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# Increased Hepatic Barr Body and Thymic Epithelial Cell Dysplasia Found in Chronic Immune Responses to Rat Male (H-Y) Antigen

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***Summary.*** **Chronic immune reactions to syngeneic male (H-Y) antigen were studied in female and male Lewis rats. As donor rats, non-treated Lewis rats and pre-treated Lewis rats were selected. Heterochromatic bodies stained with aceto-orcein were counted in the liver cells of these rats. The Barr body percentage of female liver cells ranged from 5.2±1.8% to 13.0±2.4%, while inactive Y (Y body) percentages of male liver cells ranged from 2.8±1.0% to 5.3±0.8%. Heterochromatic changes of liver cells increased to more than 9.0% in females immunized with non-treated male cells. The livers of the females, which were stimulated by male spleen cells, showed focal lymphocyte and eosinophil infiltrations into portal areas. Mild allograft rejection to H-Y antigen was characterized by the increased Barr body of hepatocytes. The injection of either male cells preimmunized with female cells or once pregnant female cells induced more aggressive chronic responses to H-Y antigen. Additionally added monoclonal antibodies (mAb), rat class I (anti-I-A) mAb and class II (anti-Ia) mAb reacted to accelerate the immune responses to H-Y antigen. The aggressive immune responses observed 260 days after immune induction were characterized by weight loss and reversible thymic dysplasia. The numbers of cortical nurse cells and medullary Hassall's bodies were decreased in the dysplastic thymuses. The cytoplasm of thymic tissue basophils was stained with both NaF-sensitive esterase and keratin.**

## INTRODUCTION

The thymus is the organ where T cells with $\alpha\beta$ T cell receptor (TCR) are generated. It is said that the differentiation of $CD4^+8^+$ thymocytes into male (H-Y) antigen-specific $CD4^-8^+$ T cells is strictly dependent on the TCR interactions with the molecules of class I major histocompatibility complex (MHC).[1-4] $CD8^+$ T cells respond to antigen in association with MHC class I molecules. It has been demonstrated that the $\alpha_3$ domain of MHC class I molecules, which is bound to CD8, is analogous to a region of the MHC class II $\beta$-chain, $\beta_2$ domain which is critical for CD4.[5] $CD8^+$ T cells specific for H-Y antigen proliferated considerably in response to H-Y antigen when $CD8^+$ mature T cells were transferred into male nude mice, but not into female nude mice.[2,3] On the other hand, reversible thymic dysplasia has been reported to occur after the induction of graft-versus-host (GVH) disease.[6,7] The protein Lck ($p56^{lck}$), Src family of thyrosoine kinases, associates specifically with the cytoplasmic domains of both CD4 and CD8 T cells. Deficiency of the protein Lck leads to considerable thymic atrophy and a dramatic reduction in $CD4^+8^+$, $CD4^+8^-$ and $CD4^-8^+$ thymocyte populations.[8]

This paper describes allograft rejective reactions of $CD4^-8^+$ T cells to H-Y antigen and GVH reactions of $CD4^-8^+$ T cells to H-Y antigen, which were recognized in Lewis rats. Heterochromatic body percentages of liver cells and dysplastic changes of thymus were examined in these rats.

## MATERIALS AND METHODS

Lewis ($RT1^l$) rats 6.5-9 weeks old were used as hosts. The body weights of female hosts and male hosts were 83-101 g and 104-146 g, respectively, at the first immunization. Donor cells were obtained from syngeneic Lewis rats. Once pregnent or preimmunized

**Table 1.** Immune systems used in this study.

| Exp. No. | Lewis host T. No. | Sex | Wt (g) | Lewis donor cell Sex | Organ ($\times 10^8$ cells/rat) | Days after immunization |
|---|---|---|---|---|---|---|
| A | 5 | F | 101 | M | Thymus (T) (4.6) i.v. | 461 |
| B | 5 | F | 94 | M | Spleen (S) (4.8) i.v. | 339 |
| C | 5 | F | 83 | M | PBMC (0.2) i.v., S (1.2)+ Anti-Ia mAb (4 $\mu$g/rat) s.c. | 317-328 |
| D | 8 | F | 91 | M | T (4.6)+S (11.6) i.v. | 130 |
| E | 4 | M | 104 | F* | T (3.2) i.v. | 306 |
| F | 5 | M | 135 | F* | S (3.4) i.v. | 329 |
| G | 5 | M | 121 | F* | PBMC (0.02) i.v., S (0.8)+ Anti-I-A mAb (4 $\mu$g/rat) s.c. | 294 |
| H | 5 | M | 112 | F | T (5.7)+S (4.5) i.v. | 346 |
| I | 5 | M | 109 | F* | T (5.1)+S (4.5) i.v. | 367 |
| J | 7 | M | 146 | F* | T (1.2)+S (0.8)+LN (1.3) i.v. | 210 |
| K | 4 | M | 111 | F** | T (1.7)+S (3.8) i.v. | 260 |
| L | 5 | F | 100 | F** | T (1.7)+S (3.8) i.v., S (0.4)+ Anti-Ia mAb (4 $\mu$g/rat) s.c. | 268 38 |
| M | 5 | F | 87 | M*** | T (0.5)+S (5.4) i.v. | 263 |

