Studies of Immune Functions of Patients with Chronic Hepatitis

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NONOMURA, A., TANINO, M., KURUMAYA, H., OHTA, G., KATO, Y. and KOBAYASHI, K. *Studies of Immune Functions of Patients with Chronic Hepatitis*. Tohoku J. exp. Med., 1982, **137** (2), 163-177 — Peripheral T cells from patients with chronic active hepatitis (CAH) showed a significantly decreased suppressor effect (or increased helper effect) on allogeneic B cell differentiation into Ig-producing cells (Ig-PC) ($p < 0.05$). After irradiation of T cells to eliminate suppressor influences, mean spontaneous helper activity of CAH was not different from that of healthy subjects, indicating that spontaneous helper activity of CAH was normal. Concanavalin A (Con A)-induced suppressor cell activity was significantly decreased in CAH ($p < 0.01$, 9 defective cases out of 18 patients). Minor defect of Con A-induced suppressor activity was also found in some patients with chronic persistent hepatitis (CPH) (2 defective cases out of 14 patients). Autologous mixed lymphocyte reaction (AMLR) was significantly decreased in patients with CAH ($p < 0.005$). Spontaneous suppressor or Con A-induced suppressor activity was not different statistically between HBsAg-positive and HBsAg-negative cases. Finally, we demonstrated a presence of a serum factor(s) that can decrease Con A-induced suppressor cell function of healthy subjects in 7 of 21 patients with CAH and 2 of 14 CPH. Our results suggest that defective suppressor cell function likely attributable to serum factor(s) may reflect altered immune responses of CAH. ——— suppressor cell; helper cell; autologous MLR; CAH

In some patients with chronic active hepatitis (CAH), circulating autoantibodies to smooth muscles, nuclei and mitochondria are occasionally present in their sera, and these are considered to represent a triad of makers of the autoimmune process associated with continuing liver injury (Doniach and Walker 1969). Using various in vitro tests of cellular immunity, lymphocytes from patients with CAH have been shown to be sensitized to liver antigens (Tobias et al. 1967; Bacon et al. 1972; Miller et al. 1972; Smith et al. 1972; Meyer zum Büschenfelde et al. 1974; Lee et al. 1975), and to be directly cytotoxic to autologous hepatocytes (Paronetto and Vernace 1975; Wands and Isselbacher 1975; Geubel et al. 1976; Vergani et al. 1979), and it has been suggested that these cell-mediated autoimmunities play a role in pathogenesis of CAH. In addition, the presence of antibodies to hepatocyte-surface membrane antigens in sera from patients with CAH (Tage-Jensen et al. 1977; Jensen et al. 1978; Kakumu et al. 1979; Kawanishi and MacDermott 1979) and on

hepatocytes obtained from CAH patients (Hopf et al. 1975, 1976; Alberti et al. 1976) has led to speculation that a humoral autoimmune mechanism also may participate in the pathogenesis of CAH.

Recent experimental data showed that both T cells and B cells capable of reacting with many self-antigens are present even in the healthy subjects and waiting to be triggered (Rose 1978). A maintenance of immunological homeostasis regulated by suppressor and helper T cell function is considered to be one of the regulatory mechanisms to prevent autoimmune responses (Gershon 1974; Dutton 1975; Rose 1978). Abnormality in the function of suppressor T cells has been reported in systemic lupus erythematosus (SLE) (Abdou et al. 1976; Bresnihan and Jasin 1977; Horowitz et al. 1977), ulcerative colitis and Crohn's disease (Hodgson et al. 1978) and in juvenile rheumatoid arthritis (Strelkauskas et al. 1978). The association between the decreased suppressor T cell function and the presence of serum inhibitory factor has been documented in SLE (Sagawa and Abdou 1979) and in juvenile rheumatoid arthritis (Strelkauskas et al. 1978). Decreased suppressor T cell function was also postulated in development of CAH (Eddleston and Williams 1974). Recently, autologous mixed lymphocyte reaction (AMLR), in which human T cells respond with increased DNA synthesis to mitomycin-C treated or x-irradiated non-T cells, is employed to investigate the immunoregulatory function of T cells (Kuntz et al. 1976; Sakane et al. 1978). AMLR was found to be decreased in SLE (Sakane et al. 1978) and in Sjögren's syndrome (Miyasaka et al. 1980).

