Low Level of Immunoglobulin G2a Subclass Correlates with a Deficiency in T Helper Cell Function in LEC Mutant Rats

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ABSTRACT. Long-Evans Cinnamon (LEC) rats bear a congenital deficiency in CD4⁺8⁻ thymocytes and consequently a deficiency in helper T cell function. This mutation is caused by a single recessive gene referred to as thiid (T helper immunodeficiency). It has been reported that rat immunoglobulin Ig G2a subclass is a counterpart of the mouse IgG1. Serum IgG2a levels in LEC rats were ten-fold lower than those of normal rats. To identify a cause of low IgG2a levels in LEC rats, we made backcross rats, (F344 x LEC)F₁ x LEC, and examined linkage to the thiid mutation. The serum IgG2a levels of rats showing thiid/thiid phenotype were much lower than those of rats showing +/thiid phenotype. This indicates that the thiid mutation correlates with low level of IgG2a subclass. Furthermore, LEC rat B cells were shown to secrete IgG2a normally when these were stimulated with LPS and IL-4, suggesting that a cause of low level of IgG2a was due to defect of T cell function but not due to B cell dysfunction in LEC rats. These results confirm the idea that T helper (Th) function is necessary for the class switch to IgG2a subclass in rats. — Key words: immunoglobulin G2a subclass, LEC rat, T helper immunodeficiency.


The LEC rat was originally established as a mutant strain that develop hepatic injury and hepatocarcinoma [9, 11, 15]. The gene responsible for these hepatic diseases was designated as hts [20]. Besides hts mutation, LEC rats exhibit a single recessive mutation, thiid (T helper immunodeficiency), which causes a maturational arrest from CD4⁺8⁻ to CD4⁺8⁺ thymocytes and consequently causes a defect to T helper (Th) function [1, 2, 13, 20].

Serum levels of immunoglobulin (Ig) G1 is markedly elevated in interleukin (IL)-4 transgenic mice [17], whereas as they are decreased in IL-4 gene-disrupted mice [10]. Furthermore, in vitro study shows that IL-4 strikingly enhances the secretion of IgG1 [16]. From these studies, IL-4 is believed to play an important role in the class switch from IgM to IgG1 subclass in mice. LEC rat lymph node (LN) cells exhibit a defect of cytokine production, including IL-2 and IL-4 [1, 13]. These facts led us to examine the serum IgG1 subclass in LEC rats, since the class switch of IgG1 subclass needs a help of Th cells, especially cytokines secreted by Th cells. It has been reported that rat IgG2a is homologous to mouse IgG1 with respect to structural and antigenic characteristics [3, 5]. Recently, it has been shown that serum IgG2a levels are selectively suppressed in LEC rats during development [8]. Therefore, in this paper, we examined the relationship between serum IgG2a level and the thiid mutation.

Rats: Inbred LEC/TJ and F344/TJ rats were bred in our laboratory under specific pathogen-free conditions. Backcross rats were produced by mating (F344 x LEC)F₁ with LEC rats.

Antibodies: Anti-rat CD4 mAb, W3/25 [18] and anti-rat CD8 mAb, OX8 [4] were gifts from Dr. M. Miyasaka (Tokyo Metropolitan Institute for Medical Science, Tokyo, Japan). Anti-CD4 mAb was purified from the supernatants of W3/25 hybridoma cell line and labeled with fluorescence isothiocyanate (FITC) by the standard procedure. Anti-rat IgG2a mAb was purchased from Zymed Laboratories, Inc. (California, U.S.A.).

Enzyme-linked immunosorbent assay (ELISA): Sera were obtained from rats at 12 weeks of age. Serum IgG2a level was measured by ELISA, using microculture wells coated with 5 µg/ml mouse anti-rat IgG2a. Serially diluted serum ranging from 1:1,000 to 1:24,000 were added to the wells, incubated at 37°C for 60 min, and then washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20. Biotin-conjugated goat anti-rat IgG was diluted 50,000-fold, added to the wells, and then incubated for 60 min at 37°C. Then, horseradish peroxidase-conjugated avidin was added after diluting 5,000-fold with PBS containing 1% BSA. After washing, enzymatic activity was visualized using the substrate, 2,2'-azino di-(3-ethylbenzthiazoline sulphonate). Optical density was measured using a test wavelength of 405 nm and a reference wavelength of 620 nm.

Determination of thiid phenotype: Heparin-treated peripheral blood samples were collected from the tail vein. Blood cells were stained with FITC-conjugated anti-CD4 mAb, W3/25. After washing the cells with PBS containing 0.1% BSA, the red blood cells were lysed by treating them with 10-fold-diluted FACS lysing solution. Stained cells were analyzed by a FACScan. The thiid phenotype was determined based on the percentage of CD4⁺ cells as described in previous report [14].

Serum IgG2a levels were quantified by ELISA in LEC and normal (F344) rats. The levels of serum IgG2a in LEC rats were approximately ten-fold lower than those of F344 rats (Fig. 1). It has been reported that lacking major histocompatibility class II (MHC class II) molecules results in an elimination of CD4⁺ cells, a defect of Th function, and a low level of serum IgG1 [7]. Furthermore, IgG1 levels were reduced in mice disrupted the IL-4 gene [10]. These studies suggest that the class switch from IgM to IgG1 requires Th function. To examine whether the thiid mutation correlates with the low IgG2a level, we produced backcross rats by mating (F344 x LEC)F₁ with
LEC rats and examined relationship between the serum IgG2a level and the \textit{thid} mutation in these rats. When peripheral blood lymphocytes from backcross rats were examined, normal and Th deficiency phenotype were clearly distinguished as already shown in previous paper [14]. As shown in Fig. 2, the serum levels of IgG2a in rats showing thid/thid phenotype were lower than those of rat showing -thid phenotype. The mean O.D. of rats showing -thid and thid/thid phenotypes were 0.635±0.094 and 0.431±0.081, respectively. Statistically significant difference was observed between the two groups (p<0.01). Serum levels of IgG1 in LEC rats were reported to be also lower than those of normal rats [12]. However, this phenotype was shown to be independent of the thid mutation [14].

LEC rat B cells were shown to normally respond to T-cell-independent antigen [1]. But we could not exclude a possibility that the low levels of IgG2a in LEC rats might be caused by disfunction of B cells. To examine this possibility, purified LEC rat B cells were stimulated with lipopolysaccharide (LPS) and IL-4 and the level of IgG2a in the supernatants was quantified by ELISA. As shown in Fig. 3, LEC rat B cells secreted comparable level of IgG2a upon LPS and IL-4 stimulation. This result suggests that the low level of IgG2a in LEC rats is not due to disfunction of B cells but due to a lack of Th function.

In conclusion, we showed that serum level of IgG2a in LEC rats is congenially low and the low level of IgG2a is caused by congenital defect of Th function.

REFERENCES