PBMC: peripheral blood mononuclear cell, LN: Lymph node.
*Once pregnant female, **Exp. No. D female, ***Exp. No. J male.

Lewis rats were also used as donor rats. All animals were bred and mainteined in our animal colony.

### Immunization

Single Lewis cells were suspended in Hanks' balanced salt solution (HBSS) (Nissui Pharmaceutical Co., LTD). The donor cells were washed with the HBSS 2-3 times. Two kinds of monoclonal antibodies (mAb) were purchased from the Japan Scientific Instrument Co., LTD. One was the lgG1 mAb recognizing a monomorphic determinant of rat class I MHC antigens (anti-I-A mAb); the other was the IgG1 mAb recognizing a determinant on the $\alpha$ chain of rat Ia antigen (anti-Ia mAb). Experimental systems were classified into 13 groups. Experimental numbers (Exp. Nos.) ranged from Exp. Nos. A to M. In Exp. Nos. A, B, C, D, L and M, host rats were Lewis females, while in Exp. Nos. E, F, G, H, I, J and K, host rats were Lewis males. Exp. Nos. A, B, C, D and M rats were immunized with Lewis male cells intravenously. Exp. Nos. E, F, G, H, I, J, K and L rats were immunized with Lewis female cells intravenously. Among them, Exp. Nos. C and L rats were injected with 4 $\mu$g anti-Ia mAb per rat subcutaneously. Exp. No. G rats were injected with 4 $\mu$g anti-I-A mAb per rat subcutaneously. Table 1 summarizes the immune systems in detail.

### Assessment

*Body weight*: All rats were checked for weight changes. *Aceto-orcein staining*:[9] Orcein was purchased from Katayama Chemical Co., LTD. Single liver cells were spread over the slide. The slides were stained with aceto-orcein by placing 4 to 5 drops of 0.1% stain solution on them for 10 min. Following fixation, the slides were placed in Mayer's hematoxylin solution for 20 sec. More than 1,000 liver cells were counted to determine the frequencies of the Barr body and inactive Y (Y body). *Histopathology*: Livers and thymuses were fixed in 20% formalin. Paraffin-embedded sections were cut and stained with Hematoxylin-Eosin (H-E). Cortical nurse cells enclosing viable T cells were calculated in 15 fields under a microscope of 50 magnifications.[10] Total numbers of Hassall's boby were counted in either the right or left lobe. Cytochemical NaF-sensitive esterase staining (Schmalzl's method) and keratin staining (Martinotti's method) were applied to some thymuses.

## RESULTS

**Body weight changes**: Fig. 1 indicates the weight changes of Exp. Nos.—Rat Nos. E-4, F-1, G-3, 4 & 5, I-1, C-2 and M-3 & 5 rats. Except for Exp. No.—Rat

