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Effects of Whey Protein Concentrate (WPC) on the Distributions of Lymphocyte Subpopulations in Rats with Excessive Alcohol Intake

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To investigate the effects of whey protein concentrate (WPC) on antioxidant statuses and the lymphocyte subpopulations in the rats with alcohol intake, the antioxidant statuses in the peripheral blood (PB) and the lymphocyte subpopulations in the PB, spleen, and bone marrow (BM) of the rats fed with WPC (0.334 g/kg) and alcohol (6 g/kg) for 3 months were analyzed. Results showed that the effects of WPC on the glutathione peroxidase and glutathione in the PB, the T and B cells in the spleen, and the B cells in the BM were more apparent in the rats with alcohol intake; however, they are not apparent in the controls. Taken together, our results indicated that the immunity of rats might be enhanced by the increased antioxidant ability after WPC supplementation and the effects of WPC on the lymphocyte subpopulations were mainly in the spleen and BM and not in the PB.

KEYWORDS: Alcohol; antioxidants; rat; whey protein concentrate (WPC); lymphocyte subpopulation

INTRODUCTION

Excessive intake of alcohol can adversely affect many tissues, such as the liver and spleen, as well as the hematopoietic and immunological systems (*1*−*3*). In addition, several previous studies have indicated that alcohol could also promote the generation of reactive oxygen species (ROS) and interfere with the normal defense systems of the body (*4*, *5*). Some of the reasons why excessive alcohol intake can damage cells include the production of carcinogens and alteration of normal hematopoietic and immunological functions during alcohol metabolism (*6*−*8*). Toxic effects of excessive alcohol intake on the hematologic system can be found in the spleen, peripheral blood (PB) streams, and bone marrow (BM), where the production of BM cells is depressed (*9*). Therefore, abnormalities in the numbers and functions of lymphocytes in these locations indicate that there would be impaired cellular immunity after excessive alcohol intake (*10*). In addition, the study by Diaz-Romero et al. indicated that the immune status could be evaluated by measuring T-cell subpopulations (*11*). On the basis of the above-mentioned research, abnormal distributions of lymphocyte subpopulations may be used as a biomarker for the evaluation of impaired cellular immunity after excessive alcohol intake.

Glutathione (GSH), is a tripeptide composed of glutamate, cysteine, and glycine and a ubiquitous molecule virtually produced in all of the mammalian tissues. It is the major non-protein thiol component of the antioxidant defense system against xenobiotics and naturally occurring deleterious compounds, such as free radicals in both prokaryotes and eukaryotes (*12*, *13*). GSH can directly bind to the acetaldehyde derived from alcohol to reduce the alcohol-induced oxidative damage of alcohol; meanwhile, lower intracellular levels of GSH were reported to be associated with various disorders in lymphocyte functions (*14*). GSH peroxidase (GPx), one of the most important antioxidant enzymes in humans, can reduce hydrogen peroxide (H_2O_2) to water, and GSH reductase (GRx) can catalyze the recycling of reduced GSH from oxidized GSH, which is involved in the metabolism of the GSH cycle (*15*). Whey protein concentrate (WPC), which is prepared to keep the native forms of the cysteine-rich protein found in whey, has been found to be an effective and safe donor of cysteine when fed to GSH-depleted animals in an immune-deficiency state (*16*). WPC has also been found to selectively manipulate the GSH levels in normal or cancer cells and potently modulate cellular immune functions (*17*−*19*). In our previous study, we found that supplementation with WPC could enhance the antioxidant statuses of human peripheral blood mononuclear cells (PBMCs) treated with high doses of alcohol (*20*). Additionally, Clemens et al. indicated that the imbalance of the antioxidant statuses may result in abnormal distributions of hematological components, such as T cells, B cells, natural killer (NK) cells, and activated T cells (*21*, *22*).

