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# Adoptive transfer of experimental autoimmune hepatitis in mice —cellular interaction between donor and recipient mice

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## SUMMARY

This report extends our previous study on experimental autoimmune hepatitis in C57BL/6 (B6) mice. Cellular immunity involved in the induction of liver injury in this model was studied by transfer of primed spleen cells from hepatitis donor mice to syngeneic normal recipient mice.

The most prominent liver damage in recipient B6 mice was induced by transfer of nylon wool adherent spleen cells from hepatitis donor mice, and T cells in this fraction were the essential requirement for the liver damage in the recipient mice. Nylon wool adherent spleen cells from hepatitis donor mice after depletion of the suppressor T-cell function by low-dose (300 rad) irradiation induced more severe liver injury compared to the same cells without irradiation. When the recipient mice were depleted of lymphocytes by low or high dose (700 rad) whole body irradiation, transfer of primed spleen cells from hepatitis donor mice did not induce liver lesion in the lymphocyte-depleted mice. This low susceptibility of lymphocyte-depleted recipient mice to primed spleen cells of hepatitis mice was no longer demonstrated after reconstitution with normal spleen cells. In a cell-migration study using $^{51}$Cr-labelled spleen cells, it was shown that a considerable number of infiltrating cells in the liver of recipient mice were derived from recipient mice themselves. These results seem to indicate that cell-to-cell interaction between radiosensitive precursor cells of recipient mice and liver-antigen-primed T cells from hepatitis donor mice play an essential role in the induction of liver injury in the recipient mice.

**Keywords** Experimental hepatitis adoptive transfer whole body irradiation cellular interaction

## INTRODUCTION

A number of *in vitro* studies have demonstrated that a cellular immune mechanism plays an important role in the induction and progression of certain liver diseases (Thomson *et al.*, 1974; Cochrane *et al.*, 1976; Vergani *et al.*, 1979). It has recently been suggested that interactions between functionally different types of cells are required for generation of cytotoxic reactions in liver disease (Barnaba *et al.*, 1986; Stefanini *et al.*, 1986). However, the precise role of these interactions *in vivo* is not known.

Collaborative interactions between several types of cells in cellular immune response have been demonstrated *in vivo* using several experimental systems (Cantor & Ashofsky, 1970; Cantor & Boyse, 1975; Glaser & Law, 1978). A similar phenomenon has been observed in experimental allergic encephalomyelitis (EAE) in rats (Holda, Silberg & Swanborg, 1983). We were able to produce experimental autoimmune hepatitis in C57BL/6 mice and have studied cellular immunity in this model (Mori Y. *et al.*, 1984; Mori T. *et al.*, 1985). It was shown that liver damage was inducible in normal recipient B6 mice by transfer of sensitized spleen T-cells from hepatitis donor mice (Mori Y. *et al.*, 1985). In these experimental models, the cellular interaction among helper, suppressor and cytotoxic effector T cells seems to play a critical role in the induction of organ injury. It is known that various types of cells can be distinguished from their radiosensitivity; antigen-primed helper T cells are radioresistant whereas pre-killer T cells and suppressor T cells are radiosensitive (Anderson & Werner, 1976; Doria, Agarossi & Adorini, 1982). In the current study, cellular components involved in liver injury were investigated taking advantage of the difference in radiosensitivity, extending our previous observations with the transfer study in this experimental hepatitis model (Mori Y. *et al.*, 1985).

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**Table 1.** The design of transfer experiments

| Group | Treatment of donor mice or spleen cells: Immunization with: | Treatment after immunization | Treatment of recipient mice: Depletion of lymphocytes | Reconstitution |
|---|---|---|---|---|
| I. (i) | Liver antigen + FCA | ND | ND | ND |
| | Liver antigen + FCA | Depletion *in vitro* of T-lymphocytes by anti-Thy 1,2 antibody + complement | ND | ND |
| (ii) | FCA alone (control) | ND | ND | ND |
| II. | Liver antigen + FCA | Depletion *in vivo* of radiosensitive lymphocytes by low dose (300 rad) whole body irradiation | ND | ND |
| III. (i) | Liver antigen + FCA | ND | Depletion of radiosensitive lymphocytes by low dose (300 rad) whole body irradiation | ND |
| (ii) | Liver antigen + FCA | ND | Depletion of total lymphocytes by high dose (700 rad) whole body irradiation | ND |
| IV. | Liver antigen + FCA | ND | Depletion of total lymphocytes by high dose (700 rad) whole body irradiation | Reconstitution with normal spleen cells |

ND: Not done.