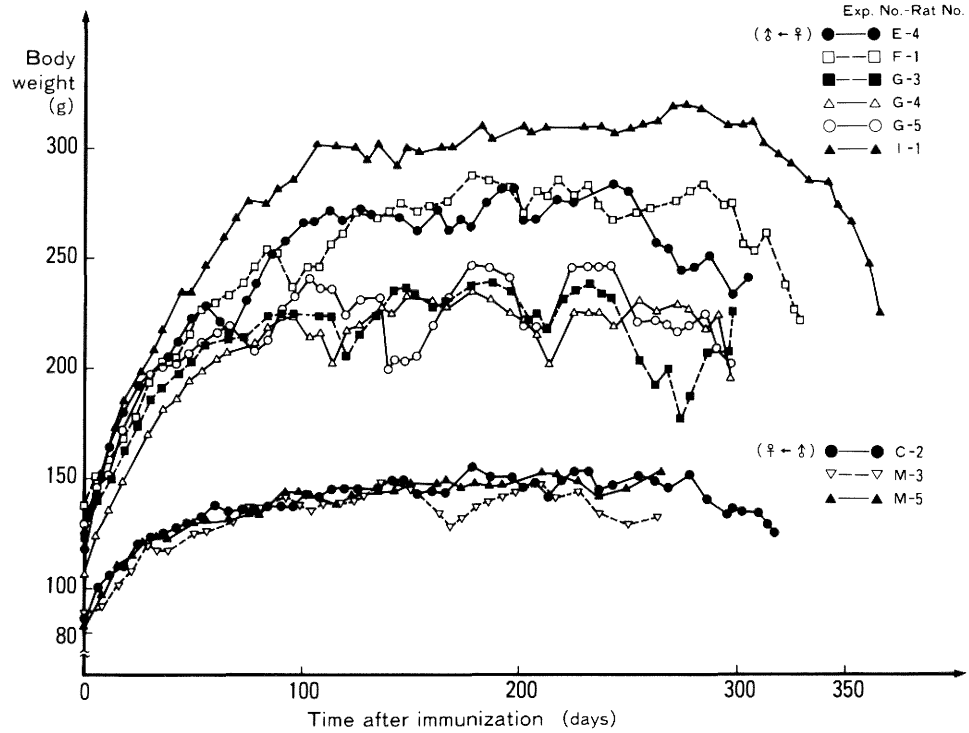


**Fig. 1.** Body weight changes after immune induction. Body weight changes of 9 Lewis rats are shown. Exp. No.—Rat No. is described at the left side of the figure together with host and donor sexes. Except for Exp. No. —Rat No. M-5 rat, the other 8 rats had obvious weight loss.

No. M-5 rat, all these rats lost weight for about 260 days after immune induction. Exp. Nos.—Rat Nos. E-1 and H-5 rats that are not shown in Fig. 1 died from weakness 305 and 228 days after cell injection, respectively. Since day 40 of postinduction, weight gain was suppressed in Exp. No.—Rat Nos. G-3, 4 & 5 male rats that were injected with anti-I-A mAb together with female cells. Exp. No.—Rat No. C-2 female rat was injected with anti-Ia mAb together with male cells. All the other rats of this study did not show any clear weight loss.

**Liver changes**: Table 2 summarizes frequencies of rat liver cells with a Barr body or an inactive Y (Y body). More than 9% of Barr bodies were regarded as higher values than normal. Exp. No.—Rat Nos. A-1 & 2 rats had 11.6±1.3% Barr bodies. Exp. No.—Rat No. C-4 rat had 9.1% Barr bodies. Exp. No.—Rat Nos. D-2, 5 & 8 had 13.0±2.4% Barr bodies. In male hosts, Y bodies stained with aceto-orcein were generally less than 5%. Fig. 2 demonstrates the Barr body obtained from Exp. No.—Rat No. D-5 female and the Y body prepared from normal male. The Barr body was released from the uncleus and the Y body in the uncleus was stained with aceto-orcein. Fig. 3 is the liver section of Exp. No.—Rat No. B-2 rat. Focal

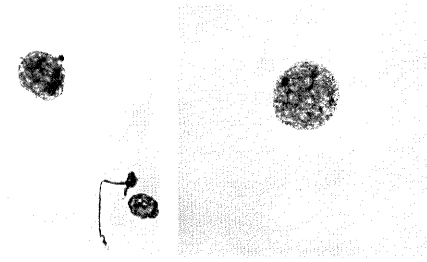

**Fig. 2.** Barr body and inactive Y (Y body) in liver nucleus. This figure shows dark stained Barr body released from the nuclear membrane and dark stained Y body in nucleus. The Barr body was observed in the liver cells of Exp. No.—Rat No. D-5 female. The Y body was observed in the liver cells of 2 m.o. normal male. Aceto-orcein stain, ×480.

