PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 174, 392–400 (1983)

Acute Hepatotoxin Exposure Effects Lymphoid and Accessory Cell Types in Inbred Mice (41753)

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Abstract. The effect of acute hepatotoxin exposure on *in vivo* and *in vitro* immune responses were investigated in inbred mice. Splenic anti-SRBC PFC responses were slightly enhanced by carbon tetrachloride or galactosamine administration 5 hr prior to immunization. Whereas splenic anti-SRBC PFC responses were slightly enhanced in euthymic mice exposed to carbon tetrachloride 5 hr prior to immunization, immune responses to the TI antigens, Fl-LPS, Fl-Ficoll, and TNP-LPS, were significantly suppressed. Athymic mice receiving similar hepatotoxin exposure elicited enhanced immune responses to the TI immunogens, thereby suggesting that the activities of B cells and macrophages are enhanced in treated animals and in euthymic mice, T suppressor cells are also activated. By admixture of purified B- and T-cell and macrophage populations from either carbon tetrachloride-treated or control animals, it was demonstrated that hepatotoxin exposure also induces suppressor T cells regulating immune responses to the T-dependent antigen, SRBC, and that macrophages from treated animals are more functional. Further, B-cell responsiveness is enhanced. In addition to these observations, an active factor could be demonstrated in sera from hepatotoxin-treated animals which augments immune responses to SRBC in normal mice and promotes immune responses to this antigen in athymic mice. These findings indicate that the effects of acute hepatotoxin exposure are multifocal, influencing the activity of lymphoid and accessory cells.

Exposure of animals to hepatotoxins, such as carbon tetrachloride, has commonly been used as an experimental model for studying liver disease. Such treatment can lead to hepatic injury (1, 2), depressed Kupffer cell function (3), and certain immunoregulatory effects commonly associated with liver disease (4–6). These include increased suppressor cell activity (7), diminished cell-mediated immunity (7), hypergammaglobulinemia (8, 9), and adjuvanticity (10, 11). All of these effects have been attributed to an increased antigenic stimulation of splenic lymphoid tissue which is believed to occur due to an inability of the injured liver to filter out intestinally derived substances in the blood. In addition, adjuvant effects associated with hepatic injury have also been explained on the basis of a general increased reactivity of the immune system (12). In support of this latter theory, many substances in the intestine, such as bacterial lipopolysaccharide, are known to stimulate lymphoid cells and can cause adjuvant effects (13). Thus, impaired clearance of these substances would contribute to their persistence in the circulation and subsequent activation of the immune system.

The plurality of effects manifested following hepatic injury obviously reflects the complex nature of the processes which occur. Thus, the present investigation was undertaken to elucidate the lymphoid cell populations influenced by acute hepatotoxin exposure. In this communication, we present evidence that carbon tetrachloride and galactosamine can influence the activity of three lymphoid and accessory cell types in the spleen, namely the T and B lymphocytes and macrophage. Further, a humoral factor can be demonstrated in hepatotoxin-treated animals which augments immune responses to T-dependent antigens in normal animals.

Materials and Methods. *Mice.* Seven- to twelve-week-old lipid A-responsive C3H/HeN (original breeding colony from the National Institutes of Health, Bethesda, Md.), Balb/c AnN and athymic Balb/c AnN (nu/nu) (original breeding colony from the National Cancer Institute, National Institutes of Health, Be-

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0037-9727/83 $1.50

thesda, Md.) mice were used in the experiments.

Antigens. Sheep erythrocytes (SRBC)[2] were obtained from the Veterinary Research Farm, University of Illinois, Urbana, Illinois. *Escherichia coli* K235 LPS was extracted by a phenol–water procedure (14) and portions were substituted with either TNP (15) or Fluorescein (Fl). Fluorescein conjugation of LPS was performed by the method described by Lopatin and Voss (16) with modifications. Equal weights of LPS and FITC (isomer I) (Molecular Probes Inc., Plano, Tex.) were mixed in distilled water to which an equal weight of K_2CO_3 was added. This mixture was allowed to react 16 hr at room temperature and subsequently dialyzed against 0.1 *M* phosphate buffer, pH 8.0. Fl-Ficoll was prepared using AECM-Ficoll (Biosearch, San Rafael, Calif.) according to the method described by Inman (17).

