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The Effect of Ionizing Radiation on Epidermal Langerhans Cells
– A Quantitative Analysis of Autopsy Cases with Radiation Therapy –

YOSHIHISA KAWASE, SHINJI NAITO, MASAHIRO ITO, ICHIRO SEKINE AND HIDEHARU FUJII*

Department of Pathology, Atomic Disease Institute, Nagasaki University School of Medicine, 12–4 Sakamoto-machi Nagasaki 852, Japan
* Nagasaki Chuo National Hospital, Nagasaki

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Langerhans cells (LCs) are dendritic cells located in the epiderm is with antigen-presenting capacities. We performed a quantitative analysis of LC density in the anterior chest skin of 286 autopsy cases, including 31 cases treated with radiation therapy. Skin specimens were stained by immunoperoxidase technique (PAP method) with an anti-S-100 protein antiserum. S-100 positive LCs were counted for comparison between non-irradiated and irradiated cases. In this study we noted that, 1) The decline in density of the LCs was age-related and dendritic processes were more prominent in younger groups. 2) The cases irradiated within one month before autopsy showed a reduction in LC density compared with age-matched controls. 3) The cases irradiated more than one month before autopsy demonstrated no consistent or definite tendency. It is suggested that ionizing irradiation as well as ultraviolet light may deplete the LC density in an acute phase. The possibility that radiation therapy alters immunological surveillance in the human skin is discussed.

INTRODUCTION

Langerhans cells (LCs) in the human epidermis were initially described in 1868 by a 21-year-old medical student, Paul Langerhans[1]. LCs are suprabasal cells in the epidermis recognized by several dendritic processes extending between keratinocytes. Despite their recognition over one century ago, only in the last two decades their role has been established. LCs are derived from bone marrow precursors[2], bear Fc and C_3 receptors[3,4] and express membrane antigens such as HLA-DR or Ia[5]. Thus LCs have important functions in the immune system as antigen-presenting cells and for the stimulation of antigen-specific T cell activation.

It is well known that malignant epithelial skin tumors are induced by chronic exposure to ultraviolet light (UV) and occur most frequently in sun-exposed regions such as the face of elderly persons. The effects of UV radiation on cutaneous immune responses have been

extensively investigated[6-8]. A great number of studies have demonstrated that UV irradiation induces a decrease in density of the LCs possessing cell surface Ia antigen and membrane ATPase. Exposure to UV radiation appears to result in an unresponsiveness to contact allergens and inability to reject highly antigenic UV-induced tumors.

But studies addressing effects of ionizing irradiation on the human cutaneous immune system have been very few in number. In the present work, we performed a quantitative analysis of LC density in human chest skin from autopsy cases treated with radiation therapy in order to determine the effects of ionizing irradiation on LCs in the epidermis.

MATERIALS AND METHODS

A total of 286 cases from the autopsy files of Nagasaki Chuo National Hospital, autopsied within 8 hours after the death were used in this quantitative study. From 31 radiation therapy cases, subject specimens of skin of the anterior median chest located within the irradiated area were studied along with 255 non-irradiated specimens from the same location serving as controls (Table 1). The irradiated cases which had been treated with radiation therapy for lung, breast, esophageal and mediastinal tumors, were divided into two groups: 10 of the 31 cases were irradiated within one month before autopsy, and the other 21 cases irradiated more than one month before autopsy. It seems well established that radiation injury trends to produce recognizable acute (days to weeks) and delayed (months to years) morphological lesions[9]. All the irradiated cases were treated with fractional and multiportal, mainly biportal, irradiation by the telecobalt (^{60}Co γ-rays). The total doses are presented in Table 3 & 4. More detailed radiological descriptions of the irradiated cases were not available. The age range of the control cases was neonate to 88-

Table 1. Age and sex distribution of the cases used in the analysis

Age range	Male		Female		Total			
	Cont.	Irradiat.	Cont.	Irradiat.	Cont.	Irradiat. <1 M	Irradiat. >1 M	Irradiat. total
0– 9	22	0	19	0	41	0	0	0
10–19	2	1	6	2	8	0	3	3
20–29	14	0	6	0	20	0	0	0
30–39	20	2	10	1	30	0	3	3
40–49	20	2	6	4	26	2	4	6
50–59	26	2	13	0	39	1	1	2
60–69	16	5	23	1	39	2	4	6
70–79	15	8	7	3	22	5	6	11
80–89	16	0	14	0	30	0	0	0
Total	151	20	104	11	255	10	21	31

