Sex Differences in the Densities of Epidermal Langerhans Cells of the Mouse

Yoh-ichi Koyama, Ph.D., Shizuko Nagao, B.H., Koji Ohashi, B.H., Hisahide Takahashi, Ph.D., and Tohru Marunouchi, Ph.D.

Division of Cell Biology (YK, KO, TM) and Center of Laboratory Animal (SN, HT), Institute for Comprehensive Medical Science, Fujita-Gakuen Health University, Toyoake, Aichi, Japan

Cutaneous immune reactions are known to show sexual dimorphism. Langerhans cells (LCs) are bone marrow–derived immune cells in the epidermis and are essential to immune reactions in the skin. In the present research, a study was made of the differences in LC density of male and female mice. Epidermal sheets were separated from the skin of the glabrous part of hind limbs and ears of specific pathogen-free (SPF) mice by ethylenediaminetetraacetic acid (EDTA) treatment and stained for adenosine triphosphatase (ATPase) activity. The density of LCs of hind limb epidermis in male C57BL/6 (823 ± 20/mm²) and BALB/c (1689 ± 66/mm²) mice was significantly less than that in females (1363 ± 52/mm², p < 0.001; 2249 ± 105/mm², p < 0.001, respectively). Langerhans cell density in the ears of male C57BL/6 (465 ± 24/mm²) mice was also significantly less than that in females (542 ± 17/mm², p < 0.02). Although ovariectomy failed to bring about any change in the LC density of hind limb epidermis in female C57BL/6 mice, the LC density in male C57BL/6 mice increased significantly at 4 weeks following ovariectomy (sham operation, 564 ± 27/mm²; castration, 1179 ± 49/mm², p < 0.001). These results indicate that mouse epidermal LC density depends on sex, i.e., male mice have fewer LCs than female mice. The reduction in LC density in males may possibly be caused by the testis.

Langerhans cells (LCs) were first discovered by Paul Langerhans in 1868 in a gold chloride-stained preparation of skin [1]. Although their origin and functions are controversial, it is now well established that LCs are bone marrow–derived immune cells in the epidermis. They express La antigens and are essential to the induction of T-cell response in the self-nonself discrimination system (reviewed in [2,3]). For example, depletion of LCs from the epidermal cell population markedly reduces T-cell response, suggesting that their density in the epidermis is important for immune reactions in the skin [4–6].

Sexual dimorphic phenomena in both humoral and cellular immunity have been observed in various experimental systems [7–9]. In most cases, females show higher immune responses than males, and orchietomy enhances immune activity of males. Graff, Lappé, and Snell reported that females reject skin allografts more consistently and rapidly than males, and that orchietomy results in accelerated rejection of skin allografts in males [8].

Assuming that LCs play a critical role in skin graft rejection, the density and functions of LCs may reflect sexual dimorphism and be influenced by castration. Although there are no reports on epidermal LC density in humans [10,11], guinea pigs

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Reprint requests to: Yoh-ichi Koyama, Ph.D., Division of Cell Biology, Institute for Comprehensive Medical Science, Fujita-Gakuen Health University, Toyoake, Aichi 470-11, Japan.

Abbreviations:
LC: Langerhans cell
PBS: phosphate-buffered saline
SPF: specific antigen-free

MATERIALS AND METHODS

Animals Eight-week-old, specific pathogen-free (SPF) C57BL/6 (C57BL/6N Crlj) and BALB/c (BALB/cAnNCrlj) mice were purchased from Charles River Japan Inc. The mice were used as soon as possible following their arrival at our laboratory or kept in a clean condition following operation.

Langerhans Cells of Hind Limb Skin Adenosine triphosphatase staining was carried out according to the method of Baker and Habowsky with some modification [13]. Skin specimens from the glabrous part of both hind limbs, proximal to the footpad, were incubated for 2 h at 37°C in a freshly prepared solution of 10 mM Na₂-EDTA-2H₂O in phosphate-buffered saline (PBS) (NaCl 6.83 g, KCl 0.2 g, Na₂HPO₄ 1.15 g, KH₂PO₄ 0.2 g per 1 liter). Epidermal sheets were separated from the dermis with fine forceps. After brief washing in PBS at room temperature, the specimens were fixed with 2% paraformaldehyde in 0.075 M cacodylate–HCl (pH 7.3) for 20 min at 4°C. The epidermal sheets were rinsed in 0.15 M NaCl at 4°C (10 min × 3) and allowed to react with an ATP-Pb solution for 15 min at 37°C. The ATP-Pb solution consisted of 42 ml of 0.2 M Trismal buffer (pH 7.3, 8.55% sucrose), 3 ml of 0.06 M Pb(NO₃)₂, 5 ml of 0.41 M MgSO₄·7H₂O. Ten milligrams of Na₂-ATP·3H₂O (WAKO, 017-
Figure 1. Epidermal sheet from the hind limb skin of a male C57BL/6 mouse showing LCs stained for ATPase activity. Bar = 10 μm.