Lightly substituted TNP-SRBC, used for the hemolytic plaque assay, were prepared as described previously (18). Fl-SRBC were obtained by mixing FITC with freshly washed SRBC in 0.05 M sodium carbonate buffer, pH 9.5, for 1 hr at room temperature (1 mg FITC/1 ml SRBC). Treated cells were subsequently washed free of unbound FITC prior to use.

Hepatotoxins. Mice were injected subcutaneously with 0.1 ml of a mixture of carbon tetrachloride (Fisher Scientific Co., Fair Lawn, N.J.) in olive oil (Humco Laboratory, Texarkana, Tex.) at a concentration of 0.33 ml carbon tetrachloride/ml solution. D-galactosamine hydrochloride, grade I (Sigma Chemical Co., St. Louis, Mo.) was dissolved in pyrogen-free PBS, filter-sterilized, and administered intravenously at a concentration of 10 mg/mouse.

In vivo immune responses. Mice were immunized intraperitoneally with either 4×10^8 SRBC in 0.2 ml PBS or various doses of TNP-LPS, Fl-LPS, or Fl-Ficoll. Direct splenic anti-SRBC or anti-hapten PFC responses were determined on appropriately diluted spleen cell suspensions employing a slide modification of the Jerne and Nordin hemolysis in gel method (19). Immunized mice were sacrificed on Day 4 for anti-SRBC PFC and on Day 3 for anti-TNP or anti-Fl PFC (during peak PFC responses, unpublished observations). Specific anti-TNP or anti-Fl PFC were assessed utilizing TNP-SRBC or Fl-SRBC, respectively, when background anti-SRBC PFC had been subtracted.

Purification of splenic lymphoid cell populations. Macrophage-depleted spleen cell preparations were prepared from carbon tetrachloride- or saline-treated mice as described previously (20) by consecutive passage through Sephadex G-10 columns (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N.J.). Effluent cells were used as the source of nonadherent lymphocytes (21).

Purified splenic T cells from mice injected 5 hr previously with saline or carbon tetrachloride were prepared from spleen cell suspensions by column fractionation (21, 22) and further enriched by treatment with anti-mouse Ig serum (a generous gift provided by Dr. E. W. Voss and Dr. D. Kranz, University of Illinois, Urbana, Ill.) and complement (23).

T-cell-depleted spleens (B cells and macrophages) from control and carbon tetrachloride-treated mice were prepared by treatment with an optimal dilution of rabbit anti-mouse thymocyte serum (Microbiological Associates, Walkersville, Md.) and complement (23). Cells prepared in this manner did not respond to either PHA or Con A (data not shown). Macrophage-enriched populations were prepared by adherence (21).

In vitro immune responses. Spleen cells from normal mice or mice treated 5 hr previously with carbon tetrachloride or D-galactosamine were cultured in 16-mm multiwell trays (Linbro Chemical Co., Hamden, Conn.) by the method of Mishell *et al.* (19) with modifications (15). The constituents of the serum containing culture medium employed in these studies have been described previously (15, 19). Spleen cells were suspended at a concentration of 5×10^6 per 0.5 ml complete medium per well (19). After the addition of SRBC ($1–2 \times 10^6$/culture), trays were incubated (37°C) with rocking in a humid chamber containing an atmosphere of 7% O_2, 10% CO_2, and 83%

[2] Abbreviations used: SRBC, sheep erythrocytes; LPS, lipopolysaccharide; PFC, plaque forming cell; TNP, trinitrophenyl; Fl, fluorescein; TI, T independent; FITC, fluorescein isothiocyanate; TD, T dependent; IL-1, Interleukin 1; PHA, phytohemagglutinin; Con A, concanavalin A: MØ, macrophage; TRF, T-cell replacing factor; FCS, fetal calf serum.

N_2. Cultures were fed daily with a nutritional cocktail (19) and terminated on Day 5 for assessment of anti-SRBC PFC responses as described above.