With the above-mentioned studies, the effects of WPC on the antioxidant statuses and immunological functions were focused in the PB or cancer cells (*18*, *20*). Thus, the aim of current study was to investigate whether the effects of WPC on the distributions of lymphocyte subpopulations in the spleen and BM were

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Table 1. Effects of WPC Supplementation on the Antioxidant Statuses and Hematological Markers in the PB of the Rats with Excessive Alcohol Intake[a]

	control group		alcohol group		
	WPC (−)	WPC (+)	WPC (−)	WPC (+)	*p* value for interaction
antioxidant statuses					
GSH (μM)	785 ± 53	1024 ± 88[b]	881 ± 215	1171 ± 165[c]	0.659
GPx (units/g Hgb)	523 ± 29	505 ± 35	480 ± 21[b]	523 ± 30[c]	0.180
GRx (units/g Hgb)	3.1 ± 0.5	2.7 ± 0.2	2.6 ± 0.3	2.9 ± 0.3	0.031
WBC ($10^3/\mu$L)	8.2 ± 3.2	11.8 ± 3.1[b]	7.4 ± 4.8	10.5 ± 4.4	0.886
neutrophils (%)	19.2 ± 3.2	14.3 ± 3.9[b]	22.2 ± 8.5	22.7 ± 7.6	0.289
lymphocytes (%)	77.9 ± 3.1	83.5 ± 4.4[b]	74.6 ± 8.9	75.3 ± 8.6	0.363
monocytes (%)	2.1 ± 1.5	1.3 ± 0.9	2.2 ± 1.4	1.0 ± 1.0	0.645
mononuclear cells (%)	80.0 ± 3.1	84.8 ± 3.9[b]	76.8 ± 8.6	76.2 ± 7.7	0.289
MCV (fL)	54.9 ± 1.9	55.1 ± 1.9	57.2 ± 2.5	54.7 ± 1.2[c]	0.103
PLT ($10^3/\mu$L)	692 ± 91	792 ± 52	883 ± 96[b]	778 ± 142	0.017

[a] Data are expressed as mean ± SD of six rats for each group. WPC, whey protein concentrate; WPC (−), rats without WPC supplementation; WPC (+), rats with WPC supplementation. [b] $p < 0.05$ compared to the control group without WPC supplementation. [c] $p < 0.05$ compared to the alcohol group without WPC supplementation.

different from those in the PB and to analyze the effects of WPC on the relationship between the antioxidant statuses and the distributions of lymphocyte subpopulations in the PB of rats with excessive alcohol intake.

MATERIALS AND METHODS

Animals. Male Sprague−Dawley (SD) rats (250−300 g) were obtained from BioLASCO Taiwan Co., Ltd. The rats were allowed to acclimate for 1 week prior to different treatments and had free access to water and standard laboratory chow. They were group-housed in hanging stainless-steel cages in a room with an artificial 12/12 h light/dark cycle at 20−22 °C. The Institutional Animal Care and Use Committee at Kaohsiung Medical University approved the animal use protocol.

Preparation of WPC. Preparation of WPC followed the same procedure as our previous study (*20*). Briefly, we followed the instructions of Immunocal (Immunocal Platinum, Immunotec, Inc.) to design the concentrations of WPC in this experiment. In the instructions of Immunocal, the daily suggested dose in adults (about 60 kg) was 10−30 g; therefore, we adopted the average daily suggested dose 20 g/60 kg (about 0.334 g/kg) of WPC as the treatment dose for a rat in this study.

Experimental Procedures. The 24 rats were randomly divided into the control group (12 rats/group) and the alcohol group (12 rats/group). The rats in the control group were fed with a normal diet and water *ad libitum*. The rats in the alcohol group were treated with alcohol at a dose level of 6 g (kg of body weight)$^{-1}$ day^{-1} by an intragastric tube. To investigate the effects of WPC, the control and alcohol groups were divided into two subgroups (6 rats/subgroup), respectively. One of the subgroups in both groups was supplemented with WPC at a dose level of 0.334 g/kg of body weight by an intragastric tube. All of the treatments continued for a period of 3 months.

