B220^{lo}$) from murine bone marrow. In mice that consumed ETOH for 7 days, the numbers of mature B cells ($B220^+$ $sIgM^+$; or $B220^{hi}$) isolated from the bone marrow did not differ from those for mice that consumed control diet or laboratory chow. However, the numbers of pre-B cells, as defined with the use of B220 expression, were drastically reduced in ETOH-consuming mice. Although we do not know the mechanisms for the ETOH-induced reduction in the number of pre-B cells, it is interesting to speculate that ETOH consumption may alter the production or action of various cytokines that modulate B cell haematopoiesis, such as granulocyte-macrophage colony-stimulating factors (GM-CSF), IL-3, IL-7, or c-kit ligand [18,21–28]. Alternatively, these results may be due to a direct effect of ETOH on the resident population of pre-B cells or their progenitors in the bone marrow, or perhaps to indirect effects associated with ETOH consumption such as that induced by corticosteroids. Future studies will be designed to help resolve these issues.

Results of the current study also show that withdrawal from ETOH leads to a significant loss of bone marrow-associated

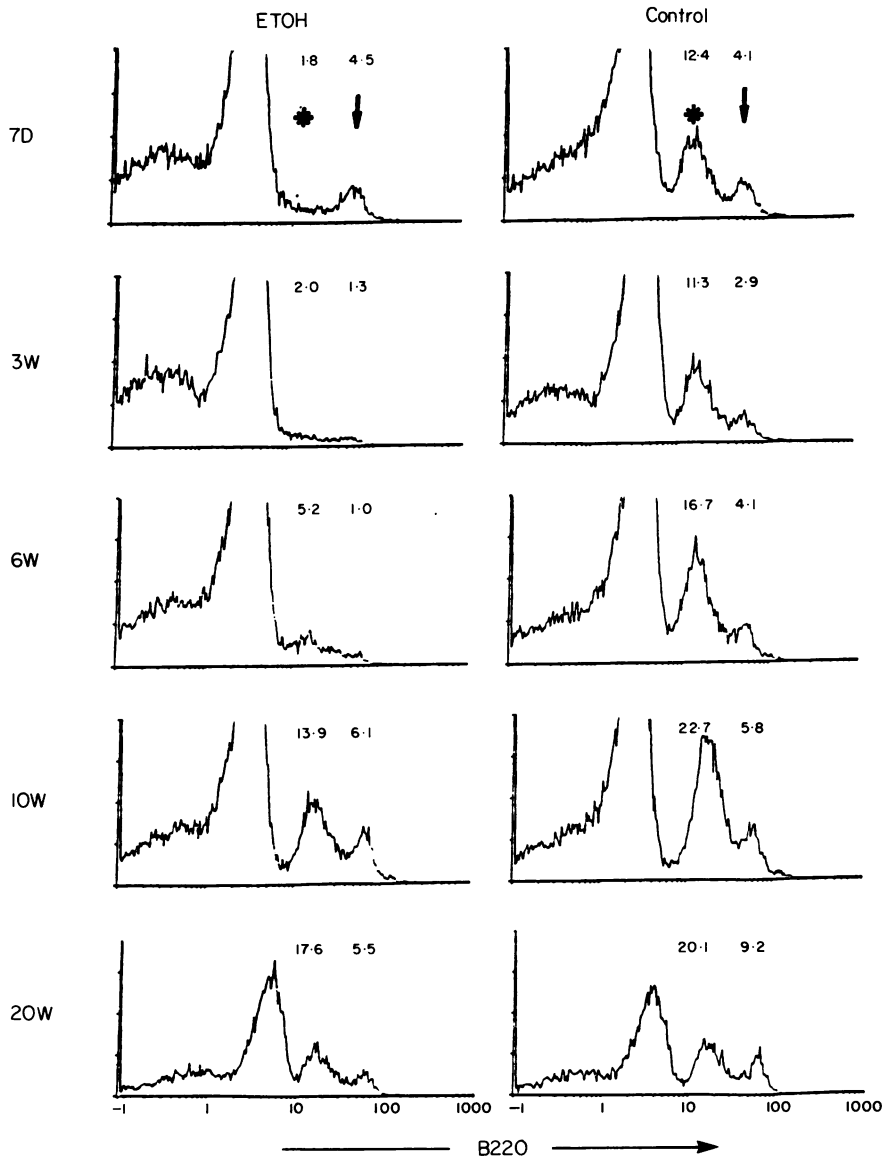

Fig. 5. Flow cytometric evaluation of $B220^{lo}$ and $B220^{hi}$ bone marrow cells of individual mice fed ethanol (ETOH) or control diet. Animals were treated as described in the legend for Fig. 1. The percentage of $B220^{lo}$ (*) and $B220^{hi}$ (arrow) cells is listed in each profile. A summary of these data from all mice analysed appears in Figs 2 and 3.