The purpose of the present investigation is to obtain more precise knowledge of immunoregulatory function in patients with CAH. In the first series of experiments we investigated the suppressor or helper cell activity in patients with CAH by measuring (a) the capacity of peripheral T cells or irradiated T cells to help differentiation of B cells into plasma cells, and (b) the capacity of T cells precultured with Concanavalin A (Con A) to suppress differentiation of B cells into plasma cells or to suppress the blast transformation responses of fresh allogeneic lymphocytes. In the second, we tested CAH sera for their ability to inhibit suppressor cell activity of normal human lymphocytes. Finally, we also employed AMLR to study the immunoregulatory function in CAH.

MATERIALS AND METHODS

Patients

The study was performed on 91 patients with chronic hepatitis without clinical or morphological evidence of alcohol abuse, ingestion of known hepatotoxic medication, primary biliary cirrhosis and Wilson's disease. Chronic hepatitis was classified into chronic active hepatitis (CAH) and chronic persistent hepatitis (CPH) according to its morphological pattern based upon Fogarty's histological criteria (Chalmers et al. 1974). Fifty-one patients were classified as CAH and 40 as CPH. Among those with CAH, 31 cases were positive in serum for hepatitis B surface antigen (HBsAg) (26 males and 5 females, mean serum glutamic-oxaloacetic transaminase (SGOT) 84.6 ± 66.4 (mean $\pm$ s.d.) IU/liter), 3 were positive for anti-HBs (1 male and 2 females, mean SGOT 76.0 ± 36.1 IU/liter) and 17 were negative for both HBsAg and anti-HBs (12 males and 5 females, mean

SGOT 89.8 ± 59.2 IU/liter). In these subgroups of CAH, SGOT levels were not statistically different. Nine patients with CPH were positive for HBsAg (8 males and 1 female, mean SGOT 48.5 ± 13.9 IU/liter), 6 were positive for anti-HBs (3 males and 3 females, mean SGOT 53.4 ± 22.3 IU/liter) and 25 were negative for both HBsAg and anti-HBs (21 males and 4 females, mean SGOT 34.9 ± 23.0 IU/liter). In these subgroups of CPH, SGOT levels were not different. HBsAg and anti-HBs were determined by the hemagglutination method (Vyas and Shulman 1970). Fifty-six HBsAg-negative healthy persons with normal liver function tests and without a history of liver disease were served as control. None had received corticosteroid or other immunosuppressive drugs.

Lymphocyte preparation

Whole lymphocyte fraction (WLF) was obtained from 30 ml of heparinized venous blood by Ficoll-Hypaque (F-H) gradient centrifugation (Böyum 1968), washed twice with phosphate buffered physiological saline (PBS, pH 7.4) and resuspended in RPMI 1640 medium (Nissui Seiyaku Co., Ltd., Tokyo) enriched with 10% inactivated fetal calf serum (Microbiological Associates, Bethesda, USA), 100 U/ml penicillin and 100 μ g/ml streptomycin (RPMI 1640-FCS). For AMLR macrophage-monocyte-depleted WLF (less than 3%) was obtained by F-H gradient centrifugation after incubation of venous blood with silica (1 hr at 37°C, 10% KAC-II suspension, Japan Immunoresearch Laboratories Co., Ltd.). From WLF lymphocytes forming rosettes with sheep erythrocytes (E) previously treated with 2-S-aminoethylisothiuronium bromide (AET) as described by Kaplan et al. (1976) were separated by F-H gradient centrifugation as described previously (Nonomura et al. 1978). Lymphocytes lying at the bottom of the tubes were referred to as T-enriched fraction (T) and were recovered by lysis of the E with a 0.83% NH_4Cl in 20 mM Tris buffer (pH 7.4) for 5 min, washed twice with PBS and resuspended in RPMI 1640-FCS. Lymphocytes remaining at the top of F-H were referred to as B-enriched fraction (B). They were collected, washed and resuspended in RPMI 1640-FCS.

