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# SUPPRESSION OF ANTIBODY RESPONSES IN VITRO BY BONE MARROW CELLS IN RATS WITH HEREDITARY LIVER INJURY

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## INDEXING WORDS

LEC rats; bone marrow; copper-deprivation

## SYNOPSIS

The primary antibody responses of spleen cell cultures were investigated for LEC rats, a mutant strain of LEA rats, which are known to develop spontaneous hepatic injuries. Compared to LEA rats, LEC rats were significantly low in responding not only to a T-dependent antigen, SRBC, but also to a B-cell stimulant, LPS. The antibody responses of LEC rats having fed a diet deprived of Cu metal (LEC/Cu-) showed values intermediate between LEA and LEC rats, thus suggesting a recovery from an immuno-deficient state as well as liver damage in LEC/Cu- rats. The addition of LEA bone marrow cells prior to culture to LEA or LEC spleen cells, strikingly enhanced the antibody response, whereas LEC bone marrow cells were totally ineffective, or sometimes depressed the response.

These results have suggested a newer concept for a possible

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correlation between liver-dysfunction and bone marrow cell-functions in LEC rats, both encoded by autosomal genes.

## INTRODUCTION

Long Evans Cinamon(LEC) rats, originating from a mutant rat of the Long Evans Agouti(LEA) strain by means of coat-color difference, have shown a high incidence of spontaneous hepatic injury and hepatocarcinoma[10]. These hepatic defects were reported to be due to a deficiency of $CD4^+$ T helper cell function relating to the recessive *thid* gene[5, 6]. However, exact relationships between helper T-cell deficiency and the incidence of hepatocarcinoma have not been established. Recently, a chromosomal abnormality has been known as to bone marrow cells in LEC rats[4]. This suggests an alteration in phenotypic properties of bonemarrow cells in LEC rats.

The present study aims to elucidate functional differences in bone marrow cells between LEC and LEA rats.

## MATERIALS AND METHODS

### *Rats*

Inbred strains of LEA/K (Long Evans agouti)(RT1.A) and its mutant strain, LEC/Mk (Long Evans cinamon) (RT1. Au) of exclusively female and 4 weeks old rats maintained in our laboratory under careful pathogen-free conditions were employed in this study.

### *Tissue culture and hemolytic plaque assay*

Bone marrows and spleens taken from LEA and LEC rats were separated into free cells by the method reported previously[8]. Eagle's minimal essential medium (MEM, GIBCO Laboratories, Inc.,Grand Island,N.Y.) supplemented with amphotericin B solution (250 μg/ml), penicillin G (5000 I.U/ml) (Flow Laboratories, Inc., North Ryde, N.S.W. Australia), and 10% fetal calf serum (Hyclone Laboratories, Inc, Logan, Utah) was employed throughout the present experiment. One ml of cell suspension containing varying amounts of spleen or bone marrow cells was added to a 1 ml of culture containing $1x10^7$ spleen cells (basic cell culture) and 0.1 ml of 50% SRBC (sheep erythrocytes, Nihon Biotest Lab., Inc.,

Tokyo) for anti-SRBC response, or 10 μg/ml LPS (lipopolysaccharide W, Difco Laboratories, Inc., U.S.A.) for B cell response. The antigenic and mitogenic doses were determined preliminarily by a number of experiments designed as reported previously in mice[7, 8]. These cultures were incubated stationarily at 37°C in a humid atmosphere of 5% $CO_2$ in air.

The plaque-forming cell (PFC) responses of cultured cells were assayed by a modified slide method of Jerne and Nordin[3]. The cells recovered from cultures were suspended in MEM by adjusting the cell density to $1x10^7$ cells/ml, and mixed with a 400 μl aliquot supplemented with 50 μl of 50% SRBC and 50 μl of guinea pig complement (Nihon Biotest, Tokyo). Three chambers were prepared for one culture. They were left at 37°C under a humidified atmosphere of 5% $CO_2$ in air. For B cell responses, trinitrobenzen-sulphonic acid (TNP)-coupled SRBC(TNP-SRBC) was used instead of SRBC based on the method of Rittenberg and Pratt[9]. The PFC numbers were counted under a microscope, and expressed as mean PFC/culture for three cultures ± standard error (SE) of the mean. Each experiment was repeated twice or thrice, and representative results are shown in figures. P values were determined by Student's t-test, and $p<0.05$ was regarded as significant.