For cell mixing experiments, 5×10^6 T-cell-depleted spleen cells (from control animals or mice treated with carbon tetrachloride 5 hr previously) were mixed with 3×10^6 purified splenic T cells (from treated or control mice) and cultured *in vitro* as described above. In other experiments, *in vitro* cultures were prepared by addition of 3×10^6 splenic T and B lymphocytes to adherent cell populations. Direct anti-SRBC PFC were subsequently assessed on Day 5.

In vivo serum transfer. Five hours after injection of carbon tetrachloride, D-galactosamine, or saline, serum was obtained from mice by retroorbital bleeding, pooled, and heat-inactivated at 56°C for 30 min. These sera were injected intraperitoneally into mice (0.3 ml/mouse) along with 4×10^8 SRBC. Direct splenic anti-SRBC PFC were assessed on Day 4.

Irradiation. Spleen cells were pelleted by centrifugation and irradiated with 1500 rad (710 rad/min) using a ^{137}Cs Mark I Model 22 Irradiator (J. L. Sheperd and Associates, Glendale, Calif.) (21).

Statistics. Results are expressed as the mean PFC response per culture ± standard error.

Results. *Adjuvant effects of carbon tetrachloride on in vivo immune responses to sheep erythrocytes.* Previous studies demonstrated that administration of carbon tetrachloride to animals could enhance serum anti-SRBC levels (10, 11). These adjuvant effects were obtained when the hepatotoxin was given several hours prior to immunization and subsequently at specific intervals throughout the course of the response. The present investigation has substantiated these findings employing the hemolytic plaque assay, Table I. Animals treated with carbon tetrachloride beginning 5 hr prior to immunization with SRBC displayed elevated direct splenic anti-SRBC PFC responses when compared to those of saline-treated control animals. Under these experimental conditions enhancement was approximately 180% of control responses. Thus, this data indicates that carbon tetrachloride, when administered prior to immunization, can induce adjuvant effects on TD responses albeit of limited magnitude.

Immunomodulating effect of carbon tetrachloride on in vivo immune responses to TI antigens in euthymic and athymic mice. Whereas acute carbon tetrachloride exposure enhanced anti-SRBC PFC responses, its effects on host responsiveness to TI antigens was undefined. Therefore, animals treated with the hepatotoxin 5 hr previously were immunized with various TI immunogens. In euthymic mice, suppression rather than enhancement was observed, see Table I. The suppressive effects were not restricted to hapten specificity since similar results were observed using Fl-LPS or TNP-LPS. Further, suppression was

TABLE I. IMMUNOMODULATING EFFECTS OF CARBON TETRACHLORIDE TREATMENT ON *IN VIVO* IMMUNE RESPONSES TO ANTIGENS IN EUTHYMIC AND ATHYMIC MICE[a]

	Euthymic mice		Athymic mice	
Immunogen	Saline treated[b]	Carbon tetrachloride treated[b]	Saline treated[b]	Carbon tetrachloride treated[b]
SRBC	700 ± 33[c]	1129 ± 63 ($P \leq 0.05$)	123 ± 30	160 ± 20
Fl-LPS	1243 ± 67	673 ± 60 ($P \leq 0.01$)	1617 ± 174	2553 ± 563 ($P \leq 0.05$)
TNP-LPS	1314 ± 69	1001 ± 75 ($P \leq 0.05$)	N.D.[d]	N.D.
Fl-Ficoll	530 ± 16	452 ± 6 ($P \leq 0.05$)	631 ± 12	842 ± 30 ($P \leq 0.05$)

[a] Antigens were given ip at the following concentrations: SRBC, 4×10^8; Fl-LPS, 1.0 μg/mouse; TNP-LPS, 1.0 μg/mouse; Fl-Ficoll, 2.0 μg/mouse.

[b] Balb/c AnN mice were given carbon tetrachloride or saline subcutaneously 5 hr before immunogen challenge.