## MATERIALS AND METHODS

*Animals*

Six-week-old male C57BL/6 (B6) mice obtained from Shizuoka Laboratory Center (Shizuoka, Japan) were used in this study.

*Preparation of sensitized spleen cells from hepatitis mice and fractionation*

Fresh livers from normal B6 mice were homogenized, mixed, centrifuged at 100,000 *g* for 1 h at 4°C and the supernatant was used as liver antigen. As described previously (Mori Y. *et al.*, 1984), experimental hepatitis with mononuclear cell infiltrate in the liver was induced by immunizing B6 mice intramuscularly with the supernatant and Freund's complete adjuvant (FCA) four times at weekly intervals. Control B6 mice were injected with FCA alone four times in the same way. On day 7 after the last immunization, the mice were killed and spleens were aseptically removed. These spleen cells were passed through a stainless steel mesh (No. 200) using the RPMI 1640 medium (RPMI, Gibco, Grand Island, NY) that contained 10% fetal calf serum (FCS, Gibco). A portion of these cells was separated by a nylon wool (NW) column into the nylon wool adherent (NWA) fraction and the nylon wool non-adherent (NWNA) fraction, according to Julius, Simpson & Herzenberg, (1973).

*T cell population in the spleen cells from immunized and control donor mice*

The T cell population in each fraction of spleen cells was studied by the complement-mediated cytolysis method. Each fraction of spleen cells separated by a NW column was suspended in FCS to yield a concentration of $1 \times 10^6$/ml. One millilitre of this suspension was incubated with $^{51}$Cr-labelled sodium chromate (Amersham Radiochemicals Co., Ltd., Amersham, UK) (40 $\mu$Ci/37°C/1 h), washed three times, and resuspended with 1 ml of Dulbecco's modified Eagle's medium (DMEM, GIBCO) containing 1·0% FCS. 0·1 ml of the labelled cell suspension was placed in each well of the microtest plate to be treated with anti-Thy 1,2 antibody (clone F7D5, Olac 1976 Ltd., Blackthorn, Bicester, Oxon, UK) and low-Tox-M rabbit complement (Cedarlane Laboratories Ltd., Ontario, Canada). As a control, 0·1 ml of the cell suspension without treatment (spontaneous release) or with 0·1 ml of a 2% saponin solution (as maximum release) was placed in each well. After the treatment, 0·1 ml of culture supernatant was collected from each well and measured for $^{51}$Cr release from damaged spleen cells. T-cell population:

$$\frac{\text{ct/min by experimental release} - \text{ct/min by spontaneous release}}{\text{ct/min by maximum release} - \text{ct/min by spontaneous release}} \times 100$$

*Transfer of sensitized spleen cells with or without pre-treatment by irradiation*

The design of transfer study is summarized in Table 1, Group I. Spleen cells from control or hepatitis donor mice were transferred to untreated syngeneic recipient mice. After fractionation of spleen cells with a NW column, cells in each fraction were resuspended in Hanks's balanced salt solution (HBSS) and $1 \times 10^7$ cells were injected to recipient mice from the tail vein. All mice were sacrificed on day 7 after the adoptive cell transfer, and the liver, kidney and other organs were removed for histological examination. Some recipient mice were injected with primed NWA spleen cells which had been depleted of T cells by anti-Thy 1,2 antibody and complement as described above.