**Table 2.** Frequencies of either Barr bodies or inactive Y bodies in rat liver nuclei.

| Exp. No.-Rat No. (Female host) | Barr body % ($\bar{M} \pm SD$) | Exp. No.-Rat No. (Male host) | Y body % ($\bar{M} \pm SD$) |
|---|---|---|---|
| A-1, 2 | 11.6 ± 1.3 | E*2, 3, 4 | 4.5 ± 0.8 |
| -3, 4, 5 | 6.4 ± 0.7 | F-1, 2, 3, 4, 5 | 5.0 ± 1.4 |
| B-1, 2, 3, 4, 5 | 5.7 ± 0.3 | G-1, 2, 3, 4, 5 | 4.4 ± 0.6 |
| C-1, 2, 3, 5 | 5.3 ± 1.4 | H*1, 2, 3, 4 | 2.8 ± 1.0 |
| -4 | 9.1 | I-1, 2, 3, 4, 5 | 3.4 ± 1.3 |
| D-1, 3, 4, 6, 7 | 5.2 ± 1.8 | J-1, 2, 3, 4, 5, 6, 7 | 3.9 ± 1.2 |
| -2, 5, 8 | 13.0 ± 2.4 | K-1, 2, 3, 4 | 5.3 ± 0.8 |
| L-1, 2, 3, 4, 5 | 5.8 ± 0.9 | Cont. (2m.o.) | |
| M-1, 2, 3, 4, 5 | 7.2 ± 0.9 | -1, 2, 3, 4 | 4.5 ± 1.1 |
| Cont. (4-8m.o.)** | | | |
| -1, 2, 3, 4, 5, 6 | 4.6 ± 0.8 | | |
| Cont. (13m.o.) | | | |
| -1, 2, 3, 4, 5, 6, 7 | 5.6 ± 0.8 | | |

*E-1 and H-5 rats were not examined. **Once pregnant female rats.

**Table 3.** Numbers of cortical nurse cells and medullary Hassall's bodies in rat thymus.

| Exp. No. (total ♀ rat No.) | Nurse cell (per 15 fields) ($\bar{M} \pm SD$) | Hassall's body (a lobe) ($\bar{M} \pm SD$) | Exp. No. (total ♂ rat No.) | Nurse cell (per 15 fields) ($\bar{M} \pm SD$) | Hassall's body (a lobe) ($\bar{M} \pm SD$) |
|---|---|---|---|---|---|
| A (n=5) | 56 ± 8 | 26 ± 4 | E (n=2) | 60 ± 3 | 78 ± 7 |
| B (n=5) | 63 ± 4 | 45 ± 18 | E-4* (n=1) | 54 | 8 |
| C (n=4) | 63 ± 6 | 43 ± 29 | F (n=4) | 74 ± 6 | 56 ± 8 |
| C-2* (n=1) | 43 | 27 | F-1* (n=1) | 40 | 20 |
| D (n=8) | 63 ± 8 | 76 ± 15 | G (n=2) | 65 ± 5 | 57 ± 1 |
| L (n=5) | 66 ± 14 | 49 ± 11 | G-3* (n=1) | 72 | 77 |
| M (n=3) | 80 ± 2 | 52 ± 18 | G-4* (n=1) | ND but atrophic | |
| M-3* (n=1) | 42 | 34 | G-5* (n=1) | 45 | 4 |
| M-5* (n=1) | 84 | 13 | H (n=4) | 64 ± 8 | 30 ± 9 |
| Control (4-8m.o.)** | | | I (n=4) | 54 ± 7 | 45 ± 5 |
| (n=6) | 85 ± 12 | 42 ±7 | I-1* (n=1) | ND but atrophic | |
| Control (13m.o.) | | | J (n=7) | 72 ± 6 | 53 ± 18 |
| | 82 ± 9 | 45 ± 17 | K (n=4) | 78 ± 13 | 51 ± 22 |
| | | | Control (2m.o.) | | |
| | | | (n=4) | 88 ± 9 | 139 ± 41 |

(Exp. Nos.-Rat Nos. E-1 and H-5 rats were not examined.)
*Body weight changes are shown in Fig. 1. **Once pregnant females.

infiltrations of many eosinophils and lymphocytes were observed in a portal space.

**Thymic changes**: Histopathological findings of thymuses are summarized in Table 3. Nurse cell numbers were decreased to less than 45 in Exp. Nos.—Rat Nos. C-2, M-3, F-1 and G-5 rats. Hassall's body numbers were decreased to less than 13 in Exp. Nos.—Rat Nos. M-5, E-4 and G-5 rats. Thymic dysplasia was found in Exp. Nos.—Rat Nos. C-2, M-3 & 5, E-4, F-1 and G-5 rats. Weight change was apparent in all these rats, as shown in Fig. 1. Exp. No.—Rat No. G-3 rat that recovered from weight loss had normal

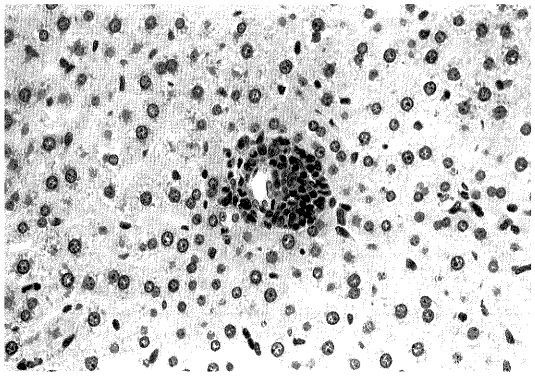

**Fig. 3.** Allograft rejective reaction found in the liver of Exp. No.—Rat No. B-2 rat. This figure shows focal infiltrations of eosinophils and lymphocytes into a portal space. Kupffer cells are enlarged in the liver. Liver cell regenerations are often recognized by two nuclear hepatocytes. H-E stain, ×200.