[c] Splenic anti-SRBC and anti-hapten responses were determined on Days 4 and 3, respectively. Values represent the mean PFC per 10^6 spleen cells from three separate experiments using three to six mice per experimental group. Anti-hapten PFC in carbon tetrachloride-treated mice only (no immunogen) were 42 ± 4 for Fl and 10 ± 2 for TNP; anti-SRBC PFC in treated mice only (no SRBC immunization) was 0 ± 0.

[d] Not determined.

TABLE II. EFFECT OF GALACTOSAMINE TREATMENT ON IMMUNE RESPONSES TO SHEEP ERYTHROCYTES

Group	Treatment[a]	*In vivo*[a] Anti-SRBC/10^6 spleen cells	*In vitro*[b] Anti-SRBC PFC/culture
A	Saline only −5 hr	756 ± 32	475 ± 95
B	Galactosamine −5 hr	1085 ± 61 ($P \leq 0.01$)	767 ± 96 ($P \leq 0.05$)

[a] Galactosamine (500 mg/kg) was administered iv into Balb/c AnN mice at specified times relative to immunization. Mice were immunized as detailed in Table I. *In vivo* responses were determined as detailed in Table I.

[b] Galactosamine was administered iv as described in footnote *a* at times relative to animal sacrifice. Spleen cell cultures (5×10^6/well) were incubated with $1\text{–}2 \times 10^6$ SRBC. *In vitro* anti-SRBC PFC in cultures without antigen, i.e., background ranged from 25–40. Values are expressed as the mean of triplicate cultures per experiment when background PFC were subtracted ± 1 SEM. A minimum of three experiments were performed per assay group. Anti-SRBC PFC responses of spleen cells cultured with galactosamine concentrations ranging from 0.01 mg to 1 mg per culture were equal to or less than control values (52 ± 13).

observed with different carriers, i.e., Fl-LPS and Fl-Ficoll.

In the absence of functional T cells, i.e., congenitally athymic nude mice, suppression was not evident. In fact, enhancement of the anti-Fl PFC response was observed (Table I). Therefore, these findings indicate that acute carbon tetrachloride exposure induces a population of suppressor T cells which regulate immune responses to TI antigens. Further, the response pattern in athymic mice suggests that the hepatotoxin treatment results in a hyperreactivity of the B cell and/or the macrophage.

Effect of D-galactosamine on in vivo and in vitro immune responses to sheep erythrocytes. Since carbon tetrachloride treatment caused immunomodulatory effects, it was important to establish whether another hepatotoxin, such as galactosamine, acted similarly. Using injection regimens similar to those used for carbon tetrachloride, *in vivo* anti-SRBC PFC responses could be modulated with galactosamine treatment. These findings were substantiated *in vitro,* see Table II. Spleen cell cultures prepared from mice which had received a single dose of galactosamine 5 hr previously elicited anti-SRBC PFC responses that were approximately 160% higher than control animals. Thus, immunomodulating effects could be induced by two different hepatotoxins and could be demonstrated *in vivo* as well as *in vitro.*

Characterization of lymphoid and accessory cells responsible for carbon tetrachloride-induced immunoregulation. The *in vivo* findings using euthymic and athymic mice implied that acute hepatotoxin treatment influenced not only a T-cell population but possibly B-cell and/or macrophage activity. Therefore, a series of experiments was performed to assess the effect of cell source on subsequent *in vitro* immune responses to sheep erythrocytes. In these studies, purified lymphoid cell populations were prepared from mice which had received a single injection of either saline or carbon tetrachloride 5 hr prior to sacrifice. As indicated in Table III, anti-SRBC PFC responses were greater in cultures containing all cell types derived from carbon tetrachloride-treated animals when compared to those in cultures containing all control lymphoid cell types. Moreover, enhancement was greatest in cultures containing control T cells and T-cell-depleted splenocytes from hepatotoxin-treated mice. In contrast, the addition of T cells from carbon tetrachloride-treated animals to control B cells and macrophages resulted in suppressed anti-SRBC PFC responses. Thus, these findings corraborated the *in vivo* observations and indicated that suppressor T cells regulating immune responses to a T-dependent antigen are induced after acute carbon tetrachloride exposure. Further, the hyperreactivity of the B cell and/or macrophage was evident *in vitro.*