A portion of donor mice were subjected to whole body irradiation at a dose of 300 rad for depletion of radiosensitive cells just before the final immunization (Group II). A set of recipient mice were treated by 300 rad irradiation to deplete radiosensitive cells (Group III (i)) and another set of recipient mice were treated by high dose (700 rad) irradiation for total lymphocyte depletion (Group III (ii)). In Group IV, recipient mice depleted of total lymphocytes by 700 rad irradiation were supplemented with whole or NW fractionated spleen cells obtained from syngeneic normal mice on day 3 before the adoptive transfer of donor spleen cells.

Mice were irradiated using the MBR-1505 (Hitachi Medico) X-ray apparatus. The focal skin distance was 50 cm and the absorbed dose rate was 10 rad/min. The machine was operated under the condition of 150 kv and 5 mA with a 1 mm aluminium and a 0·2 mm copper inherent filter. Irradiated mice were nursed in a box with laminar air flow; all these mice survived the experiment period.

*Histological examination of liver*

Liver specimens were taken and fixed with 10% buffered formalin, cut and stained with haematoxylin and eosin. Histological changes of the liver were scored as follows: −, no morphological changes; +, diffuse but mild infiltration of mononuclear cells around the portal area and in the lobules; ++, moderate infiltration of mononuclear cells and hepatocyte necrosis.

*Migration study of transferred spleen cells*

Migration of transferred cells in the recipient mice was studied according to Carroll & Sousa, 1984. A portion of the NWA spleen cells from hepatitis donor mice and unfractionated spleen cells from normal B6 mice were radiolabelled by incubating them in RPMI (10% FCS) containing 100 $\mu$Ci $^{51}$Cr-sodium chromate/$10^8$ cells/ml for 45 min at 37°C in 5% $CO_2$. After labelling, cells were washed three times and resuspended in HBSS at a concentration of $5 \times 10^7$ cells/ml. Standard aliquots of the labelled cell suspension were used for the determination of total radioactivity injection.

*Transfer of $^{51}$Cr-chromate labelled spleen cells obtained from hepatitis donor mice to normal and lymphocyte-depleted recipient B6 mice.* Ten of the untreated mice and another 10 of the mice depleted of total lymphocytes by 700 rad irradiation were injected with $^{51}$Cr-labelled NWA spleen cells from hepatitis donor mice.

*Reconstitution of totally lymphocyte-depleted recipient mice with $^{51}$Cr-labelled normal spleen cells.* Twenty recipient mice depleted of total lymphocytes by 700 rad whole body irradiation were reconstituted with $1 \times 10^7$/mouse of $^{51}$Cr-labelled normal spleen cells on day 3 before the adoptive transfer of primed spleen cells from hepatitis donor mice. Ten of the treated mice were injected with $1 \times 10^7$ of liver-antigen primed NWA spleen cells from hepatitis donor mice and the other 10 were injected with unprimed spleen cells from normal B6 mice as a control.

*Assessment of the distribution of transferred cells.* On day 7 after the adoptive transfer, all the mice were killed and the biggest mesenteric lymphnode, spleen, liver and the kidney were removed along with a 0·5 ml blood aliquot. The radioactivity of these tissue samples and standard aliquots of the injected cell suspension were counted by a $\gamma$-scintillation counter. Results were calculated as the percentage of radioactivity localized within each organ or tissue sample. Student's $t$-test was used to analyse the difference.

## RESULTS

*Group I: transfer of primed spleen cells from hepatitis donor mice to normal recipient mice*

Figure 1(a) shows the histological findings of the liver from a hepatitis donor mouse immunized with crude syngeneic liver proteins and FCA. Intensive infiltration of mononuclear cells in the periportal tracts and spotty hepatocyte necrosis in the lobule are seen. No such alternations were seen in the mice injected with FCA alone. In Table 2(I), the T-cell population of spleen cells from the hepatitis or control donor mice and histological examination of the liver from recipient mice transferred with the spleen cells from hepatitis donor mice are shown. The population of T cells in the unfractionated spleen cells of hepatitis mice was 36%. After fractionation of these spleen cells with NW, the T cell population was 72% in the NWNA fraction and 31% in the NWA fraction. Histological changes of the liver were most prominent in the recipient mice transferred with NWA spleen