Table 2. PAP procedure

Step No.	Procedure
1	3% hydrogen peroxide for 5 min., then rinse in 0.05 M Tris buffer pH7.6
2	Normal swine serum for 20 min.
3	Rabbit antiserum to S-100 proteins incubated overnight at 4°C Rinse Tris buffer-Bath Tris buffer
4	Swine antiserum to rabbit immunoglobulins for 20 min. Rinse Tris buffer-Bath Tris buffer
5	PAP (soluble horseradish peroxidase-rabbit antihorseradish peroxidase) complex for 20 min. Rinse Tris buffer-Bath Tris buffer
6	DAB (3,3'-Diaminobenzidene tetrahydrochloride) in Tris buffer H_2O_2 0.005% Wash in tap water
7	Counterstain with methyl green

year-old. The autopsy cases of younger persons (0 to 30 yrs) included congenital heart disease; 11 cases, leukemia; 10, while the elderly cases included hepatoma; 25, lung cancer; 23, gastric cancer; 15, liver cirrhosis; 14.

All autopsy specimens were fixed in 10% phosphate buffered formalin and after routine processing, embedded in paraffin wax. Paraffin sections 3 μ thick were cut and stained with hematoxylin-eosin and LCs were identified with anti-S-100 protein antiserum (Dako company, Denmark) using a modified immunoperoxidase technique (PAP method) according to the procedure described in Table 2. It has been shown that immunoreacitive S-100 protein is detected in intraepidermal LCs[10].

LCs were observed microscopically in vertical skin sections stained with hematoxylin-eosin stain and immunostain using anti-S-100 antibody. S-100 positive dendritic cells in the epidermis were counted as intraepidermal LCs when fulfilling the following criteria; located in the suprabasal layer of the epidermis, the nuclei being visible, with at least one dendritic process and without melanin pigment. The LC density was expressed as number of LC per unit epidermal surface area (cell number/mm^2). The average of each ten-year stage was calculated in control cases.

RESULTS

Immunoperoxidase staining (PAP method) of the paraffin sections with antibody to S-100 protein demonstrated the characteristic dendritic feature of the epidermal LCs (Fig. 1). LCs were located mainly in the suprabasal layer of the epidermis and distributed with approximately uniform densities in each case. Morphologically S-100 positive dendritic

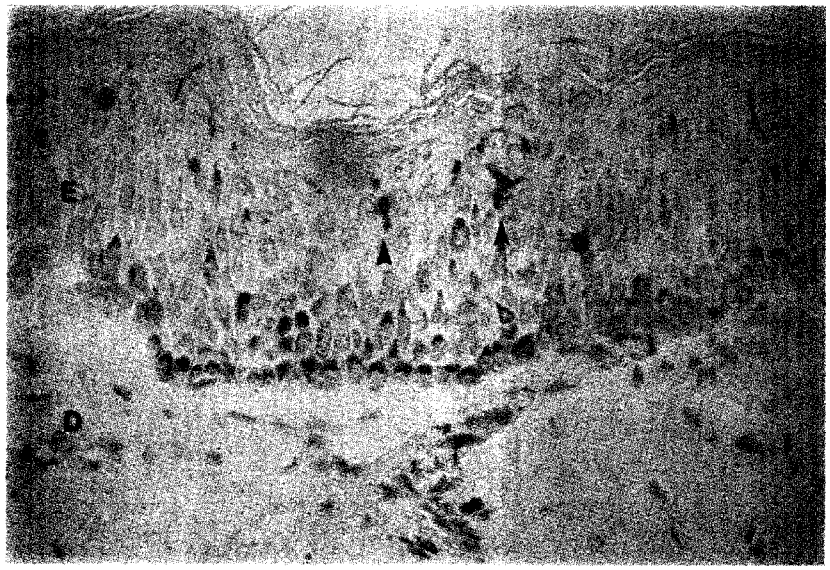

Fig. 1. 47 y.o. Male. The non-irradiated case.
Many S-100 positive Langerhans cells with processes which are detected by brown coloration (arrow head) due to PAP method, are seen in the middle layer of epidermis. E: epidermis, D: dermis (Immunostain, ×400)