mature B cells ($B220^+$ $sIgM^+$; or $B220^{hi}$) and pre-B cells. Animals that were fed control diet and had their isocaloric liquid diets discontinued after 7 days did not exhibit a withdrawal effect on their bone marrow B cells. Alterations in the cellularity of spleen and thymus during ETOH withdrawal may be due in part to increased serum levels of corticosteroids. Analysis of adrenalectomized rats, however, suggested that corticosteroids are not solely responsible for the observed withdrawal effects in peripheral lymphoid tissues [13]. It is possible that the withdrawal effect of ETOH noted in the present study is partially or exclusively mediated by corticosteroids.

Although there were no statistically significant differences between control diet- and laboratory chow-fed animals in this study, there were some dietary effects on the B cell lineages in the bone marrow. As shown in Fig. 4 at day 7 of diet administration, the percentage of $B220^+$ $sIgM^+$ B cells in both control diet- and ETOH diet-fed mice was lower than the typical range (8–10%) reported elsewhere [15,17,21,29], as well as that observed in our laboratory chow-fed animals that had a mean of $7{\cdot}3 \pm 1{\cdot}7\%$ $B220^+$ $sIgM^+$ B cells. Of greater importance, however, are the relative changes that occurred throughout the study between ETOH diet- and control diet-fed mice.

Results of the current study demonstrate control levels of mature B cells in the bone marrow of mice after consuming ETOH diet for 7 days, but findings in previous studies show that splenic B cells are markedly depleted at this time. Both the pre-B and B cell populations (lost during ETOH consumption and withdrawal, respectively) in the bone marrow of

ETOH-consuming animals returned to control levels 10 days after withdrawal from ETOH. In previous studies demonstrating ETOH-induced depletion of B cells from the spleens of ETOH-consuming mice, the cellularity of the spleen returned to normal levels by day 7 after withdrawal [11,14]. In light of these observations, it is interesting to speculate that ETOH consumption interferes with normal homeostatic feedback mechanisms from peripheral lymphoid tissues, which alters the release of mature B cells from the bone marrow, changes trafficking to the peripheral lymphoid organs, or both. Other investigators have shown both stimulatory and inhibitory effects of exogenous stimuli, such as antigens, macrophage-derived cytokines, and serum immunoglobulins, on the genesis of B cells in murine bone marrow [30–35]. The possible role of these factors in the bone marrow changes reported here are currently under study.

Taken together, the results of this study support the suggestion that ETOH consumption and withdrawal result in both central and peripheral depletion of pre-B and B cells, possibly limiting the antibody repertoire available for fighting certain infectious diseases.