Due to the hyperreactivity of the macrophage and/or the B cell that was evident in the previous studies as well as the known role of the macrophage in adjuvant effects (21, 24), a second series of experiments was performed to investigate macrophage function. In these studies, splenic adherent cells from either carbon tetrachloride-treated (5 hr prior to sac-

TABLE III. DEMONSTRATION OF GREATER B CELL AND/OR MACROPHAGE REACTIVITY AND INCREASED SUPPRESSOR T-CELL ACTIVITY IN CARBON TETRACHLORIDE-TREATED MICE

	Anti-SRBC PFC/culture[a] (source of splenic B cell + MØ)[b]	
Source of splenic T cell[c]	Saline treated	Carbon tetrachloride treated
Saline treated	358 ± 108	877 ± 131 ($P \leq 0.001$)[d]
Carbon tetrachloride treated	85 ± 0 ($P \leq 0.001$)[d]	717 ± 48 ($P \leq 0.05$)[d]

[a] Five-day anti-SRBC PFC/culture when background PFC are subtracted; mean ± 1 SEM. Anti-SRBC PFC responses of spleen cells cultured with carbon tetrachloride concentrations ranging from 0.005 to 3 μl/culture were less than control values.

[b] C3H/HeN spleen cell cultures (prepared from five to six animals treated with saline or carbon tetrachloride 5 hr prior to sacrifice) were T-cell depleted with anti-thymocyte plus complement treatment; cell concentration was adjusted to 5×10^6/culture. No anti-SRBC PFC were detected in T-cell-depleted spleen cultures from either saline or hepatotoxin-treated mice.

[c] C3H/HeN splenic T cells (prepared from five to six mice treated with saline or carbon tetrachloride 5 hr prior to sacrifice) were purified by passage over glass and nylon-wool columns followed by anti-Ig plus complement treatment and used at a cell concentration of 3×10^6/culture. No anti-SRBC PFC were detected in either purified T-cell preparation.

[d] Statistical significance to saline control.

rifice) or control mice were cultured with Sephadex G-10 passed T and B lymphocytes (from either hepatotoxin-treated or control mice) and *in vitro* anti-SRBC PFC were assessed, see Table IV. Cultures containing macrophages from hepatotoxin treated mice elicited greater anti-SRBC PFC responses than those with control macrophages. In contrast, T- and B-cell source had little influence on the magnitude of the adjuvant effect. Thus, these findings imply that macrophages are more functional as the result of the hepatotoxin treatment and can cooperate equally well with T and B cells from either hepatotoxin-treated or control animals.

Adjuvant effect of sera from acute hepatotoxin treated mice on in vivo immune responses of normal mice. Activated macrophages as well as T cells are known to secrete soluble factors which have immunoregulatory effects. In this regard, recent studies from several laboratories have demonstrated that adjuvant effects may be mediated by soluble (humoral) factors (24, 25). Thus, the ability of sera from 5-hr hepatotoxin-treated mice to alter *in vivo* anti-SRBC PFC responses of normal untreated mice was assessed. As can be seen in Table V, heat-inactivated sera from either carbon tetrachloride- or galactosamine-treated animals enhanced anti-SRBC PFC responses ap-

TABLE IV. DEMONSTRATION OF GREATER MACROPHAGE REACTIVITY IN CARBON TETRACHLORIDE-TREATED MICE IRRESPECTIVE OF T- AND B-CELL SOURCE

	Anti-SRBC PFC/culture[a]	
	Source of splenic MØ[b]	
Source of splenic B + T cells[c]	Saline treated	Carbon tetrachloride treated
Saline treated	449 ± 74	747 ± 128 ($P \leq 0.05$)[d]
Carbon tetrachloride treated	297 ± 62 ($P \leq 0.05$)[d]	809 ± 133 ($P \leq 0.05$)[d]

[a] Five-day anti-SRBC PFC/culture when background PFC are subtracted; mean ± 1 SEM.