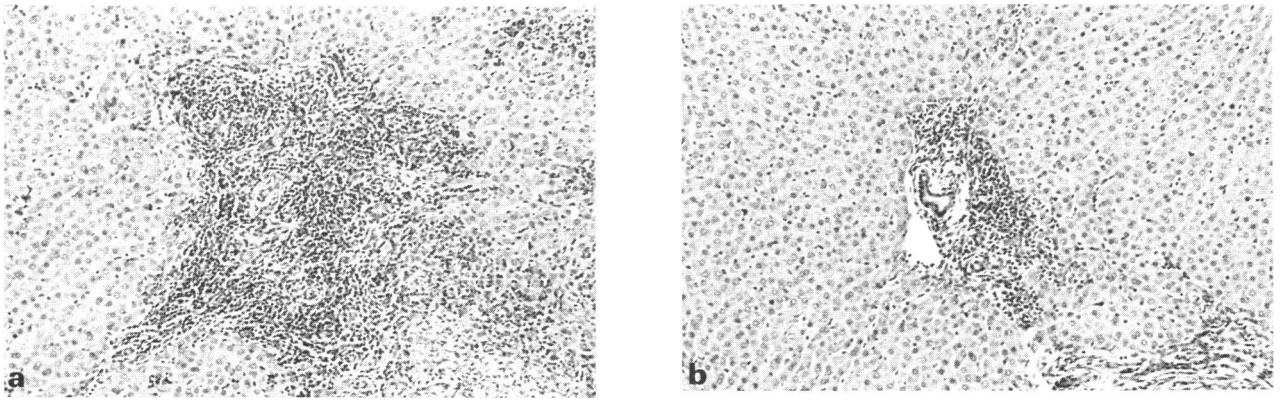


**Fig. 1** (a). Liver of C57BL/6 mouse immunized with crude liver antigens. Marked mononuclear cell infiltration in the periportal tracts and spotty necrosis in the hepatic lobule are apparent (H & E × 250).
(b). Liver of C57BL/6 mouse injected with nylon wool column adherent spleen cell from hepatitis donor mice. Mononuclear cell infiltration in the portal area and focal hepatocyte necrosis are seen (H & E × 250).

**Table 2.** Histological changes of the liver in recipient mice transferred with spleen cells obtained from hepatitis or control donor mice

| Group | Fraction of injected spleen cells from donor mice | T cell population | Frequency of histological liver changes in recipient mice: No. examined | (−) | (+) | (++) |
|---|---|---|---|---|---|---|
| I. | Donor mice without pre-treatment of irradiation | | | | | |
| | (i) Hepatitis donor mice immunized four times with liver antigen and FCA | | | | | |
| | Unfractionated cells | 36·2±4·2% | 20 | 11/20(55%) | 9/20(45%) | |
| | Nylon wool non-adherent cells | 72·3±3·7 | 20 | 14/20(70%) | 6/20(30%) | |
| | Nylon wool adherent cells treated with: | | | | | |
| | None | 33·5±2·6 | 20 | 5/20(25%) | 10/20(50%) | 5/20(25%) |
| | Anti-Thy 1,2 antibody alone | 32·3±2·2 | 20 | 6/20(30%) | 9/20(45%) | 5/20(25%) |
| | Complement alone | 30·5±3·2 | 20 | 7/20(35%) | 9/20(45%) | 4/20(20%) |
| | Anti-Thy 1,2 antibody+complement | 0 | 20 | 15/20(75%) | 5/20(25%) | |
| | (ii) Control donor mice immunized with FCA alone | | | | | |
| | Unfractionated cells | 30·3±4·2 | 20 | 20/20 | | |
| | Nylon wool non-adherent cells | 74·5±5·5 | 20 | 20/20 | | |
| | Nylon wool adherent cells | 25·1±3·3 | 20 | 20/20 | | |
| II. | Donor mice treated by whole body irradiation | | | | | |
| | (i) Hepatitis donor mice treated by 300 rad irradiation just after the last immunization with liver antigen and FCA | | | | | |
| | Unfractionated cells | | 20 | 7/20(35%) | 7/20(35%) | 6/20(30%) |
| | Nylon wool non-adherent cells | ND | 20 | 12/20(60%) | 7/20(35%) | 1/20(5%) |
| | Nylon wool adherent cells | | 20 | 3/20(15%) | 9/20(45%) | 8/20(40%) |
| | (ii) Control donor mice treated by 300 rad irradiation just after the last injection of FCA | | | | | |
| | Unfractionated cells | | 20 | 20/20 | | |
| | Nylon wool non-adherent cells | ND | 20 | 20/20 | | |
| | Nylon wool adherent cells | | 20 | 20/20 | | |

