Systemic Cytotoxic Drugs Depletes Epidermal Langerhans Cells in Guinea Pigs

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The systemic effects of the cytotoxic drugs, cyclophosphamide (Cy) and methotrexate (MTX), which are known to suppress the bone marrow cells, on epidermal Langerhans cells (LC) in guinea pigs were investigated. Cy and MTX were intraperitoneally injected either once (300 mg/kg for Cy and 350 mg/kg for MTX) or 10 consecutive days (30 mg/kg/day for Cy and 35 mg/kg/day for MTX) in 4 groups of animals consisting of 5 guinea pigs each.

This investigation was also sought the correlation between the number of LC and the total count of peripheral white blood cells. A significant LC reduction was noted in Cy-injected groups compared to little change in the number of LC in MTX-injected groups. The LC reduction and subsequent recovery after the cytotoxic drug treatment was correlated with the bone marrow suppression assessed by the total count of peripheral white blood cells.
(Ann Dermatol 1:10−15, 1989)

Langerhans cells (LC) constitute a morphologically well characterized subpopulation of mammalian epidermal cells. It is generally accepted that LC originate in the bone marrow and that they are functionally and immunologically related to the monocyte-macrophage-histiocyte series.[1]

LC have abundant surface adenosine triphosphatase (ATPase) activity, as well as murine Ia or human HLA-DR antigens.[2] Among the histoenzymologic methods, the method for ATPase has proved the most helpful for studies of LC in human and guinea pig skin.[3] The specificity of the ATPase method formed a basis for the study of the variations of LC density according to their epidermal site.[4]

Through transplantation and chimerization studies, it has been demonstrated that epidermal LC are derived from and are continuously repopulated by precursor cells originating in the bone marrow.[5,6] Thymidine labeling or culture studies, however, have

indicated that a small population of epidermal LC is capable of mitotic division[7,8] and can be stimulated to proliferate under experimental conditions.[9] Applying the technique based on the specific incorporation of bromodeoxyuridine, Czernielewski & Demarchez[10] showed evidence of LC being an actively cycling, stable, and self-reproducing cell population in normal epidermis.

This study was attempted to investigate the possible role of bone marrow cells in maintaining the epidermal LC population. Cytotoxic drugs, which are known to suppress the bone marrow cells, were systemically administered in guinea pigs for this purpose.

MATERIALS AND METHODS

Cytotoxic drug administration

Twenty female albino guinea pigs (300 to 400g of the body weight) were used in this experiment. They were fed liberally a pellet diet (Samyang Co., Korea) and water supplemented with cabbage. Cyclophosphamide (Cy) (Choongwae Pharm. Co., Korea) and methotrexate (MTX) (Yuhan Pharm. Co., Korea) were

obtained commercially. They were freshly prepared and injected intraperitoneally in aqueous solution. The animals were randomly divided into 4 groups (A, B, C, and D) according to the different dosage scheme of the two cytotoxic drugs. Animals of group A and B were given a single injection of Cy (300 mg/kg) and MTX (350 mg/kg), respectively, on the first day. Group C and D were injected daily Cy (30 mg/kg) and MTX (35 mg/kg), respectively, from the first day to 10th day.

Epidermal sheet preparation and ATPase staining

·Guinea pig skin samples were obtained with 6-mm punch from the depilated back of the animals. Anesthesia was achieved by subepidermal injection of a 2% solution of lidocaine. The samples were immediately incubated for 2 hrs at 37°C in a buffered EDTA tetrasodium salt solution (pH 7.3) according to Juhlin & Shelly.[11] The epidermis could then be separated from the dermis by simple traction with fine forceps. The staining with ATPase was performed as described by Juhlin & Shelly.[11] Briefly, the EDTA-separated epidermal sheet is placed immediately in saline solution for 30 min at room temperature. It is then immersed and fixed in cacodylate-formaldehyde solution containing 6.85g of sucrose, 10ml of 40% formaldehyde, 40 ml of 0.2 M cacodylic acid (Sigma, USA), and 50 ml of distilled water (4°C, 20 min). After rinsing, it is immersed in a solution containing the substrate, ATP, at 37°C for 20 min. Stock ATP solution was prepared with 50 mg of adenosine 5'triphosphate disodium salt (Sigma, USA), 5 g of glucose, 50 ml of distilled water, 40 ml of Trismal buffer, and 10 ml of 0.1 M $MgSO_4 \cdot 7H_2O$. The final ATP-Pb staining solution contained 2.7 ml of stock ATP solution and 0.3 ml of 2% lead nitrate. After rinsing, it is immersed in 5% ammonium sulfide solution for 20 min at room temperature. After rinsing, the sample is mounted dermal side up in glycerine jelly. Trismal buffer (pH 7.3) was used as the rinsing solution.