Tissue Collection and Cell Preparation. PB was collected from the tail veins of the rats under light ether anesthesia after a period of 3 months, and then the animals were sacrificed by CO_2 asphyxiation. The spleen and femur were harvested separately. A total of 200 μL of the whole blood was transferred to a sterile 5 mL tube with 2 mL of ammonium lysing solution and mixed thoroughly. The blood tubes stood at room temperature for 10 min or until the suspension became clear and then were centrifuged for 10 min at 300*g*. The cell pellets were washed twice with phosphate-buffered saline (PBS) and centrifuged for 5 min at 300*g*. The cells were resuspended (approximately 2×10^7 cells/mL) in a PBS buffer for the following experiments. The excised spleen was cut into four to six parts and then homogenized with a homogenizer. BM cells were isolated from the femur, and then both ends of the bone were cut to expose the bone cavity. A 23 gauge needle attached to a 5 mL syringe filled with PBS was inserted into the cavity, and the marrow was flushed with PBS until it was completely evacuated. A single-cell suspension was created from spleens and BM cells by loading it through a cell strainer with 40 μm of nylon mesh (BD Falcon). The cell suspensions were washed twice with a PBS buffer and centrifuged for 10 min at 300*g*. The cells were resuspended (approximately 2×10^7 cells/mL) in a PBS buffer for the following experiments.

Assay of Hematological Markers and Biomarkers. We adopted an automated hematology analyzer (XE 2100, Sysmex Co., Japan) to analyze complete blood counts (CBCs), including the counts of red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs), the level of mean corpuscular volume (MCV), and percentages of neutrophils, lymphocytes, and monocytes.

Assay of GRx and GPx Activity. The whole blood was centrifuged at 500*g* for 5 min. The supernatant was removed, and the RBC pellet was remixed with 200 μL of PBS. Among the cell suspensions, 100 μL was assayed for the assay of the activities of GRx and GPx. In brief, a 100 μL RBC suspension was mixed with 0.1% Triton X-100 by a 1:2 volumetric ratio and then centrifuged at 500*g* for 10 min after being adequately mixed by a vortex mixer. The supernatant was assayed for the activities of GPx and GRx on an autoanalyzer (Synchron CX 7, Beckman Coulter, Brea, CA).

Assay of GSH. The 100 μL RBC suspension, as indicated above, in an amber microcentrifuged tube was mixed with 0.1% MPA by a 1:2 volumetric ratio, and then it was centrifuged at 500*g* for 10 min after being adequately mixed by a vortex mixer. The supernatant was assayed on a capillary electrophoresis analyzer (P/ACE MDQ, Beckman Coulter) after being filtered with a 0.2 μm syringe set. The analysis was performed at a constant temperature (28 °C) and 300 mM borate running buffer (pH 7.8) equipped with a UV absorbance detector set to 200 nm.

Assay of Lymphocyte Subpopulations. The BD FACSArray bioanalyzer (BD Biosciences) was used to analyze the lymphocyte subpopulations. The Rat T Cell Cocktail (BD Pharmingen, BD Biosciences) is a three-color reagent cocktail designed to identify T cells in rats, such as total T cells ($CD3^+$), T helper cells ($CD4^+$), and T suppressor/cytotoxic cells ($CD8^+$) by direct immunofluorescence staining with flow cytometric analysis. The Rat T/B/NK Cocktail (BD Pharmingen, BD Biosciences) is designed to identify rat T cells, B cells ($CD45RA^+$), and NK cells ($CD161_a^+$), and the Rat Lymphocyte Activation Cocktail (BD Pharmingen, BD Biosciences) is designed to identify activated lymphocytes, such as the α chain of the IL-2 receptor on T lymphoblasts and thymic and splenic dendritic cells ($CD25^+$) and non-polymorphic determinants of the Rat MHC class II antigens, I-A equivalent ($RT1B^+$). Each sample of 2 mL of PB, splenic cells, and BM cells was stained with 12 μL of cocktail antibodies and stood for 30 min at 4 °C in the dark. After incubation, the stained cells were washed twice with PBS and the 100−200 μL cell suspension was transferred to a 96-well round-bottomed microtiter plate and analyzed by a BD FACSArray bioanalyzer.