ND: Not done.

cells from hepatitis donor mice. Figure 1(b) shows the liver histology of the recipient mice transferred with NWA spleen cells from hepatitis donor mice. Mild to moderate infiltration of mononuclear cells was seen in the portal tract. The ability of primed spleen cells to induce liver changes in recipient mice was lost after depletion of T cells using anti-Thy 1,2 antibody and complement. In the liver of recipient mice transferred with unfractionated or NWNA spleen cells from hepatitis donor mice, histological changes were milder than those seen in recipient mice transferred with NWA spleen cells from hepatitis donor mice. No histological change was seen in the other organs of any recipient mice examined. Spleen cells from control mice induced no histological alternation in the liver nor in the other organs of recipient mice.

*Group II: transfer of spleen cells obtained from hepatitis donor mice treated with low dose whole body irradiation*

In parallel with Group I experiment, some of B6 mice were transferred with spleen cells from hepatitis donor mice which had been pretreated with low dose (300 rad) irradiation (Table 2(II)). Histological liver changes induced by the transfer of fractionated spleen cells following 300 rad irradiation of hepatitis donor mice (Group II) were greater than those caused by corresponding cell populations from unirradiated donor mice (Group I). The difference in the histological scores between Groups I and II was prominent with NWA or unfractionated cells, but not so obvious with NWNA cells.

*Group III: treatment of recipient mice with whole body irradiation*

Recipient mice were treated with low-dose (300 rad) and high-dose (700 rad) whole-body irradiation prior to the transfer of spleen cells from hepatitis donor mice. Histological findings of the liver from the recipient mice transferred with primed spleen cells from hepatitis donor mice were negligible.

*Group IV: total lymphocyte depletion of recipient mice and reconstitution of lymphocytes*

Some of the B6 mice that were depleted of total lymphocytes by 700-rad irradiation were reconstituted with normal spleen cells, and then used as the recipient (Table 3). Prominent liver injuries were induced by transfer of primed NWA spleen cells from hepatitis donor mice to the recipient mice that had been reconstituted with unfractionated spleen cells from normal B6 mice. Transfer of unfractionated or NWNA spleen cells from hepatitis donor mice induced only mild changes in the liver of

**Table 3.** Transfer of primed spleen cells from hepatitis donor mice to the recipient mice that had been depleted of lymphocytes and reconstituted with normal spleen cells (Group IV)

| Group | Donor mice: Source of transferred spleen cells | Lymphocyte-depleted recipient mice*: Fraction of normal spleen cells used for reconstitution | No. examined | Frequency of histological liver changes in recipient mice (−) | (+) | (++) |
|---|---|---|---|---|---|---|
| I. | Hepatitis donor mice immunized with liver antigen and FCA | | | | | |
| | Unfractionated | | 20 | 12/20(60%) | 8/20(40%) | |
| | Nylon wool non-adherent | Reconstitution with unfractionated cells | 20 | 15/20(75%) | 5/20(25%) | |
| | Nylon wool adherent | | 20 | 9/20(45%) | 7/20(35%) | 4/20(20%) |
| | Unfractionated | | 20 | 14/20(70%) | 6/20(30%) | |
| | Nylon wool non-adherent | Reconstitution with nylon wool non-adherent cells | 20 | 16/20(80%) | 4/20(20%) | |
| | Nylon wool adherent | | 20 | 13/20(65%) | 7/20(35%) | |
| | Unfractionated | | 20 | 13/20(65%) | 7/20(35%) | |
| | Nylon wool non-adherent | Reconstitution with nylon wool adherent cells | 20 | 15/20(75%) | 5/20(25%) | |
| | Nylon wool adherent | | 20 | 14/20(70%) | 6/20(30%) | |
| | Unfractionated | | 20 | 19/20(95%) | 1/20(5%) | |
| | Nylon wool non-adherent | No Reconstitution (Control) | 20 | 20/20 | | |
| | Nylon wool adherent | | 20 | 20/20 | | |
| II. | Control donor mice injected with FCA alone | | | | | |
| | Unfractionated | | 20 | 20/20 | | |
| | Nylon wool non-adherent | Reconstitution with unfractionated cells | 20 | 20/20 | | |
| | Nylon wool adherent | | 20 | 20/20 | | |