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REFERENCES

1 Breeden JH. Alcohol, alcoholism, and cancer. Med Clin North Am 1984; **68**:163–77.
2 MacGregor RR. Alcohol and immune defense. JAMA 1986; **256**:1474–9.
3 Mutchnick MG, Missirian A, Johnson AG. Lymphocyte cytotoxicity in human liver disease using rat hepatocyte monolayer cultures. Clin Immunol Immunopathol 1980; **16**:423–37.
4 Gilhus NE, Matre R. *In vitro* effect of ethanol on subpopulations of human blood mononuclear cells. Int Arch Allergy Appl Immunol 1982; **68**:382–6.
5 Morland B, Morland J. Effects of long-term ethanol consumption on rat peritoneal macrophages. Acta Pharmacol Toxicol (Copenh) 1982; **50**:221–4.
6 Bagasra O, Howeedy A, Kajdacsy-Balla A. Macrophage function in chronic experimental alcoholism. I. Modulation of surface receptors and phagocytosis. Immunology 1988; **65**:405–9.
7 McCarthy SP, Lewis CE, McGee JO. Effects of ethanol on human monocyte/macrophage lysozyme storage and release. Implications for the pathobiology of alcoholic liver disease. J Hepatol 1990; **10**:90–8.
8 Gluckman SJ, Dvorak VC, MacGregor RR. Host defenses during prolonged alcohol consumption in a controlled environment. Arch Intern Med 1977; **137**:1539–43.
9 Smith WI Jr, Van Thiel DH, Whiteside T *et al.* Altered immunity in male patients with alcoholic liver disease: evidence for defective immune regulation. Alcohol Clin Exp Res 1980; **4**:199–206.
10 Young GP, Vander Weyden MB, Rose IS *et al.* Lymphopenia and lymphocyte transformation in alcoholics. Experientia 1979; **35**:268–9.
11 Jerrells TR, Smith W, Eckardt MJ. Murine model of ethanol-induced immunosuppression. Alcohol Clin Exp Res 1990; **14**:546–50.
12 Saad AJ, Domiati-Saad R, Jerrells TR. Ethanol ingestion increases susceptibility of mice to *Listeria monocytogenes*. Alcohol Clin Exp Res 1993; **17**:75–85.
13 Jerrells TR, Marietta CA, Weight FF *et al.* Effect of adrenalectomy on ethanol-associated immunosuppression. Int J Immunopharmacol 1990; **12**:435–42.
14 Saad AJ, Jerrells TR. Flow cytometric and immunohistochemical evaluation of ethanol-induced changes in splenic and thymic lymphoid cell populations. Alcohol Clin Exp Res 1991; **15**:796–803.
15 Park YH, Osmond DG. Regulation of early precursor B cell proliferation in mouse bone marrow: stimulation by exogenous agents mediated by macrophages in the spleen. Cell Immunol 1991; **135**:168–83.
16 Osmond DG. Proliferation kinetics and the lifespan of B cells in central and peripheral lymphoid organs. Curr Opin Immunol 1991; **3**:179–85.
17 Hardy RR, Carmack CE, Shinton SA *et al.* Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. J Exp Med 1991; **173**:1213–25.
18 Morrissey PJ, Conlon P, Charrier K *et al.* Administration of IL-7 to normal mice stimulates B-lymphopoiesis and peripheral lymphadenopathy. J Immunol 1991; **147**:561–8.
19 Sundblad A, Marcos M, Huetz F *et al.* Normal serum immunoglobulins influence the numbers of bone marrow pre-B and B cells. Eur J Immunol 1991; **21**:1155–61.
20 Mishell BB, Shiigi SM, eds. Selected methods in cellular immunology. New York: WH Freeman, 1980:23–24.
21 Dorshkind K. *In vivo* administration of recombinant granulocyte-macrophage colony-stimulating factor results in a reversible inhibition of primary B lymphopoiesis. J Immunol 1991; **146**: 4204–8.
22 Sudo T, Ito M, Ogawa Y *et al.* Interleukin 7 production and function in stromal cell-dependent B cell development. J Exp Med 1989; **170**:333–8.
23 Hayashi S, Kunisada T, Ogawa M *et al.* Stepwise progression of B lineage differentiation supported by interleukin 7 and other stromal cell molecules. J Exp Med 1990; **171**:1683–95.
24 Henney CS. Interleukin 7: effects on early events in lymphopoiesis. Immunol Today 1989; **10**:170–3.
25 Zsebo KM, Williams DA, Geissler EN *et al.* Stem cell factor is encoded at the *Sl* locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell 1990; **63**:213–24.
26 Huang E, Nocka K, Beier DR *et al.* The hematopoietic growth factor KL is encoded by the *Sl* locus and is the ligand of the c-*kit* receptor, the gene product of *W* locus. Cell 1990; **63**:225–33.
27 Anderson DM, Lyman SD, Baird A *et al.* Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell 1990; **63**:235–43.
28 Martin FH, Suggs SV, Langley KE *et al.* Primary structure and functional expression of rat and human stem cell factor DNAs. Cell 1990; **63**:203–11.
29 Forster I, Rajewsky K. The bulk of the peripheral B-cell pool in mice is stable and not rapidly renewed from the bone marrow. Proc Natl Acad Sci USA 1990; **87**:4781–4.
30 Fulop GM, Osmond DG. Regulation of bone marrow lymphocyte production. III. Increased production of B and non-B lymphocytes after administering systemic antigens. Cell Immunol 1983; **75**:80–90.
31 Fulop GM, Osmond DG. Regulation of bone marrow lymphocyte production. IV. Cells mediating the stimulation of marrow lymphocyte production by sheep red blood cells: studies in anti-IgM-suppressed mice, athymic mice, and silica-treated mice. Cell Immunol 1983; **75**:91–102.
32 Pietrangeli CE, Osmond DG. Regulation of B-lymphocyte production in the bone marrow: role of macrophages and the spleen in mediating responses to exogenous agents. Cell Immunol 1985; **94**:147–58.

33 Pietrangeli CE, Osmond DG. Regulation of B-lymphocyte production in the bone marrow: mediation of the effects of exogenous stimulants by adoptively transferred spleen cells. Cell Immunol 1987; **107**:348–57.

34 Kincade PW, Lee G, Paige CJ *et al.* Cellular interactions affecting the maturation of murine B lymphocyte precursors *in vitro*. J Immunol 1981; **127**:255–60.

35 Jyonouchi H, Kincade PW, Good RA. Immunosuppression of marrow B lymphocytes by administration of *Corynebacterium parvum* in mice. J Immunol 1981; **127**:2502–7.