## RESULTS

*Comparison of anti-SRBC and anti-TNP-SRBC PFC responses between LEA and LEC rats*

LEA or LEC spleen cells were cultured at various cell densities shown in Fig.1, and dose response curves were determined by assays made 3, 4 and 5 days after culture. Under the present experimental conditions, a peak PFC response of LEA spleen cultures was observed day 3 after culture (Fig.3). So far as revealed by the dose response curves, the peak PFC responses of LEC rats were apparently lower than those of LEA rats (Fig.1). However such difference was somewhat variable for the cultures taken day 4.

Anti-TNP-SRBC responses were employed for assaying LPS-induced B cell response in vitro. The B cell responses of LEC spleen cultures were strikingly low compared to those of LEA spleen cultures (Fig.2). These results strongly suggested that LEA rats are responders in terms of both T-dependent and T-independent antibody responses *in vitro*, whereas

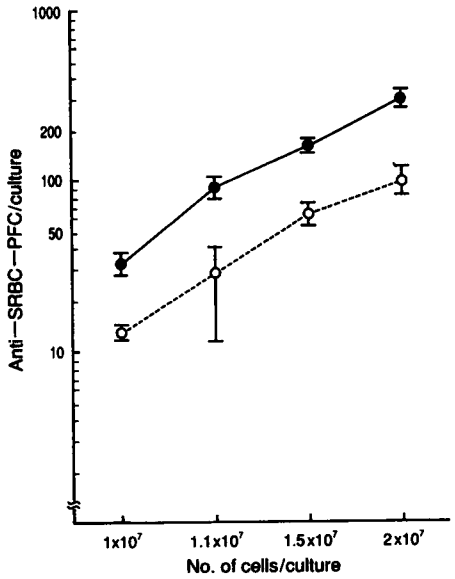


Fig. 1

Cultures of spleen cells ($2x10^7$, $1.5x10^7$, $1.1x10^7$, $1x10^7$) from LEA (A-Sp) or LEC (C-Sp) rats were incubated with SRBC. Anti-SRBC responses were assayed on day 3 after culture. Bars indicate SE of the mean for three chambers. (—●—) A-Sp; (--○--) C-Sp.

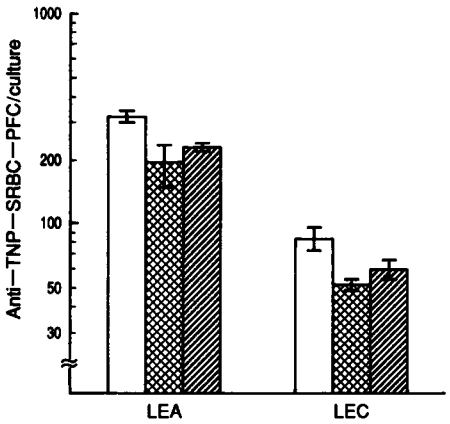


Fig. 2

Varying amounts of spleen cells ($2x10^7$, $1.5x10^7$, $1x10^7$) from LEA or LEC rats were cultured with LPS (10 μg/ml). LPS-induced B cell responses were determined in the PFC responses to TNP-SRBC on day 3. Bars indicate SE of the mean for three chambers.
(□) $2x10^7$ cells; (▩) $1.5x10^7$ cells;
(▨) $1x10^7$ cells.

LEC rats are low responders in this respect

*Recovery of antibody response by feeding diet lacking of Cu*

LEC rats which had been fed with diet deprived of Cu metal, LEC (Cu-) rats were shown to develop low incidence of hepatocarcinoma compared to those LEC rats fed with normal diet[4]. Based on this finding, splenic PFC responses to SRBC were studied in vitro by comparing LEA, LEC, and LEC (Cu-) rats. Varying amounts of spleen cells from these rats were cultured respectively in the presence of SRBC and assayed for their capability to produce PFC on 3, 4 and 5 days after culture. Among the rats compared, LEA rats showed the highest PFC responses, LEC rats were the lowest, and LEC (Cu-) rats showed levels intermediate between LEA and LEC rats (Fig.3, 4). Therefore, the immuno-deficient state of LEC rats also considerably improved by feeding diet deprived of Cu metal.