*Recipient mice were pre-treated by 700 rad whole body irradiated for total lymphocyte depletion.

**Table 4.** Distribution of $^{51}$Cr-labelled spleen cells injected to the recipient mice treated variously before and after the injection

| Group | Treatment of recipient mice | No. examined | Tissue localization (% injected radioactivity): Liver | Spleen | Kidney | Lymphnodes | Blood* |
|---|---|---|---|---|---|---|---|
| I. | Distribution of $^{51}$Cr-labelled NWA spleen cells transferred from hepatitis donor mice to recipient mice | | | | | | |
| | Normal B6 mice | 10 | 31·0±6·4 | 16·5±3·5 | 2·7±0·8 | 0·9±0·5 | 0·4±0·3 |
| | B6 mice depleted of total lymphocytes† | 10 | 27·5±5·1 | 18·9±4·4 | 2·8±0·3 | 1·2±0·7 | 0·3±0·1 |
| II. | The effects of primed NWA spleen cells of hepatitis donor mice on distribution of $^{51}$Cr-labelled normal spleen cells reconstituting the total lymphocyte-depleted recipient mice | | | | | | |
| | B6 mice transferred with primed NWA spleen cells from hepatitis donor mice | 10 | 38·4±5·6‡ | 15·2±2·5 | 2·5±0·7 | 1·0±0·7 | 0·2±0·2 |
| | Control (B6 mice transferred with unprimed NWA spleen cells from normal B6 mice) | 10 | 25·2±3·5‡ | 21·4±3·4 | 2·8±0·5 | 1·3±0·4 | 0·2±0·1 |

Results are expressed as mean ± s.d. percentage of the injected radioactivity localized within a whole organ or tissue sample as described in 'Materials and Methods'.

*0·5 ml.

†B6 mice were pre-treated by 700 rad whole body irradiation for total lymphocyte depletion.

‡Difference was significant at $P < 0·01$.

the reconstituted recipient mice. Liver injury was also very mild in recipient mice reconstituted with the NWNA or the NWA fraction of normal spleen cells.

*Tissue localization of $^{51}$Cr-labelled cells*

The *in vivo* distribution of $^{51}$Cr-labelled spleen cells in the recipient mice is provided in Table 4. $^{51}$Cr-labelled NWA spleen cells obtained from hepatitis donor mice were transferred to normal recipient mice, and the liver and other organs of these recipient mice were removed 7 days after the transfer. The radioactivity in these livers was higher than that in the spleen or any other organs (Table 4(I)). However, there was no significant difference in the tissue distribution of $^{51}$Cr-labelled primed spleen cells whether the recipient mice had been depleted of total lymphocytes by irradiation or not. In the second experiment, the totally lymphocyte-depleted recipient mice were reconstituted with $^{51}$Cr-labelled spleen cells from normal B6 mice. When primed NWA spleen cells from hepatitis donor mice were transferred to these reconstituted recipient mice, the radioactivity of liver was significantly higher compared to that in the recipient mice transferred with unprimed NWA spleen cells from normal B6 mice (Table 4(II)).