Testing for helper function of peripheral blood lymphocytes

Methods described by Yata (1977) was used. T obtained from patients (Tp) and healthy persons (Tn) were suspended in RPMI 1640-FCS (1×10^6 cells/ml). Responder B was prepared from a normal donor and suspended in RPMI 1640-FCS (1×10^6 cells/ml). One donor served as the same source of responder B in all experiments. 0.2 ml (2×10^5 cells) of B was mixed with 0.8 ml (8×10^5 cells) of Tp (B+Tp) or 0.8 ml (8×10^5 cells) of Tn (B+Tn) to yield a ratio of B to T of 1:4, together with 10 μ g/ml of PWM, and incubated for 7 days in a humidified atmosphere of 95% air and 5% CO_2 . One ml of responder B (2×10^5 cells) only with PWM was also set up. In some experiments, the test was performed simultaneously at B to T ratios of 1 to 1, 4 and 8. After incubation, aliquots were cytocentrifuged onto glass slides, fixed in a solution of 95% ethanol and 5% acetic acid, and stained with fluorescent polyvalent anti-human immunoglobulin (Behring Institute, W. Germany). Cells with brightly stained cytoplasmic immunoglobulins were regarded as immunoglobulin producing cells (Ig-PC) (Siegal et al. 1976). The number of Ig-PC was counted and helper activity was determined by the number of Ig-PC obtained at T/B ratio of 4:1 as follows:

$$\text{Helper activity} = \frac{\text{Number of Ig-PC in (B+Tp)} - \text{Number of Ig-PC in B}}{\text{Number of Ig-PC in (B+Tn)} - \text{Number of Ig-PC in B}} \times 100$$

When Tn was used as Tp, helper activity was 100.8 ± 22.1 (mean $\pm$ s.d., $n=21$).

Testing for genuine helper function

In certain experiments directed to test the genuine helper function of T, T was previously x-irradiated with 2,000 rads to eliminate suppressor cell influences (Broder et al. 1978), since there were extensive data that suppressor T cells were relatively radiosensitive, whereas helper T cells were relative radioresistant (Rich and Pierce 1973; Dutton 1975; Keightley

et al. 1976; Siegal and Siegal 1977). It has been reported that suppressor T cells are sensitive to this dose of x-ray and their activities are nullified, whereas helper T cells are resistant (Broder et al. 1978). After irradiation, helper activity was tested as described above.

Generation of suppressor cells by Con A

Lymphocytes of WLF and T were divided respectively into two groups each containing 2×10^6 cells/ml in RPMI 1640-FCS, which were incubated with or without (WLF-Con A, T-Con A, WLF-C, and T-C, respectively) $10 \mu\text{g/ml}$ of Con A (Sigma Chemical Company, Saint Louis, USA) for 48 hr at 37°C in a humidified incubator. Lymphocytes incubated without Con A (WLF-C, T-C) were used as control cells.

Testing for Con A-induced suppressor function

Suppression on differentiation of B cells into Ig-PC. After incubation for 48 hr, both T-Con A and T-C were washed once, suspended in fresh Eagle's minimum essential medium (MEM) (GIBCO, New York, USA) containing $0.1 \text{ M } \alpha$ -methyl-D-mannoside (P-L Biochemicals Inc., Milwaukee, USA) and incubated for 30 min at 37°C to remove Con A from lymphocytes. After incubation, cells were washed three times with MEM containing 5% FCS, tested for viability by trypan blue exclusion and finally resuspended in RPMI 1640-FCS at a concentration of 2×10^6 cells/ml. Fresh responder B was prepared from a normal donor as described above and adjusted to a concentration of 2×10^6 cells/ml in RPMI 1640-FCS. One donor served as the source of responder B in all experiments. 0.2 ml of responder B and 0.2 ml of T-Con A or T-C were mixed, together with $10 \mu\text{g/ml}$ of PWM (GIBCO, New York, USA) and incubated for 7 days at 37°C in a humidified atmosphere of 95% air and 5% CO_2 . Cells were harvested, and counted. Ig-PC were counted as described above. The suppressor activity was determined as follows:

$$\% \text{ suppression} = 100 \times \left(1 - \frac{\text{Number of Ig-PC in the presence of T-Con A}}{\text{Number of Ig-PC in the presence of T-C}} \right)$$