[b] C3H/HeN splenic macrophage cultures were prepared from mice treated 5 hr prior to sacrifice by adherence of irradiated (1500 rad) splenic cells for 2–3 hr at 37°C. No anti-SRBC PFC were detected in purified macrophage cultures from either saline- or hepatotoxin-treated mice.

[c] C3H/HeN spleen cell cultures from mice treated 5 hr prior to sacrifice were depleted of macrophages by twice passage of cells through a Sephadex G-10 column. No anti-SRBC PFC were detected in B + T-cell cultures from either saline- or hepatotoxin-treated mice.

[d] Statistical significance to saline control.

TABLE V. ADJUVANT EFFECT OF SERUM FROM HEPATOTOXIN-TREATED MICE ON *IN VIVO* IMMUNE RESPONSES OF NORMAL MICE

Group	Treatment[a]	Splenic anti-SRBC PFC/10^6 Spleen cells[b]	
		Euthymic mice	Athymic mice
A	SRBC + saline-serum	419 ± 41	187 ± 28
B	SRBC + carbon tetrachloride-serum	602 ± 18 ($P \leq 0.01$)	723 ± 56 ($P \leq 0.001$)
C	SRBC + saline-serum	698 ± 10	
D	SRBC + galactosamine-serum	1085 ± 61 ($P \leq 0.005$)	N.D.[c]

[a] Sera (0.3 ml) from control animals (Balb/c AnN) or mice exposed to hepatotoxins 5 hr previously were collected, pooled and heat-inactivated, mixed with 4×10^8 SRBC, and injected ip.
[b] Splenic anti-SRBC PFC were determined on Day 4 and values represent mean ± 1 SEM.
[c] Not determined.

proximately 150% in euthymic mice. Further, sera from carbon tetrachloride-treated mice promoted *in vivo* anti-SRBC PFC responses in athymic nude mice. Thus, these results suggest that an initial exposure to hepatotoxin causes the release of humoral factors which augment immune responses to T-dependent antigens.

Discussion. Immunological disorders associated with liver injury are well documented, however, the cellular basis and underlying mechanisms involved are poorly understood. Thus, a systematic investigation was begun to define these effects. In this study, a regimen for hepatotoxin administration was selected similar to one which had previously been shown to enhance immune responses to heterologous erythrocytes (10). This treatment results in mild inflammation, hepatic lipid accumulation, minimal hepatic necrosis, and reticuloendothelial proliferation (2). Therefore, the studies reported in this communication are designed to elucidate immunomodulatory effects of acute rather than chronic hepatic injury.

Data has been provided by several investigations including the study described in this communication which indicates that acute hepatotoxin exposure can modulate *in vivo* immune responses (10, 11, 26, 27). Immunization regimens, routes of hepatotoxin administration, duration of exposure, and animal species can all affect the magnitude and type of modulation that occurs. If multiple lymphoid cell populations are activated, variable effects may also be manifested. Immunoregulation by substances, such as LPS, are dependent on the net effect of both positive and negative regulatory cells (28). Thus, the limited adjuvant effects observed in this investigation could be attributed to an induction of several cell types. This gains additional support from the *in vitro* experiments in which adjuvant effects were greater in cultures containing normal T cells and T-cell-depleted spleen cells from carbon tetrachloride-treated mice (Table III). Further, it is interesting to note that the levels of enhancement (150–200%) are of the same magnitude as the hypergammaglobulinemia that is observed in clinical conditions (4).

Acute carbon tetrachloride exposure influenced the activity of all three lymphoid and accessory cell types, namely the T and B lymphocytes as well as the macrophage. The most evident effect on T-cell activity is the induction of suppressor cells which regulate immune responses to TI as well as TD antigens. The enhanced *in vivo* immune responses in athymic mice to Fl-LPS and Fl-Ficoll relative to euthymic mice (Table I) and the *in vitro* cell mixing experiments (Table IV) suggest that suppression is, in fact, mediated by T lymphocytes. However, from the results reported here, it cannot be determined whether the same population of suppressor T cells governs immune responses to both TI and TD antigens. The action of these suppressor cells may also partially explain a discrepancy between the present study and the findings of others regarding hepatotoxin-induced effects on TI immune responses. Thomas (7) reported that rats chronically treated with phenobarbitone and carbon tetrachloride elicited enhanced

antibody responses to the TI antigen flagellin, when compared to control animals. Whereas this may be ascribed to differences between the duration of hepatotoxin exposure, antigen composition may equally be important. Exposure of germfree animals to environmental antigens induces a population of T cells which suppress host immune responsiveness to haptenated LPS (29). Thus, the discrepancy between the two studies may represent a carrier effect.