Total white blood cell (TWBC) counts and Langerhans cell (LC) counts

A TWBC count was performed on the first day to the 10th day and when the LC count was done It was performed on capillary blood samples using a hemocytometer.

The LC count was done on 5 occasions in each group, i.e., on the first day and 5, 15, 25, and 35 days after the injection of the cytotoxic drugs. Each stained specimen was examined under the calibrated ocular grid of a light microscope and assessed blind by two people to minimize error; comparable results were obtained. The LC were counted in 4 interfollicular fields at a magnification of ×400 and expressed as the mean number of cells per mm^2 of the skin surface. Cells with indefinitely stained dendrites were also included in the count.

The one-way ANOVA for repeated measurements and Scheffe method of multiple comparison were used for statistical analysis.

RESULTS

The effect on total white blood cell (TWBC) (Table 1)

A singificant reduction in TWBC count was observed after administration of the cytotoxic drugs in group A, C, and D. Maximum reduction of the TWBC count was seen 5 days after the injection of the cytotoxic drug in group A (95%), 7 days in group C (82%), and 9 days in group D (58%). Recovery of the TWBC count followed the reduction and reached the basal value 15 days after the injection of the cytotoxic drugs.

The effect on epidermal Langerhans cell (LC) (Table 2, Fig. 1-5)

In each group, a variable degree of reduction in LC count was observed following injection of the cytotoxic drugs. A significant LC reduction was seen on 5, 15, and 25 days after the injection of the cytotoxic drug in group A ($P < 0.01$, $P < 0.01$, $P < 0.05$). Group C also showed significant LC reduction 5 and 15 days after the injection ($P < 0.01$, $P < 0.05$). The reduction in group B and D was not significant. Recovery of the LC reduction was observed 25 days after the injection of the cytotoxic drug in both group A and C and the LC count returned to basal value 35 days after the injection in group A and 25 days after in group C. Group A showed a more severe degree of LC reduction than group C (15, 25, and 35 days after the injection of the cytotoxic drugs, $P < 0.01$).

Table 1. The number of total white blood cell (TWBC) determined before and after the injection of cytotoxic drugs

Time of TWBC Count	Group A	Group B	Group C	Group D
Basal value	9480±3532(n=5)	8760±2977(n=5)	11860±4033(n=5)	9700±3851(n=5)
5	470±182**(n=5)	8300±4221(n=5)	8650±1073*(n=4)	6470±1034*(n=5)
15	8425±2734(n=4)	8470±2318(n=5)	13020±2971(n=4)	9219±3316(n=5)
25	9375±4047(n=4)	7810±3304(n=5)	13688±1639(n=4)	10490±3312(n=5)
35	8838±4002(n=4)	9170±3170(n=5)	13020±2971(n=4)	10520±3840(n=5)

Data expressed as the mean number of cells/ul±S.D.
Time of TWBC expressed as days after last injection of drug.
Group A: Single injection of cyclophosphamide (Cy) (300 mg/kg).
Group B: Single injection of methotrexate (MTX) (350 mg/kg).
Group C: 10 daily injections of Cy (30 mg/kg).
Group D: 10 daily injections of MTX (35 mg/kg).
*$p<0.05$, **$p<0.01$
n: Number of animals.

Table 2. The number of epidermal Langerhans' cells (LC) after the injection of cytotoxic drugs

Group	No. of Animals	Time of LC Count*	No. of LC
A	5	Basal value	952± 73
	5	5	504± 95
	4	15	478± 45
	4	25	727± 30
	4	35	878± 41
B	5	Basal value	913±102
	5	5	735±136
	5	15	811± 43
	5	25	878± 69
	5	35	900±100
C	5	Basal value	844±115
	4	5	560±125
	4	15	704±197
	4	25	758±171
	4	35	837±149
D	5	Basal value	908±173
	5	5	802± 62
	5	15	832±146
	5	25	868±140
	5	35	898±171