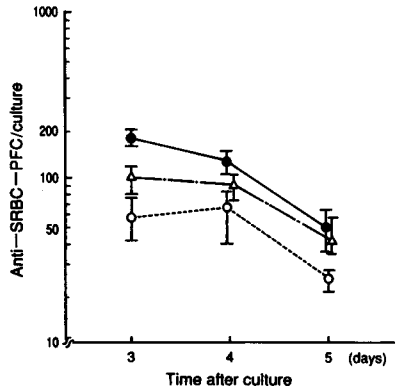


Fig. 3

Comparative anti-SRBC PFC responses of spleen cell cultures ($2x10^7$/2 ml) prepared from LEA (—●—), LEC (--○--), or LEC rats fed with CU-diet (LEC(CU-)(—Δ—)). Assay of PFC were made day 3, 4, and 5. Bars indicate standard errors of the mean for three chambers.

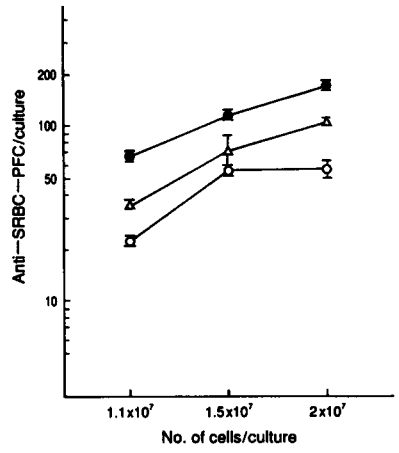


Fig. 4

Dose response curves of spleen cell cultures from LEA, LEC, or LEC(CU-) rats. Varying amounts of spleen cells ($2x10^7$, $1.5x10^7$, $1.1x10^7$) from LEA (●), LEC (○), or LEC (CU-)(Δ) were cultured with SRBC for 3 days. Bars indicate SE of the mean for three chambers.z

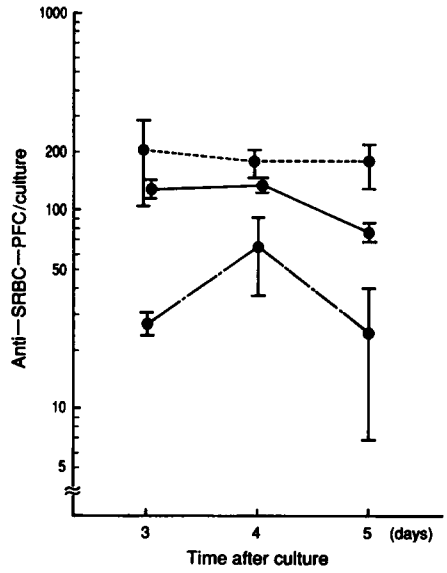


Fig. 5

LEA spleen cells ($2x10^7$) were cultured with bone marrow cells ($1x10^7$) from LEA or LEC rats. PFC responses were assayed on day 3, 4, and 5. Bars indicate SE of the mean for three chambers. (—●—) A-Sp; (--●--) A-Sp+A-BM; (-·-●-·-) A-Sp+C-BM.

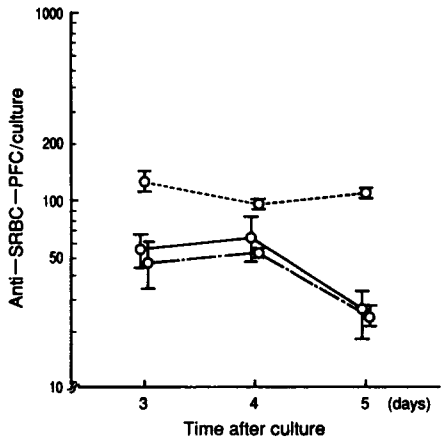


Fig. 6

LEC spleen cells ($2x10^7$) were cultured with bone marrow cells ($1x10^7$) from LEA or LEC rats. PFC responses were assayed on day 3, 4, and 5. Bars indicate SE of the mean for three chambers. (—○—) C-Sp; (--○--) C-Sp+A-BM; (-·-○-·-) C-Sp+C-BM.