Suppression on the transformation responses of fresh allogeneic lymphocytes. After incubation for 48 hr, both WLF-Con A and WLF-C were treated with $50 \mu\text{g/ml}$ of mitomycin C (Sigma Chemical Company, Saint Louis, USA) for 30 min at 37°C to block DNA synthesis, and then washed three times with $30 \text{ mM } \alpha$ -methyl-D-mannoside in RPMI-1640 medium. Cells were suspended in RPMI 1640-FCS, tested for viability by trypan blue exclusion and finally resuspended in RPMI 1640-FCS (1×10^6 cells/ml).

Fresh responder WLF were prepared from a normal donor as described above and adjusted to a concentration of 1×10^6 cells/ml. One donor served as the source of responder cells in all experiments. One ml of responder WLF and one ml of WLF-Con A or WLF-C were mixed, together with $10 \mu\text{g/ml}$ of Con A, and incubated for 72 hr at 37°C in a humidified incubator. 24 hr before harvesting, $2 \mu\text{Ci}$ of [^3H]thymidine (sp. act. 20 Ci/mmole , the Radio Chemical Centre, Amersham, England) were added to each culture tube. After incubation cells were harvested and their radioactivities were measured for 2 min in a Beckman liquid scintillation counter. Tests were performed in quadruplicate. The suppressor activity was expressed in the following manner:

$$\% \text{ suppression} = 100 \times \left(1 - \frac{[\text{H}] \text{thymidine incorporation in the presence of WLF-Con A}}{[\text{H}] \text{thymidine incorporation in the presence of WLF-C}} \right)$$

Effect of CAH sera on Con A-induced suppressor function of healthy lymphocytes

WLF from healthy subjects was preincubated with heat-inactivated test serum (56°C for 30 min) for 12 hr at 37°C in a humidified atmosphere of 95% air and 5% CO_2 (2×10^6 cells/ml serum), then washed and resuspended in RPMI 1640-FCS (2×10^6 cells/ml). The suspension was divided into two groups and incubated for 48 hr with or without Con A to generate suppressor cells, and then Con A-induced suppressor function on blast transformation responses of allogeneic lymphocytes was studied as described above.

Autologous MLR

T and B were obtained from macrophage-monocyte-depleted WLF as described above. B was treated with mitomycin C (50 $\mu\text{g/ml}$) (Sigma Chemicals Co.) for 30 min at 37°C, then washed and resuspended in RPMI 1640-FCS. 0.5 ml of responding T (1×10^5 cells) and 0.5 ml of stimulating B (1×10^5 cells) were mixed and incubated for 168 hr at 37°C in a 5% $\text{CO}_2/95\%$ air humidified atmosphere. At 24 hr before the termination of the incubation period, 1 μCi of [^3H]thymidine (sp. act. 20 Ci/mmol, the Radio Chemical Centre, Amersham, England) was added to each culture tube. After incubation cells were harvested and their radioactivities were measured for 2 min in a Beckman liquid scintillation counter. Tests were performed in triplicate. Results were recorded as the difference in cpm in stimulated and unstimulated culture (responding T cell alone).

RESULTS

Spontaneous helper function

Helper activity. The number of Ig-PC after 7 day-incubation of B with T at various T/B ratios, together with PWM, is shown in Fig. 1 (open column). The number of Ig-PC at all respective T/B ratios tested in patients with CAH was significantly increased in comparison with corresponding results of healthy subjects ($p < 0.01$), whereas those of CPH were not significant. The spontaneous helper activity was calculated from the data tested at T/B ratio of 4/1 (a physiological T/B ratio in peripheral blood lymphocytes in healthy subjects). As

Fig. 1. Numbers of Ig-producing cells after 7 day-incubation of B cells from a normal subject with T cells (open columns) or irradiated T cells (2,000 rads, shaded columns) from healthy subjects ($n=11$), patients with chronic active hepatitis (CAH, $n=11$) and patients with chronic persistent hepatitis (CPH, $n=11$) at various T or irradiated T to B ratios, together with 10 $\mu\text{g/ml}$ of PWM. Results and bars shown represent means $\pm$ S.D.