Statistical Analysis. Data were expressed as mean ± standard deviation (SD), and statistical results were analyzed with two-way analysis of variation (ANOVA) and Student's *t* test to assess the significant differences between the groups. The Pearson correlation coefficient was used to determine the correlation between the antioxidant status and lymphocyte subpopulations in the PB. Statistical analyses were performed using SPSS 17.0 software. $p < 0.05$ was considered statistically significant.

RESULTS

Effects of WPC on the Antioxidant Statuses of PB in the Rats. In this study, when the rats were supplemented with WPC in either the control group or alcohol group, their GSH levels of PB were

significantly increased (**Table 1**). In the alcohol group, there was higher GPx activity in the rats with WPC than those without WPC ($p < 0.05$); however, there were no significant differences of GRx activity between these two groups. In the control group, the activities of GPx and GRx were not significantly different, regardless of whether they had been supplemented with WPC. Furthermore, we found that there was an interaction effect in the GRx activity between WPC and alcohol.

Effects of WPC on the Hematological Markers of PB in the Rats. As shown in **Table 1**, in the control groups, the counts of WBC and the percentages of lymphocytes and mononuclear cells in the rats with WPC supplementation were higher than those without WPC supplementation; however, the percentages of neutrophils showed opposite results. In the alcohol groups, the MCV level in the rats with WPC supplementation was significantly lower than that without WPC supplementation. Furthermore, we found that there was an interaction effect in the PLT count between WPC and alcohol.

Effects of WPC on the Distributions of Lymphocyte Subpopulation in the PB, Spleen, and BM of the Rats with Excessive Alcohol Intake. To further investigate possible effects of WPC on the distributions of lymphocyte subpopulations in the rats with or without excessive alcohol intake, we assessed their percentages of lymphocyte subpopulations (T and B cells, NK cells, and activated T cells) in the PB, spleen, and BM. **Table 2** showed that the effects of WPC supplementation on the distributions of lymphocyte subpopulations in the PB were not significantly different in both groups. However, there were more $CD3^+$ T cells in the spleen of the alcohol group with WPC supplementation than those without WPC supplementation; on the contrary, there were fewer B cells in the spleen and BM of the alcohol group with WPC supplementation. Additionally, there were fewer NK cells in the BM of the alcohol group with WPC supplementation than those without WPC supplementation, and there were also fewer $CD25^+$ cells in the BM of the control group with WPC supplementation than those without WPC supplementation. Interestingly, in all of the groups, both $CD25^+$ cells in the PB and $RT1B^+$ cells in the spleen and BM were extremely low.

Correlations between the Antioxidant Statuses and the Distributions of Lymphocyte Subpopulations in the PB of the Rats. To investigate the relationship of the antioxidant statuses and the distributions of the lymphocyte subpopulations, which demonstrated significant correlations, as shown in **Figure 1**, the correlations between the activities of GPx and GRx as well as the level of GSH and the distributions of the lymphocyte subpopulations in the PB of the rats were analyzed (**Table 3**). In the control group without WPC supplementation, there was no significant correlation between the antioxidant statuses and the distributions of the lymphocyte subpopulations; however, when they were supplemented with WPC, their activities of GPx were positively correlated with the percentages of cytotoxic T cells (**Figure 1A**). In the alcohol group without WPC supplementation, the levels of GSH were positively correlated with the percentages of NK cells (**Figure 1B**); however, the relationship turned into a negative correlation when they were supplemented with WPC additionally (**Figure 1C**). Furthermore, the activities of GRx were positively correlated with the percentages of NK cells and cytotoxic T cells when they were supplemented with WPC (panels **D** and **E** of **Figure 1**).