Figure 2. Epidermal sheet from the hind limb skin of a female C57BL/6 mouse showing LCs stained for ATPase activity. Bar = 10 μm.

**Langerhans Cells of Dorsal Ear Skin** Both ears were cut off and cut up into square pieces (4 x 4 mm) with a knife. The dorsal skin was separated from underlying cartilage with fine forceps. For ATPase staining of ear epidermis, the method was modified in some respects to improve the reaction. Na₂ADP-H₂O (WAKO, 306-50501) was used in place of ATP according to Chaker et al. [14]. Skin pieces were treated with EDTA for 3 h instead of 2 h, and the concentration of the MgSO₄·7H₂O solution added to the ADP-Pb solution was increased to 1.93 M.

**Enumeration of LCs** The LCs were counted with a reticle at x200. One field outlined by the reticle corresponded to an area of 0.016 mm². One skin piece was obtained from each hind limb and ear, and the number of LCs was counted in 2 randomly selected fields of each sample (a total area of 0.032 mm²). The mean of 4 values obtained for both hind limbs and ears of each mouse was calculated and used as the LC density of each animal for calculation of the mean ± SEM of each group. Cell counting was carried out by the blind test.

**Castration** Eight-week-old C57BL/6 mice were ovariectomized or orchietomized under Nembutal anesthesia. The number of LCs of the hind limb skin was counted at 4 weeks following the operation. Control animals were sham operated.

**Statistical Methods** The Student t-test was used to assess differences in LC densities. A p value of less than 0.05 was considered significant.

**RESULTS**

**Langerhans Cells of Hind Limb Skin** Adenosine triphosphatase staining of the epidermal sheet indicated the LCs to be reaction-positive. The LCs in both male (Fig 1) and female (Fig 2) C57BL/6 mice showed regular distribution, but cell density was less and dendrites were longer in males than in females (males, 823 ± 20/mm²; females, 1363 ± 52/mm², Table I). In BALB/c mice, sex differences in LC density were also significant (males, 1689 ± 66/mm²; females, 2249 ± 105/mm², Table I). Although the LCs were regularly spaced as in C57BL/6, the dendrites were rather short and differences in dendrite length were not apparent between the two sexes. Male/female LC ratios were 0.63 for C57BL/6 and 0.75 for BALB/c.

**Langerhans Cells of Dorsal Ear Skin** The LC density of ear epidermis in male C57BL/6 mice (465 ± 24/mm²) was significantly less than that in females (542 ± 17/mm²) (Table I). Although the density in BALB/c female mice was higher than that in males, the difference was not significant (males, 702 ± 17/mm²; females, 809 ± 44/mm², Table I). In both strains, regularly spaced LCs of the ear had lower densities and longer dendrites than those in the hind limb skin. The male/female LC ratio was 0.86 for C57BL/6.

**Effects of Castration** Since sex differences in LC density were distinct in the hind limb skin of C57BL/6 mice, the effects of castration were examined in C57BL/6 males and females. The LC density in sham-operated males (12 weeks old) in the orchietomy experiments was 564 ± 27/mm² (Table II) but in orchietomized males, the density was significantly more (1179 ± 49/mm²) (Table II). In ovariectomized females (1237 ± 52/mm²), LC densities did not differ significantly from those in the sham-operated animals (1146 ± 49/mm²) (Table II) at 4 weeks following the operation.

**DISCUSSION**

It is evident from the data presented above that mouse epidermal LC density shows sex differences since male mice had fewer LCs than females. From the castration experiments, it is suggested that the LC density in males may possibly be reduced by the testis.

A number of papers report LC density to show species and regional specificity and genetic variation without any indication of sex differences [10–12]. The LC density values reported in this paper agree with those previously reported. For example, the LC density in the hind limb and ear of male C57BL/6 mice (hind

Table I. Densities of Epidermal Langerhans Cells (LCs) in the Mouse

<table>
<thead>
<tr>
<th>Region</th>
<th>Strain</th>
<th>Sex</th>
<th>LCs/mm² (mean ± SEM) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hind limb</td>
<td>C57BL/6</td>
<td>Male</td>
<td>823 ± 20 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>1363 ± 52 (8)</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>Male</td>
<td>1689 ± 66 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>2249 ± 105 (8)</td>
</tr>
<tr>
<td>Ear</td>
<td>C57BL/6</td>
<td>Male</td>
<td>465 ± 24 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>542 ± 17 (8)</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>Male</td>
<td>702 ± 17 (7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>809 ± 44 (8)</td>
</tr>
</tbody>
</table>