Fig. 2. The results of spontaneous helper activity on B cell differentiation into Ig-producing cells in patients with chronic hepatitis and healthy subjects. Results are given as the mean $\pm$ s.d. Shaded area indicates the range of mean $\pm$ 2 s.d. of healthy subjects ($n=21$). Abbreviations: CAH, chronic active hepatitis; CPH, chronic persistent hepatitis; HBsAg+, HBsAg positive; HBsAg-, HBsAg negative; anti-HBs+, anti-HBs positive; anti-HBs-, anti-HBs negative.

Fig. 3. The results of genuine spontaneous helper activity in patients with chronic hepatitis and healthy subjects. Bars represent mean $\pm$ s.d. Shaded area indicates the mean $\pm$ 2 s.d. of the healthy subjects. ●, both HBsAg- and anti-HBs-negative case, ○, HBsAg-positive case; ■, anti-HBs-positive case. Abbreviations are the same as in Fig. 2.

shown in Fig. 2, mean spontaneous helper activity of HBsAg-positive CAH, anti-HBs-positive CAH or both HBsAg- and anti-HBs-negative CAH was significantly higher than that of healthy subjects ($p < 0.01$, $p < 0.001$ and $p < 0.05$, respectively), whereas those of CPH were not significant. Mean helper activity was not different in the subgroups of CAH. When normal range was taken as mean ± 2 s.d. in healthy subjects and each patient who showed a helper activity higher or lower than this value was considered respectively to be positive helper or suppressor activity, 15 (11 HBsAg-positive, 2 anti-HBs-positive, 2 both HBsAg- and anti-HBs-negative) of 30 patients with CAH and 5 (2 HBsAg-positive, 1 anti-HBs-positive, 2 both HBsAg- and anti-HBs-negative) of 25 with CPH showed positive helper activity, and one (HBsAg-positive) of 30 patients with CAH and 5 (1 anti-HBsAg-positive, 4 both HBsAg- and anti-HBs-negative) of 25 with CPH showed positive suppressor activity (Fig. 2).

Genuine helper activity. The number of Ig-PC on 7 day-incubation of B with increasing numbers of x-ray irradiated T cells is shown in Fig. 1 (shaded column). A sharp increase in number of Ig-PC was observed with increasing numbers of x-ray irradiated T cells in all groups tested and the mean number of Ig-PC at the same T/B ratio was significantly increased when compared to that obtained from the test using non-irradiated T cells ($p < 0.001$), but the mean number of Ig-PC at the same irradiated T/B ratio was not significantly different between groups tested. Healthy subjects and CAH or CPH were not different in genuine helper activity (Fig. 3).

Con A-induced suppressor function

Suppressor activity on lymphocyte blast transformation. The results are shown in Fig. 4. Mean Con A-induced suppressor activity of each subgroup of CAH was significantly decreased when compared to healthy subjects ($p < 0.001$, $p < 0.01$ and $p < 0.001$, respectively). Mean suppressor activities of anti-HBs-positive CPH and both HBsAg- and anti-HBs-negative CPH were also significantly decreased in lesser degrees ($p < 0.05$ and $p < 0.05$, respectively). However, no difference in mean Con A-induced suppressor activity was noted between any two subgroups of CAH or CPH. Significantly decreased suppressor activity, judged by a value less than mean -2 s.d. of the healthy subjects, was demonstrated in 4 of 10 patients with HBsAg-positive CAH, 1 of 2 with anti-HBs-positive CAH, 4 of 6 with both HBsAg- and anti-HBs-negative CAH and 2 of 9 with both HBsAg- and anti-HBs-negative CPH.

Suppressor activity on differentiation of B cells into Ig-PC. The results are shown in Fig. 5. Mean suppressor activity of HBsAg-positive CAH and both HBsAg- and anti-HBs-negative CAH was significantly decreased as compared with that of healthy subjects ($p < 0.001$ and $p < 0.001$, respectively). The mean value of both HBsAg- and anti-HBs-negative CPH was also significantly decreased ($p < 0.05$). Significantly decreased suppressor activity, judged by a value less than

Fig. 4. The results of Con-A induced suppressor cell function on lymphocyte blast transformation responses. Results and bars shown represent mean $\pm$ s.d. Shaded area indicates the range of mean $\pm$ 2s.d. of healthy subjects ($n=38$). Abbreviations are the same as in Fig. 2.

mean -2 s.d. of the healthy subjects, was found in 7 of 10 patients with CAH and 1 of 10 with CPH.