Several observations indicate an influence on the B cell. In the case of TD antigens, responses of normal B cells appear to be more susceptible to T-cell suppression than B cells from carbon tetrachloride-treated animals (Table III). Further, B cells from hepatotoxin-treated animals elicited enhanced *in vitro* anti-SRBC PFC responses when cultured with normal T cells. An explanation for this may involve the level of B-cell maturation. Several studies have demonstrated that biologically active substances such as LPS (30) or soluble mediators (IL-1) (31) induce B cell differentiation and upon maturity the cells cooperate more readily with helper T cells. Thus, acute hepatotoxin exposure may stimulate the B cells to differentiate into more mature states. Studies are currently in progress to substantiate this hypothesis.

Stimulation of macrophages by antigenic substances is known to result in the subsequent release of soluble immune factors which mediate numerous immune reactions including adjuvanticity (24). In this study adjuvant effects were observed following a single dose of hepatotoxin prior to antigen challenge. This phenomenon can be explained in part by a more functional macrophage. *In vitro* anti-SRBC PFC responses were greatest when cultures contained macrophages from hepatotoxin-treated mice (Table IV). T- and B-cell source was less critical for this enhancement.

Of particular interest to this investigation was the demonstration that sera from hepatotoxin treated mice augmented immune responses in normal mice to TD antigens. Several lines of evidence indicate that the adjuvanticity is not likely due to residual hepatotoxin present in the serum. The first is that carbon tetrachloride and galactosamine are rapidly cleared from the blood by the liver (32, 33). Second, spleen cells cultured *in vitro* with various concentrations of either hepatotoxin and sheep erythrocytes yielded anti-SRBC responses which were equal to or less than control cultures (Tables II and III). Last, this factor facilitated athymic mice to respond to a T-dependent antigen, a property very unlikely for a simple organic compound. Whereas the activity of this humoral factor could account for the increased B-cell reactivity of hepatotoxin-treated animals, further studies will be required for its characterization and mode of action. Thus, a serum factor is implicated which appears to be very similar to those reported to be induced by LPS (34, 35), to IL-1 (36), and to the T-cell product, TRF (37). The effects of acute hepatotoxin exposure are clearly multifocal. Lymphoid and accessory cells are influenced resulting in suppressed as well as enhanced expressions. The adjuvant effects do not appear to be due to polyclonal activation. Carbon tetrachloride exposure alone does not facilitate the induction of anti-SRBC PFC responses in immunized athymic mice nor in immunized T-cell-depleted spleen cell cultures. Further, anti-SRBC PFC responses are not observed with spleen cells from hepatotoxin-treated mice when cultured *in vitro* without antigen. It is important to note that the effects described in this communication are those associated with acute hepatic injury. The demonstration of adjuvant effects *in vitro* provides direct evidence that hepatotoxin exposure activates the immune system. This is manifested at the T- and B-cell as well as the macrophage level. Currently, it is unknown how long these effects persist as well as their role in the immunological disorders associated with chronic liver disease. However, further characterization of these cell effects using this animal model will contribute to the understanding of the immunological events associated with hepatic injury.

This work was supported by a VA Medical Research Service grant. We thank Dr. E. W. Voss, Jr., Dr. J. R. McGhee, and Dr. J. A. Rudbach for critical assessment of this work. Our thanks also to J. Worthington, B. Quinlan, R. Wiegand, R. Lawlyes, and M. Shawchuck for their contribution to this research effort.

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Received April 11, 1983. P.S.E.B.M. 1983, Vol. 174.