Results expressed as the mean number of cells per mm² of the skin surface±S.D.
*: Days after last injection of drug.
Group A: Single injecton of cyclophosphamide (Cy) (300 mg/kg).
Group B: Single injection of methotrexate (MTX) (350 mg/kg).
Group C: 10 daily injections of Cy (30 mg/kg.)
Group D: 10 daily injections of MTX (35 mg/kg).
p 0.05: Between basal value and day 25, day 5 and 25 in group A; Between basal value and day 15 in group C,
p 0.01: Between basal value and day 5, basal value and day 15, day 15 and 25, day 15 and 35, and day 25 and 35 in group A; Between basal value and day 5, day 5 and 25, day 5 and 35 in group C; Between group A and C (basal value and day 15 in group A, basal value and day 15 in group C; day 15 and 25 in group A, day 15 and 25 in group C; day 25 and 35 in group A, day 25 and 35 in group C).
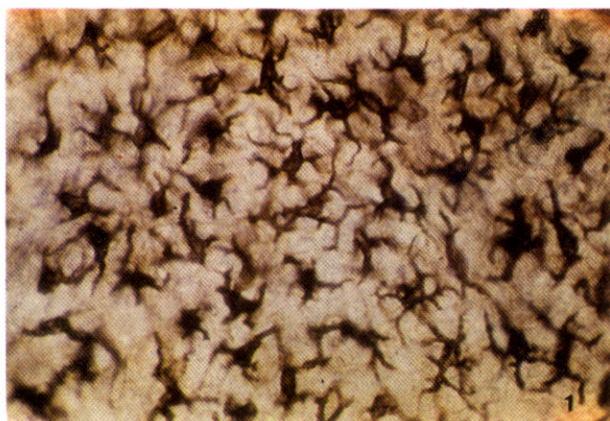
Fig. 1. Epidermal Langerhans cells(LC) of guinea pig skin just before single injection of cyclophosphamide(Cy) (300 mg/kg). LC are shown as dendritic dark brown cells (ATPase staining, ×400).
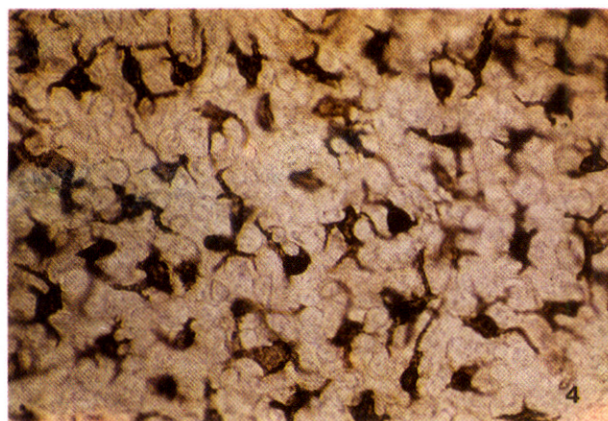

Fig. 4. Epidermal LC 25 days after single injection of Cy (300 mg/kg). Moderate recovery of LC numbers is shown (ATPase staining, ×400).
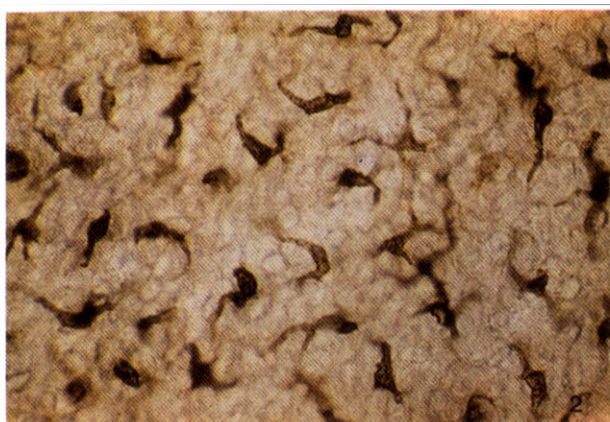

Fig. 2. Epidermal LC 5 days after single injection of Cy (300 mg/kg). Marked reduction of LC numbers is shown (ATPase staining, ×400).
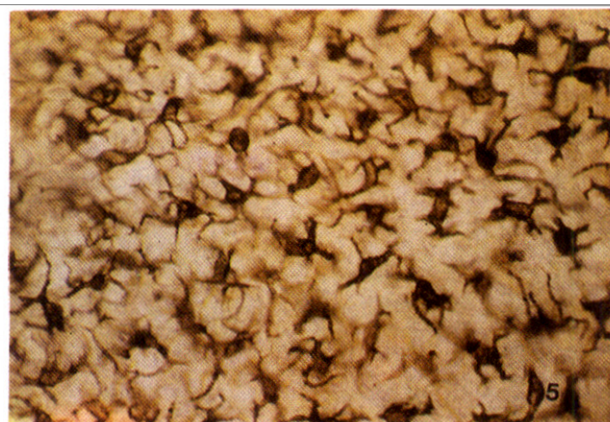

Fig. 5. Epidermal LC 35 days after single injection of Cy (300 mg/kg). Nearly complete recovery of LC numbers is shown (ATPase staining, ×400).