Effects of CAH sera on Con A-induced suppressor activity of healthy subjects

The results are shown in Fig. 6. Con A-induced suppressor activity of healthy subjects was significantly decreased after preincubation of lymphocytes with HBsAg-positive CAH sera or both HBsAg- and anti-HBs-negative CAH sera ($p < 0.01$ and $p < 0.005$, respectively). Sera from patients with both HBsAg- and anti-HBs-negative CPH also inhibited suppressor cell function of healthy subjects ($p < 0.005$). Significantly inhibitory sera, judged by a value less than mean -2 s.d. of the healthy subjects, was found in 5 of 13 patients with HBsAg-positive CAH, 2 of 8 both HBsAg- and anti-HBs-negative CAH, 1 of 7 HBsAg-positive CPH and 1 of 7 both HBsAg- and anti-HBs-negative CPH.

Autologous mixed lymphocyte reaction (AMLR)

The results are shown in Fig. 7. The test was performed in 22 patients with CAH (14 HBsAg-positive, 8 both HBsAg- and anti-HBs-negative) and 13 with CPH (2 HBsAg-positive, 2 anti-HBs-positive and 9 both HBsAg- and anti-HBs-negative). Mean AMLR of patients with CAH was significantly decreased as

Fig. 5. The results of Con A-induced suppressor cell function on B cell differentiation into Ig-producing cells in patients with chronic hepatitis and healthy subjects. Results and bars shown represent mean $\pm$ s.d. Shaded area indicates the range of mean $\pm$ 2s.d. of healthy subjects ($n=10$). Abbreviations are the same as in Fig. 2.

compared with that of healthy subjects ($p<0.005$), whereas that of CPH was not significant. Mean AMLR was not different in patients with HBsAg-positive CAH and those with both HBsAg- and anti-HBs-negative CAH ($2,610.3 \pm 1,352.2$ vs. $4,297.9 \pm 2,647.8$). Mean AMLR of patients with both HBsAg- and anti-HBs-negative CPH ($n=9$, $4,319.2 \pm 1,522.2$) was also significantly decreased as compared with that of healthy subjects ($p<0.05$).

DISCUSSION

Evidence has been accumulated that development of autoimmune diseases in animal model systems (Gerber et al. 1974; Krakauer et al. 1976; Eardley et al. 1978; Cantor et al. 1978) and in human beings (Abdou et al. 1976; Bresnihan and Jasin 1977; Horowitz et al. 1977; Sakane et al. 1978; Strelkauskas et al. 1978; Sagawa and Abdou 1979) may result from defective immunoregulatory suppressor T cell functions. The similar abnormality of suppressor T cell function was postulated to be one of the causes of CAH (Eddleston and Williams 1974). The present study demonstrated that peripheral blood lymphocytes from patients with CAH have defective suppressor cell activity and decreased AMLR. The similar suppressor cell alteration in a lesser degree was also demonstrated in patients with HBsAg-negative CPH when tested by Con A-induced suppressor systems. Although patients

Fig. 6. Effects of preincubation of healthy lymphocytes with sera of patients with chronic hepatitis on Con A-induced suppressor cell function. Results and bars shown represent mean $\pm$ s.d. Shaded area indicates the range of mean $\pm$ 2s.d. of healthy subjects ($n=12$). Abbreviations are the same as in Fig. 2.