DISCUSSION

In this study, the results showed that the supplementation of WPC could promote the antioxidant ability of the rats with an increase of the GSH level and elevate the alcohol-induced lower activities of antioxidant enzymes in the alcohol group. However,

Table 2. Effects of WPC Supplementation on the Distributions of Lymphocyte Subpopulations in the PB, Spleen, and BM of the Rats with Excessive Alcohol Intake[a]

	PB				spleen				BM			
	control		alcohol		control		alcohol		control		alcohol	
%	WPC (−)	WPC (+)	WPC (−)	WPC (+)	WPC (−)	WPC (+)	WPC (−)	WPC (+)	WPC (−)	WPC (+)	WPC (−)	WPC (+)
T cells ($CD3^+$)	74.7 ± 5.6	68.5 ± 2.8	73.6 ± 4.2	73.2 ± 3.3	46.7 ± 7.1	45.3 ± 5.6	47.2 ± 2.7	53.7 ± 4.0[b]	28.4 ± 5.9	25.5 ± 1.9	29.6 ± 4.8	26.6 ± 3.6
T helper cells ($CD4^+$)	48.4 ± 4.4	44.2 ± 1.6	46.7 ± 5.9	47.3 ± 4.6	31.9 ± 4.1	27.9 ± 3.1	31.1 ± 3.1	33.9 ± 2.3	4.6 ± 1.3	4.1 ± 1.0	6.6 ± 2.3	6.0 ± 2.1
T cytotoxic cells ($CD8^+$)	23.4 ± 3.5	23.7 ± 2.1	25.1 ± 1.9	24.4 ± 2.5	14.2 ± 3.8	14.5 ± 3.2	14.9 ± 1.5	18.3 ± 3.6	4.4 ± 1.1	3.1 ± 0.5	4.9 ± 1.7	3.8 ± 0.9
B cells ($CD45^+$)	12.9 ± 3.5	12.3 ± 1.8	13.2 ± 2.6	13.1 ± 2.3	35.5 ± 5.2	39.0 ± 3.9	34.3 ± 2.5	30.3 ± 3.6[b]	27.4 ± 7.0	18.9 ± 9.1	32.1 ± 4.1	18.1 ± 5.0[b]
NK cells ($CD161a^+$)	7.6 ± 2.3	6.7 ± 1.3	9.1 ± 2.8	5.9 ± 2.2	5.0 ± 1.5	5.4 ± 1.0	5.9 ± 1.7	6.6 ± 1.8	8.3 ± 1.3	6.9 ± 2.8	9.9 ± 2.9	6.6 ± 1.6[b]
activated T cells												
$CD25^+$ cells	<1	<1	<1	<1	35.3 ± 6.7	39.0 ± 4.1	34.7 ± 3.6	32.0 ± 2.5	5.2 ± 1.1	2.7 ± 1.0[c]	4.0 ± 1.2	4.1 ± 1.1
$RT1B^+$ cells	13.7 ± 2.5	14.9 ± 0.8	15.1 ± 3.4	11.7 ± 2.7	<1	<1	<1	<1	<1	<1	<1	<1

[a] Data are expressed as mean ± SD of six rats for each group. WPC, whey protein concentrate; WPC (−), rats without WPC supplementation; WPC (+), rats with WPC supplementation. [b] $p < 0.05$ compared to the alcohol group without WPC supplementation. [c] $p < 0.05$ compared to the control group without WPC supplementation.