## DISCUSSION

Experimental hepatitis has been produced in rabbits (Meyer zum Büschenfelde & Hopf, 1974; Uibo *et al.*, 1982) and in mice (Sceiffarth, Warnatz & Mayer, 1967; Kuriki *et al.*, 1983). It has also been shown that liver damage could be induced in recipient mice transferred with sensitized spleen cells from mice with experimentally induced hepatitis (Warnatz, Scheiffarth, Wolf & Schmidt, 1967; Kuriki *et al.*, 1983; Mori Y. *et al.*, 1985). The results of such transfer experiments seem to indicate the significance of cellular immunity in the development of liver injury in recipient mice. However, the precise functional role of the transferred cells is not known.

In our transfer study, it was shown that NWA T-cells in the spleen of hepatitis mice are responsible for the induction of hepatitis in recipient mice. With the use of low-dose (300 rad) irradiation to deplete suppressor T-cell function in hepatitis donor mice, more prominent liver damage was induced in the liver of recipient mice transferred with unfractionated or NWA spleen cells from these irradiated hepatitis donor mice. Further transfer study was performed using recipient mice pre-treated by whole body irradiation. No liver lesion was induced either in low-dose-irradiated recipient mice nor in the recipient mice totally depleted of lymphocytes by a high-dose irradiation. The reduced susceptibility of irradiated recipient mice to primed spleen cells from hepatitis donor mice was restored to normal after reconstitution with normal spleen cells. These results indicated that primed spleen cells from hepatitis donor mice would not cause liver injury in the recipient mice without recipient-derived radiosensitive cells containing precursor cells of helper and/or cytotoxic T cells. By the cell migration study, it was found that a considerable number of the infiltrating cells in the livers of recipient mice were derived from recipient mice themselves. Recipient-derived T cells which infiltrated in the liver of recipient mice may have worked as helper T cells enhancing the cytotoxic effects of donor T cells. Alternatively, it seems possible that the infiltrating cells derived from recipient mice cause liver injury in recipient mice themselves. We have already studied and reported on T cell interaction between donor and recipient mice (Mori Y. *et al.*, 1985). In this study, it was further suggested that primed spleen cells from hepatitis donor mice worked as amplifier or helper cells which transmit information to radiosensitive cells of recipient mice and that those educated T cells derived from recipient mice became, at least in part, the final effector cells responsible for liver damage in the recipient mice.

Cellular interactions in the development of organ-specific autoimmune diseases have been investigated in EAE. Encephalomyelitis can be produced in normal mice or rats by transfer of T cells sensitized with central nervous tissue antigen (Bernald, Leydon & Maccay, 1976; Holda *et al.*, 1983). Induction of EAE in recipient mice by transfer of these sensitized T-cells was inhibited when lymphocytes of the recipient mice were totally depleted by *in vivo* irradiation (Bernald, Leydon & Mackay, 1976). Holda *et al.* (1983) used a sequential transfer system for EAE and showed that T cells sensitized *in vitro* recruited EAE effector cells in normal syngeneic recipient rats (Holda *et al.*, 1983). These results are consistent with our current results which suggested the necessity of cellular interactions for the development of autoimmune disease.

It is well known that antibody-dependent cellular cytotoxicity or T cell-mediated cytotoxicity is the effector mechanism in chronic active hepatitis (CAH). However, the persistence of liver-directed autoimmune reactions in CAH is related to the abnormalities of immunoregulation (Vento & Eddleston, 1987). It has been reported that cytotoxic/suppressor T cells are predominant at the site of liver injury, but helper/inducer T cells are also demonstrable *in situ* (Corucci *et al.* 1983). Recently, Barnaba *et al.* investigated *in vitro* the role of T cells with the helper/inducer phenotype in patients with CAH (Barnaba *et al.*, 1986). Stefanini *et al.* showed *in vitro* that two mechanisms were operative in the cytotoxic reactions in patients with CAH (Stefanini *et al.*, 1986). These reports seem to indicate that several types of cells, such as antigen-presenting cells, helper T cells, suppressor and/or cytotoxic T cells are involved in the induction and progression of CAH. Further studies on the interactions of these cells in man and animal models would be desirable in the analysis of the pathogenesis of certain types of liver disease.

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