Fig. 3. Epidermal LC 15 days after single injection of Cy (300 mg/kg). Persistence of reduction of LC numbers is shown (ATPase staining, ×400).

DISCUSSION

Katz et al.[5] suggested that LC are not a stable population of the epidermis but migrate from the bone marrow into the skin, where they are continuously replaced at an as yet unknown rate. The observations of LC crossing the dermal-epidermal junction as well as their presence in the dermis has been explained by their migration into the epidermis.[12,13] However, Czernielewski & Demarches[10] showed evidence of LC being an actively cycling, stable, and self-reproducing cell population in normal epidermis and suggested that LC do not migrate from the bone marrow under circumstances other than embryogenesis, such as bone marrow reconstitution of lethally irradiated subjects.
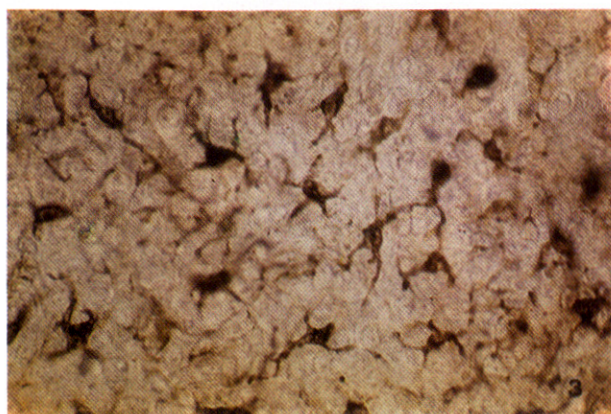
Treatment with ultraviolet light,[14-16] X-rays,[17] glucocorticoids,[18] chemical carcinogens[19] and cytotoxic drugs[20] depletes or reduces number of the epidermal LC and the resultant reduction may be followed by later repopulation. The exact mechanism by which LC repopulate in injured epidermis is unknown.

Miyauchi & Hashimoto,[21] studying the UVB-irradiated mouse skin, found evidence of mitosis of epidermal LC in irradiated epidermis and suggested that local mitosis of LC contribute to their later repopulation in the epidermis. In their study, LC repopulation occurred rapidly between day 7 and 14 after the UVB irradiation. Because the cytotoxic drugs used in our study would be sufficient to cause death of epidermal LC and since the mitotic index of LC in normal epidermis is very low,[8,10] the later phase of recovery observed in our study is unlikely to be due to cell division of those cells remaining in the epidermis. This is also evidenced by the fact that LC reduction persisted with the same magnitude from the 6th to 16th day in animals of group A and then rapidly recovered after the TWBC count returned to the basal value.

Belsito et al.[22] suggested that the loss and return of LC markers following steroid therapy in the presence of bone marrow ablation in animals may be due to a change in cell surface markers rather than loss of cells and their recovery may possibly due to resynthesis of lost markers. Following exposure of mouse skin to UV-light, Aberer et al.[14] found LC still present by electron microscopy, although they were damaged and lacking in ATPase. In contrast, Noonan et al.[23] reported UV-light to deplete LC as determined by electron microscopy and ATPase staining criteria. Since electron microscopic examination of LC was not performed in this study, it is difficult to say whether the ATPase-positive cells which reappear in the epidermis following the cytotoxic drug administration are the original population re-expressing the lost surface membrane ATPase.

Glucocorticoid-mediated loss of ATPase from the LC membrane has been observed to require 7 days for the ATPase to be re-expressed on the LC membrance.[22] Injury by UVA required 3 weeks[24] and 3-4 weeks by UVB injury.[16] DMBA-treated LC-ATPase did not return to control values until 55-64 days following cessation of the treatment.[19] In our study,

more than 15-25 days were required after Cy injection for the LC count to return to basal values.

The results of the experiment reported herein demonstrated that systemic Cy administration modulates epidermal LC-ATPase in guinea pigs. Extended studies are required to determine whether the recovery of LC is due to LC migration from the bone marrow or local recovery of LC markers.