numbers of nurse cells and Hassall's body. No histopathological study is available for Exp. Nos.—Rat Nos. G-4 and I-1 rats, because macroscopically, the rats had as much atrophic thymus as deficiency. Many tubuli of a medullary type were found in Exp. No. A rats with 26±4 Hassall's body a lobe. Histopathological findings of dysplastic thymuses showed loss of distinct cortico-medullary demarcation, reduction of cortical lymphocytes and depletion of medullary epithelial cells and Hassall's bodies. The thymic tissue basophils were stained blue by Martinotti's keratin staining. Not all but many thymic tissue basophils were stained positively by the mixture of naphthol AS acetate and fast red violet LB salt.

## DISCUSSION

This paper has described chronic immune responses to H-Y antigen. T lymphocytes derived from the thymus, spleen, lymph node and peripheral blood mononuclear cell (PBMC) were used as donor cells to investigate the inductive mechanisms of chronic reversible changes to H-Y antigen. Preimmunized donor cells and two kinds of mAb (anti-I-A mAb and anti-Ia mAb) were also applied in this study, which caused aggressive allograft rejection.

Heterochromatic changes of liver cell nucleus were studied for identification of chronic immune responses to H-Y antigen. Barr bodies and inactive Y of liver cells were stained with aceto-orcein.[9] It was shown by aceto-orcein staining that the inactive X or Barr Body remained to be condensed during the interphase stage, which was later released from the nucleus. Even if quinacrine is usually applied to detect the heterochromatic distal long arm region of the Y chromosome, in this study, aceto-orcein staining was used to detect the inactive Y (Y body) of liver cells. Inactive Y chromosome percentages did not increase to more than 5.3±0.8%. In Exp. Nos.—Rat Nos. A-1 & 2, C-4 and D-2, 5 & 8 female rats, the inactive X, Barr body, was increased to 9.1% to 13.0±2.4%. Increased Barr body percentage indicated that XO changes of the rats became faster than normal XO changes. The increased numbers of Barr bodies implied that chronic rejection to H-Y antigen made progress gradually in the female host liver. However, the regeneration of liver cells with a new nucleus was recognized at the same time in the liver section, which indicated the chronic rejection of H-Y antigen. Mild chronic rejections to H-Y antigen were characterized by an increased Barr body of liver cells as a result, when non-treated male lymphocytes had been injected into female rats. Donor T cells obtained from not only the spleen but also the thymus were concerned with the chronic rejections to H-Y antigen.

Among the 5 rats of Exp. No. G that received anti-I-A mAb, three rats showed suppressed weight gain since day 40 of post-induction. Donor $CD4^-8^+$ T cells, especially derived from PBMC, rejected host H-Y antigen chronically in the hosts that were injected with anti-I-A mAb. It was suggested that the binding of anti-I-A mAb to MHC class I antigens changed the host cells into strong target cells, or that anti-I-A mAb suppressed the thymic proliferation of host $CD8^+$ T cells. Anti-Ia mAb led one of 5 females of Exp. No. C to aggressive allograft rejection. The effect of anti-Ia mAb on allograft rejection was also observed when anti-Ia mAb was injected with PBMC. Anti-MHC mAb combined with PBMC exaggerated chronic immune responses to H-Y antigen. Female $CD4^-8^+$ T cells which had received fetomaternal immunization rejected host H-Y antigen aggressively in Exp. Nos. E, F and I rats. In Exp. No. M, two of the 5 females rejected the male cells pre-treated with once pregnant female cells. Preimmunized female T cells, which were present in donor cells, reacted severely to H-Y antigen.

Thymic epithelial cells were adequate to identify the aggressive immune responses to H-Y antigen-specific $CD4^-8^+$ T cells. Weight loss signs which were recognized about 260 days after immune induction were correlated with the reversible thymic dysplasia. As the causes of thymic dysplasia, epithelial cell dysplasia was determined based on the decreased numbers of nurse cells and Hassall's body. The lack

of protein Lck ($p56^{lck}$) was also considered to be an another cause in the aggressive immune responses to H-Y antigen.[8] An absolute increase of thymus-derived $CD8^+$ T cells was disturbed in the hosts with aggressive immune responses to H-Y antigen.

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