Fig. 7. Autologous MLR between T and non-T cells in patients with chronic hepatitis and healthy subjects. Results and bars shown represent mean $\pm$ s.d. ●, both HBsAg- and anti-HBs-negative case; ○, HBsAg-positive case; ■, anti-HBs-positive case. Abbreviations are the same as in Fig. 2.

who had poor Con A-induced suppressor activity tended to be found in HBsAg-negative cases, no statistically significant difference in mean suppressor activity was found between HsBAg-positive and HBsAg-negative cases with CAH or CPH (Fig. 4). Nor difference in spontaneous suppressor cell activity was found between HBsAg-positive and HBsAg-negative cases, either. The results are considered to be consistent with previous reports that humoral antibody and cell-mediated immunity to liver-specific membrane lipoprotein (LSP) were found with an equal frequency in HBsAg-positive and HBsAg-negative CAH (Chisari et al. 1978; Meyer zum Büschenfelde et al. 1979; Kakumu et al. 1979).

There are a number of possible explanations for the altered suppressor cell activity in chronic hepatitis. A generalized T lymphocyte hyporesponsiveness (Rössler et al. 1969; Martini et al. 1970; Giustino et al. 1972; Dudley et al. 1972; Toh et al. 1973) and a decrease in number and percentage of peripheral blood T lymphocytes (DeHoratius et al. 1974; Thomas et al. 1976; Colombo et al. 1977; Williams et al. 1980) have been reported by several investigators in patients with chronic hepatitis, particularly in CAH. Our present data suggested that a serum factor(s) present in CAH patients may alter suppressor lymphocyte function of healthy subjects. Natures of the serum factor(s) were not characterized in our experiments. However, several investigators reported the presence of serum factor which may derange the functions of T lymphocytes in patients with chronic hepatitis (Wands et al. 1975; Dehoratius et al. 1976; Chisari et al. 1977, 1978; Kakumu et al. 1978). Dehoratius et al. (1976) emphasized a presence of lymphocytotoxins in sera of chronic hepatitis in relation to decreased peripheral T lymphocytes. Chisari et al. (1977) demonstrated the presence of serum factor that inhibits the function of T lymphocytes forming rosettes with sheep erythrocytes in CAH and acute hepatitis. They termed it rosette inhibitory factor (RIF). Furthermore, Chisari et al. (1978) reported that the presence of RIF is concordant with the frequent presence of decreased suppressor activity and this association of RIF with decreased suppressor cell activity is also concordant with evidence of sensitization and cytotoxicity towards a hepatocellular autoantigen (LSP). Finally the presense of autoantibody that would appear to have a specificity against suppressor T cells has been reported in juvenile rheumatoid arthritis (Strelkauskas et al. 1978) and SLE (Sagawa and Abdou 1979). An analogous antibody has been reported in NZB and NZB/W mice (Shirai and Mellors 1971; Klassen et al. 1977) which have been well known to develop autoimmunity mimicking human SLE. Additional serum factors such as lipoproteins, alpha fetoprotein and bile acid have been reported to suppress the lymphocyte functions (Chisari et al. 1978; Gianni et al. 1980). The precise nature of serum inhibitory factor(s) related to liver diseases or its relationship to suppressor T cell function has not yet been defined and remains to be clarified.

In the present study, AMLR was significantly decreased in patients with CAH. Analogous results have been reported in patients with SLE (Sakane et al. 1978) and Sjögren's syndrome (Miyasaka et al. 1980) and in the SLE model in NZB mice

(Smith and Pasternak 1978). Although the exact biologic role of the AMLR has not been fully established, it is assumed to reflect an important immunoregulatory function (Opelz et al. 1975; Kuntz et al. 1976; Sakane et al. 1978). Recently, evidence is accumulating that AMLR and Con A-induced suppressor cell activity are closely related to each other. Sakane and Green (1979) reported that autoreactive T cells capable of responding to autologous non-T cells are particularly enriched in cells capable of becoming Con A-induced suppressor cells. Innes et al. (1979) reported that AMLR may play an important role in generation of suppressor cells by Con A. So it seems to be understandable that both Con A-induced suppressor cell activity and AMLR were decreased in patients with CAH.

Although our investigation was directed to non-specific suppressor functions, the data presented herein suggest that decreased suppressor cell activity reflects altered immune responses of chronic hepatitis and may be related to the pathogenesis of some CAH. To clarify the precise immunopathological mechanisms involved in CAH, specific helper or suppressor cell function induced by, for example, liver specific antigen and hepatitis-related viral-associated antigens are required to be evaluated.

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