*n = Total number of animals.
*p < 0.001.
*p < 0.02.
Table II. Effects of Castration on the Densities of Epidermal Langerhans Cells (LCs) of the Hind Limb Skin in C57BL/6 Mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>Operation</th>
<th>LCs/mm² (mean ± SEM)(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Sham operation</td>
<td>564 ± 27 (6)</td>
</tr>
<tr>
<td>Male</td>
<td>Orchietomy</td>
<td>1179 ± 49 (7)*</td>
</tr>
<tr>
<td>Female</td>
<td>Sham operation</td>
<td>1146 ± 49 (7)</td>
</tr>
<tr>
<td>Female</td>
<td>Ovariectomy</td>
<td>1237 ± 52 (9)</td>
</tr>
</tbody>
</table>

* n = Total number of animals.
* p < 0.001.

Ovariectomy in the present study failed to have any significant effects on the LC density of hind limb skin. In contrast, LC density and morphology in the vaginal epithelium of the mouse is reported to be affected by ovariectomy or estrogen administration [27]. This discrepancy may be explained by the fact that the kinetics of the vaginal epithelium are controlled by estrogen, which may possibly have an indirect effect on LC density and morphology. A direct effect would suggest the possible existence of LC subpopulations differing in response to estrogen.

A comparison of LC shape and density of the ear skin and the hind limb skin in both sexes suggested the possibility that LC dendrite length increases with lessening density. Such a relationship between LC morphology and density has also been observed in vaginal epithelium with epithelial change in the estrous cycle and was suggested to relate to the immunologic role of LCs [28]. It may be reasonable to speculate that LCs, when the density has decreased, extend their dendrites to cover the epithelial tissue effectively. However, further experiments should be conducted to investigate functional differences between highly dendritic and stellate LCs.

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limb, 823 ± 20/mm²; car, 465 ± 24/mm²) is in good agreement with that reported by Bergstresser et al. (foot pad, 820/mm²; car, 470/mm²) although they make no reference to the sex of their mice [4]. Our LC density for hind limb skin in BALB/c mice (males, 1689 ± 66/mm²; females, 2249 ± 105/mm²) also agrees with that reported by Mackenzie and Squier (up to 2000/mm²) [15]. In this study, however, LC density at two body sites appeared to indicate sex differences for both mouse strains. This may be evident for the following reasons. (a) In this study, age-matched mice were used. Since in mice, as well as in humans [16], LC density decreases gradually with age (to be published elsewhere), data for age-unmatched animals would show considerable variance, thus making it difficult to detect sex differences. (b) As evident from Table I, indication of sex differences varies according to body site, being less in the ear skin often used for studies on LCs. Thus, detection of sex differences without systematic study of LC density in age-matched animals may be difficult.

Epidermal LCs are considered to give critical allosensitizing signals for graft rejection. They have been implicated also as antigen-presenting cells in the induction and expression of delayed contact hypersensitivity reactions [2,3] though there is a report in conflict with this view [17]. Our data strongly indicate that sex-matched animals should be used in studies on the functions of LCs. Based on the present findings, LC density is apparently reduced by the tests. Without such action exerted by this organ (in females or orchietomized males), LC density increases. Thus, the higher LC density in females and castrated males may be one of the mechanisms responsible for higher rejection of skin allografts observed in females and orchietomized males [7,8]. Dysfunctions of the tests induced by mutations or chemicals may also result in greater LC density and altered immune reactions in males.

The H-Y antigen is a Y chromosome-associated weak histocompatibility antigen expressed in males. The transplantation of skin grafts from males to females has facilitated the investigation of H-Y antigen expression. Rejection of H-Y incompatible skin grafts by females is known to show interstrain variation (reviewed in [18]). Recent evidence indicates that LCs possibly play important roles in the rejection of H-Y incompatible skin grafts [19]. Assuming that the rejection of H-Y incompatible skin grafts is influenced by sex differences in LC densities, the interstrain variation may be explained in part by strain variation of sex difference in LC densities.

Since the tests is a major source of sex hormones, reduction in LC density may possibly be caused by androgens. The following mechanisms may possibly be operative for the control of LC density. (a) Proliferation of epidermal LCs might be reduced by testosterone. Because LCs are known to have mitotic activity [20–22] and mitosis of thymocytes induced by calcium-independent mitogens has been reported to be inhibited by testosterone [23], it is conceivable that this proliferation is reduced by testosterone. (b) Langerhans cell migration has been reported in skin graft rejection [5], contact hypersensitivity [24], and wound healing [25]. Repopulation of the epithelium by LCs may possibly occur through this migration [26]. If testosterone has inhibitory effects on LC migration, a decrease in LC density should result.