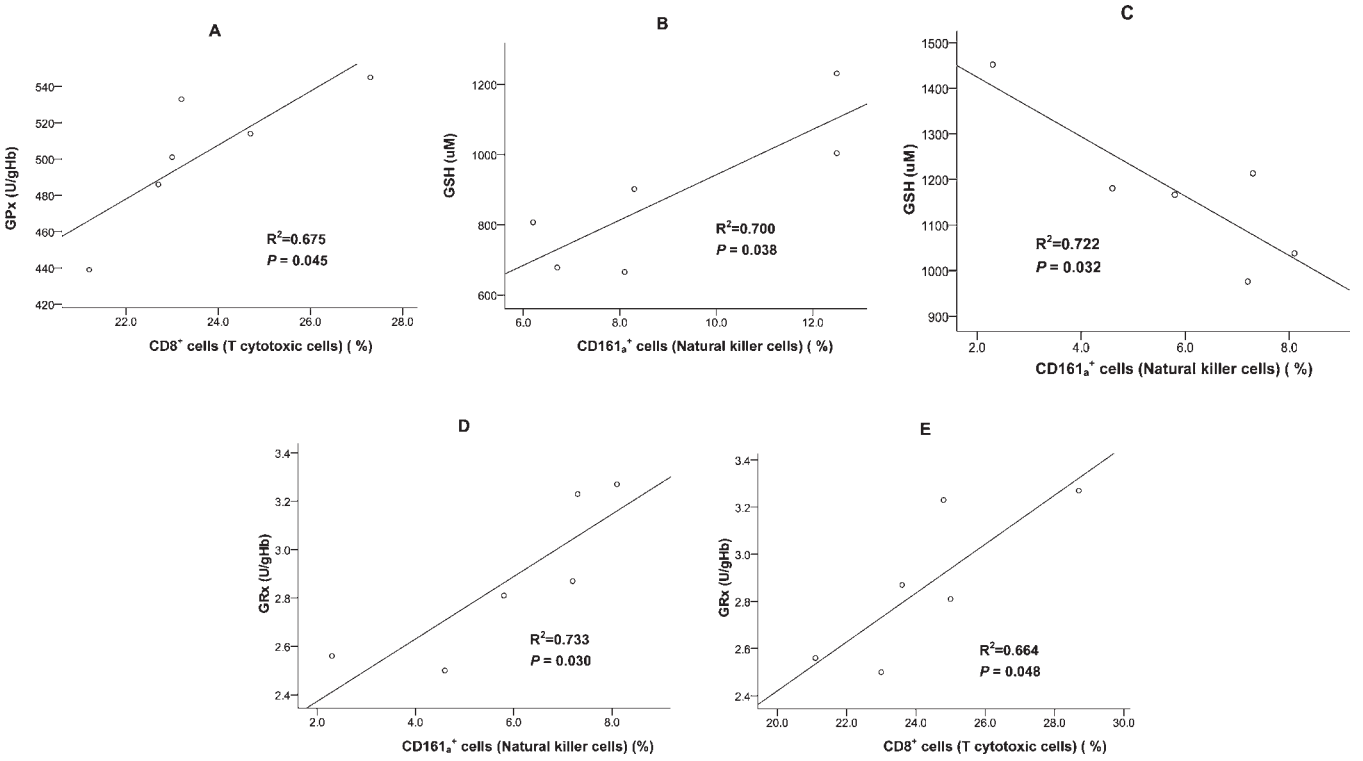

Figure 1. Significant correlations between the antioxidant statuses and the distributions of lymphocyte subpopulations in the PB of the rats in the (A) control group with WPC, (B) alcohol group without WPC, and (C, D, and E) alcohol group with WPC. WPC = whey protein concentrate.

whether the increased GSH levels attributed to the elevated activities of antioxidant enzymes requires further research to understand the related mechanisms. Additionally, the activity of antioxidant enzymes, such as GRx, was positively correlated with the percentages of NK cells in the rats supplemented with WPC. We also found that the effects of WPC on the distributions of lymphocyte subpopulations in the rats were not apparent in the control group but were apparent in the alcohol group, and the effects were mainly in the spleen and BM and not in the PB. To our knowledge, this is the first report to investigate the effects of WPC on the distributions of the lymphocyte subpopulations in the spleen and BM of rats with excessive alcohol intake and to describe the relationships between the antioxidant statuses and the distributions of the lymphocyte subpopulations in the PB of rats with excessive alcohol intake and WPC supplementation.