REFERENCES

1. Tamaki K, Stingl G, Kat SI: *The origin of Langerhans' cells. J Invest Dermatol* 74:309-311, 1980.
2. Wolff K: *Dohi memorial lecture. The Langerhans' cell and epidermis: An integrated immune system. J Dermatol (Tokyo)* 10:401-410, 1983.
3. Wolff K: *The Langerhans' cell. Curr Probl Dermatol* 4:83, 1972.
4. Bergstresser PR, Fletcher CR, Streilein JW: *Surface densities of the Langerhans' cells in relation to rodent epidermal sites with special immunologic properties. J Invest Dermatol* 74:77-80, 1980.
5. Katz SI, Tamaki K, Sachs DH: *Epidermal Langerhans' cells are derived from cells originating in bone marrow. Nature* 282:324-326, 1979.
6. Stingl G, Tamaki K, Katz SI: *Origin and function of epidermal Langerhans' cells. Immunol Rev* 53:149-174, 1980.
7. Cruickshank CND, Cooper JR: *Langerhans' cells in tissue cultures of guinea pig epidermal cells. Br J Dermatol* 97:121-130, 1975.
8. Mackenzie IC: *Labelling of murine epidermal Langerhans' cells with H³-thymidine. Am J Anat* 144:127-136, 1975.
9. Gschnait F, Brenner W: *Kinetics of epidermal Langerhans' cells. J Invest Dermatol* 73:566-569, 1979.
10. Czernielewski JM, Demarchez M: *Further evidence for the self-reproducing capacity of Langerhans' cells in human skin. J Invest Dermatol* 88:17-20, 1987.
11. Juhlin L, Shelly WB: *New staining techniques for the Langerhans' cell. Acta Dermatovenereol (Stockholm)* 57:289-296, 1977.
12. Murphy GF, Bhan AK, Harrist TJ, Mihm MC: *In situ identification of T-6 positive cells in normal human dermis by immunoelectronmicroscopy. Br J Dermatol* 108:423-431, 1983.
13. Murphy GF, Fonferko E, Flotte, T, Bhan AK: *Association of basal-lamina defects with epidermal and dermal T-6 positive cells: Evidence of Langerhans' cell migration. Acta Dermatol Res* 278:126-132, 1985.
14. Aberer W, Schuler G, Stingl G, Hoenigsmann H, Wolff K: *Ultraviolet light depletes surface markers of Langerhans' cell. J Invest Dermatol* 76:202-210, 1981.
15. Friedmann PS: *Disappearance of epidermal Langerhans' cells during PUVA therapy. Br J Dermatol* 105:219-221, 1981.
16. Humm SA, Cole S: *Changes with time in Langerhans' cell number, ATPase reactivity and morphology in murine epidermis after exposure to UVB. Photodermatology* 3:174-178, 1986.

17. Cole S, Lewkowicz SJ, Townsend KMS: *Langerhans' cell number and morphology in mouse footpad epidermis after X irradiation. Radiat Res 100:594-606, 1984.*

18. Berman B, France DS, Martinelli GP, Hass A: *Modulation of expression of epidermal Langerhans' cell properties following in situ exposure to glucocorticoids. J Invest Dermatol 80:168-171, 1983.*

19. Muller HK, Halliday GM, Knight BA: *Carcinogen-induced depletion of cutaneous Langerhans' cells. Br J Cancer 51:81-85, 1985.*

20. Pakes WL, Muller HK, Schwarz MA, Marks R: *Langerhans' cells—a reduction in numbers and their reappearance following steroid and cytotoxic therapy in humans. Clin Exp Dermatol 11:450-459, 1986.*

21. Miyauchi S, Hashimoto K: *Epidermal Langerhans cells undergo mitosis during the early recovery phase after ultraviolet-B irradiation. J Invest Dermatol 88:703-708, 1987.*

22. Belsito DV, Baer RL, Thorbecke GJ, Gigli I: *Effects of glucocorticoids and gamma radiation on epidermal Langerhans' cells. J Invest Dermatol 82:136-138, 1984.*

23. Noonan FP, Bucana C, Sauder DN, De Fabo EC: *Mechanism of systemic immune suppression by UV irradiation in vivo. J Immunol 132:2408-2416, 1984.*

24. Liu HH, Muller SA, Schroeter AL: *Quantitative and morphologic changes of Langerhans' cells after ultraviolet A irradiation of human epidermis. Acta Dermatovenereol (Stockholm) 67:411-416, 1987*