*Effects of bone marrow cells on the anti-PFC responses in spleen cultures*

To a constant amount ($1x10^7$ cells/ml) of LEA or LEC spleen cell cultures varying amounts of bone marrow cells taken from LEA or LEC rats were mixed and cultured together with SRBC. Fresh and cultured bone marrow cells did't produce detectable PFC in response to SRBC and LPS[8]. The addition of LEA spleen or bone marrow cells almost to an equal extent increased the peak PFC responses of basic ($1x10^7$ cells) LEA spleen cultures (Fig.5) and of LEC spleen cultures (Fig.6). In higher amounts of cells added, LEC spleen cells considerably increased the LEA splenic responses. However, LEC bone marrow cells were ineffective to stimulate the PFC responses of LEA spleeen cultures. Figures 5 and 6 are representatives showing increased PFC responses in LEA (Fig.5) and LEC (Fig.6) spleen cultures mixed with LEA bone marrow cells and decreased responses in those spleen cultures mixed with LEC bone marrow cells. Since bone marrow cells by themselves did not develop any countable plaque forming cells against SRBC, the increased number of PFC in splenic cultures admixed with LEA bone marrow cells were due largely to the stimulatory activity of the bone marrow cells. On the contrary, the LEC bone marrow cells were apparently lacking in this activity. In addition, it was important to notice the fact that similar addition of bone marrow cells to basic cell cultures, whether or not the bone marrow was coming from LEA or LEC rats, gave almost negligible influence on the development of splenic B cell responses.

## DISCUSSION

The present results have demonstrated that compared to LEA rats PFC responses of splenic cell cultures to SRBC are significantly low in LEC rats. The LEC rats are reported to have a T cell dysfunction, which was caused by a mutant gene, *thid*, while killer T- and B-cell functions of the rats were found to be normal[1]. Subsequently, this helper T cell-deficiency in LEC rats was reported to be due to maturation arrest of T cells in the thymus[11]. Moreover, the present results have shown that anti-TNP-SRBC antibody responses of splenic cultures were also lower in LEC rats than in LEA rats. These results suggest LEC rats being deficient not only in a T cell, but also in a B cell function. Although the hereditary hepatic injury in LEC rats is caused by an

autosomal recessive gene, *hts*, there found no linkage between the *thid* and *hts* loci[11]. This finding has suggested that the development of hepatic injury is independent of the immuno-deficiency. However, recently LEC rats are found to be lacking of an autosomal gene, which encoded for a genetic dysfunction of liver, like Wilson disease in man, manifesting an anabolic abnormality of Cu metal[12]. Thus LEC rats accumulated Cu metal in the liver causing severe liver dysfunction. As was revealed in the present experiment the immuno-deficient state of LEC rats could be corrected by feeding the LEC rats with diet deprived of Cu. It is likely, therefore, that there provides a newer concept for a relationship between liver dysfunction and immuno-deficiency, both encoded by the same autosomal genes. This fact awaits further detailed genetic investigations. The present experiments have suggested that the immuno-deficient states of LEC rats is possibly correlated with a function of bone marrow cells. Bone marrow cells cultured for 3 or more days with or without adequate mitogens or SRBC generally decreased in number of viable cells and didn't show any significant responses to mitogens, while these bone marrow cells generally have an ability to suppress in vitro antibody responses in mice[2, 7]. Thus, the addition of LEC bone marrow cells to LEA splenic cultures significantly retarded the development of PFC responses, while LEA bone marrow cells were stimulatory for both LEA and LEC spleen cell cultures. However LEC and LEA bone marrow cells were both ineffective in stimulating B cell response. The stimulatory activity of LEA bone marrow cells was significantly decreased by prior treatment of the bone marrow cells with mitomycin or UV light. Taking together, it was likely that the stimulation of T-dependent PFC responses of spleen cultures by LEA bone marrow cells might not be due to a quantitative increase of mature B cells in bone marrow population, but caused by helper activity of T cell nature existing in the population. This stimulatory activity of bone marrow cells of LEA rats were apparently lacking in LEC rats; the bone marrow cells always acted to suppress rather than to stimulate the increasing PFC responses to SRBC. Furthermore, it has been shown that certain water-soluble substances derived from LEC but not from LEA bone marrow cells, have a depressing activity for PFC responces (unpublished results). It was interesting to consider that such suppressing substance is possibly disclosed to occur relating with a genetic defect in LEC rats. Therefore, more detailed studies of the bone marrow - suppressing factor are in progress relating to the

appearance or disappearance of ceruloplasmins, which play a critical role in the Wilson disease.

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