With regard to the effects of WPC, previous studies have indicated that the supplementation with WPC could modulate the immune response against pathogens and reduce the severity of virues-induced acute gastroenteritis (*18*, *23*). In this study, we observed that the counts of WBC were elevated when the rats were supplemented with WPC in the control group. We also found that the higher counts of WBC might be attributed to the elevated counts of lymphocytes and originated from immature lymphocytes derived from hematopoietic organs. In addition, the higher MCV level in the alcohol group was decreased when the rats were supplemented with WPC. These results could demonstrate that the effects of WPC on the MCV were apparent in the rats with excessive alcohol intake. Previous studies have indicated that excessive alcohol and its metabolites could suppress the proliferation of hematopoietic progenitor cells (*24*) and alter the structures of erythrocyte constituents in heavy drinkers (*25*, *26*). A cytotoxic effect of alcohol on erythropoiesis was supported by the occurrence of erythroblast vacuolization and reticulocytosis after heavy alcohol intake (*27*). Moreover, the study of Ballard indicated that the indirect effects of alcohol, including nutritional deficiencies, impaired the production and functions of various blood cells (*1*). In addition, Szabo also indicated that alcohol could inhibit the functions of the hematological cells that ingested and destroyed invading microorganisms, i.e., neutrophils, monocytes, and macrophages, and adversely mediated the immune response against specific microorganisms (*10*). As a result, alcoholics have an increased susceptibility to diseases caused by bacterial or viral infections.

Diaz-Romero et al. indicated that measurement of the T-cell subpopulations ($CD4^+$ and $CD8^+$ cells) was a useful marker to evaluate the immune status (*11*). Furthermore, the activated T cells, such as interleukin-2 receptor cells ($CD25^+$ cells) and antigen presenting cells (APCs), played important roles in initiating the immune response to antigens (*28*, *29*). Besides, the NK cells are a subset of lymphocytes that are capable of killing tumor cells and virus-infected cells (*30*). Thus, it is important to analyze the lymphocyte subpopulations for the evaluation of immunological malfunctions derived from alcohol. In this study, we found that the supplementation of WPC resulted in higher T cells in the spleen but lower B cells in the spleen and BM and NK cells in the BM of the alcohol group. However, the distribution of lymphocyte subpopulations in the PB was not significantly different in both groups. Our finding was similar to previous studies (*31*−*34*), which indicated that the lower proportions of activated T cells in the BM reflected that the primary hematopoietic organ, such as BM, might be affected by the treatment of excessive alcohol or other substances. Furthermore, they have shown that infectious diseases were the most common causes of death in alcoholics. In addition, alcohol abuse is considered to be an important factor that interferes with the immune surveillance system of the host, as shown by an increased incidence of disease in animal and human studies (*32*, *34*). Moreover, the study by Helm et al. indicated that alcohol exposure was implicated in atrophy of the spleen and thymus, as well as an abnormal distribution of PB leukocytes (*33*). In this study, although the effects of WPC on the distribution of

Table 3. Correlations between the Antioxidant Statuses and the Distributions of Lymphocyte Subpopulations in the PB of the Rats[a]

	control group						alcohol group					
	WPC (−)			WPC (+)			WPC (−)			WPC (+)		
	GSH	GPx	GRx	GSH	GPx	GRx	GSH	GPx	GRx	GSH	GPx	GRx
$CD3^+$ cells	−0.245	0.738	0.160	−0.215	0.722	0.268	0.217	0.056	−0.325	−0.032	−0.330	0.161
$CD4^+$ cells	0.116	0.655	0.021	−0.723	0.000	−0.486	0.225	0.406	0.147	0.353	−0.355	−0.414
$CD8^+$ cells	−0.568	0.684	0.216	0.118	0.821[b]	0.680	−0.218	−0.347	−0.796	−0.637	0.383	0.815[b]
$CD45^+$ cells	0.073	−0.587	0.049	0.101	−0.719	−0.152	−0.475	−0.262	0.216	−0.485	0.329	0.043
$CD161a^+$ cells	0.534	−0.493	0.768	0.242	0.286	0.468	0.837[b]	−0.254	0.135	−0.850[b]	0.378	0.856[b]
$RT1B^+$ cells	0.300	−0.416	−0.049	−0.603	0.147	0.098	−0.127	0.556	0.021	−0.048	0.010	−0.029

[a] Data was expressed as the Pearson correlation coefficient of six rats for each group. WPC, whey protein concentrate; WPC (−), rats without WPC supplementation; WPC (+), rats with WPC supplementation. [b] Correlations were considered significant at $p < 0.05$.

lymphocyte subpopulations in the PB of the rats with alcohol intake were not apparent, the effects in the spleen and BM were significant; therefore, we suggest that the effect of WPC on the lymphocyte subpopulations was mainly in the BM and spleen and not in the PB. Therefore, whether the homeostasis of lymphocyte subpopulations of PB originated from the shift of the spleen and BM when they were supplemented with WPC will be a valuable topic to explore in the future.

In this study, the activity of GPx was decreased after excessive alcohol intake, which was consistent with the study by Afifi et al. (*35*). Afifi et al. indicated that the GSH level and the activity of GPx were significantly decreased in the mice treated with alcohol; therefore, the antioxidant ability of rats might be elevated if they were supplemented with WPC. Besides, our previous studies also indicated that the supplementation of WPC in various cells, including PC12 cells and PBMCs, could promote the antioxidant ability by GSH synthesis or elevated activities of antioxidant enzymes (*19*, *20*).

Interestingly, although the relationship between the antioxidant statuses and distributions of the lymphocyte subpopulations was not apparent in the control group, the positive correlation between the levels of GSH and the percentages of NK cells in rats with alcohol intake showed that the immunity of rats treated with alcohol might be elevated if they were supplemented with antioxidants. In addition, we found that there were positive correlations between the activities of GRx and the percentages of NK cells as well as cytotoxic T cells in the alcohol group when they were supplemented with WPC. These findings showed that the immunity of rats with excessive alcohol intake might be enhanced by the supplementation with WPC because of the increase of the percentages of NK cells and cytotoxic T cells; however, the relationships were not apparent in normal situations. With the above-mentioned results, we found that the effect of WPC on the distributions of lymphocyte subpopulations in a normal condition was not apparent, but it was apparent, except in the PB, when the rats had excessive alcohol intake. Besides, we also found that there were opposite correlations between the levels of GSH and the percentages of NK cells in the alcohol group when supplemented with WPC. These data suggest that supplementation with WPC might have different effects on the distributions of lymphocyte subpopulations in different organs in alcoholics.

In this study, our results indicated that the supplementation of WPC could promote the antioxidant ability of the rats with an increase of the GSH level, and we also found that the activity of the antioxidant enzyme, such as GRx, was positively correlated with the percentages of NK cells in the rats supplemented with WPC. In addition, the effects of WPC on the distributions of lymphocyte subpopulations were apparent in the alcohol group and were mainly in the spleen and BM and not in the PB; on the contrary, the effect was not apparent in the control group.

ABBREVIATIONS USED

WPC, whey protein concentrate; PB, peripheral blood; BM, bone marrow; GSH, glutathione; GPx, glutathione peroxidase; GRx, glutathione reductase; PBMC, peripheral blood mononuclear cell; NK, natural killer; SD, Sprague−Dawley; CBC, complete blood count; RBC, red blood cell; WBC, white blood cell; PLT, platelet; MCV, mean corpuscular volume.

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