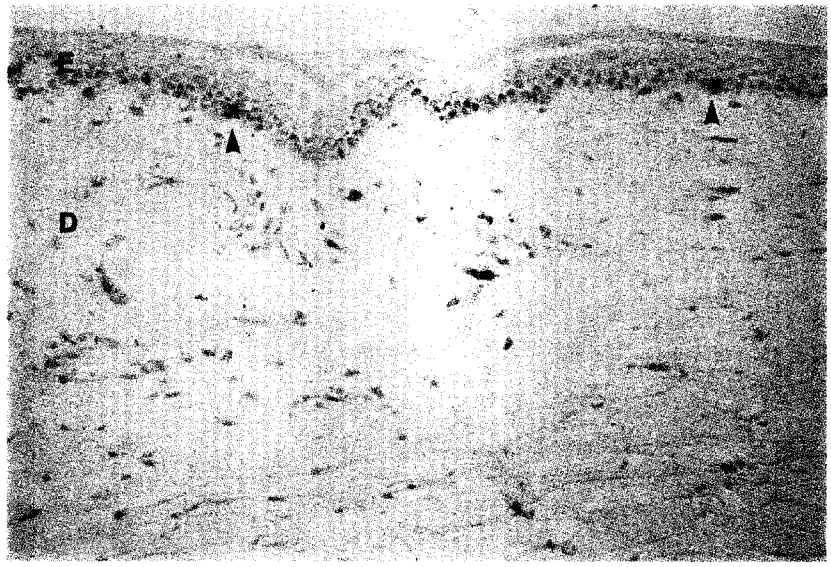

Fig. 2. 88 y.o. Female. The non-irradiated case.
S-100 positive Langerhans cells (arrow head) are very scant accompanied by the age-related thin epidermis. E: epidermis, D: dermis. (Immunostain, ×200)

processes were reduced in individuals over sixty, accompanied by age-related thinning of the epidermis (Fig. 2). Quantitatively in the non-irradiated control cases, LC densities reached their peak between teen-ages and the thirties (twenties: 302.2±45.4/mm^2, mean ± SE), and the LC densities in those from seventies to eighties decreased to less than a half of the peak (eighties: 130.1±17.5). Figure 3 illustrates age-related changes of the LC densities in the control cases.

The LC densities in the 10 cases irradiated within one month before autopsy showed a distinct reduction, compared with age-matched controls. For example the LC densities of those in the sixties and seventies were reduced to 51% and 60% of the age-matched controls

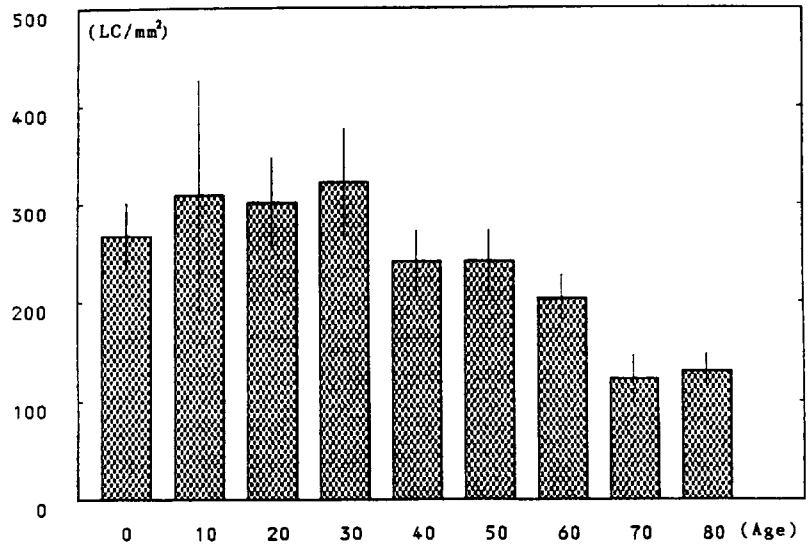

Fig. 3. Age-related changes of the densities of S-100 positive Langerhans cell.

Table 3. The cases irradiated within one month before autopsy

Age	Sex	Diagnosis	Total dose (Gy)	LC density (control)
45	M	Lung cancer	40	71.8 (241.2)
46	M	Germ cell tumor	60	230.8 (241.2)
56	M	Lung cancer	45	174.6 (240.6)
69	M	Lung cancer	28	165.4 (203.4)
69	M	Esophageal ca.	69	43.9 (203.4)
70	M	Esophageal ca.	100	24.5 (121.9)
72	F	Lung cancer	43.75	12.5 (121.9)
73	M	Thymoma	56	45.2 (121.9)
73	M	Lung cancer	7.5	130.2 (121.9)
74	M	Lung & Eso. ca.	94	156.9 (121.9)

(104.7±60.7, 73.9±29.2 versus 203.4±22.5, 121.9±22.9 respectively sixties and seventies) (Table 3, Fig. 4 and 5). On the other hand the 21 cases who underwent radiation treatment in more than one month before death showed very low LC densities in some cases and high values in other cases. Concerning the delayed phase, many cases showed higher LC densities

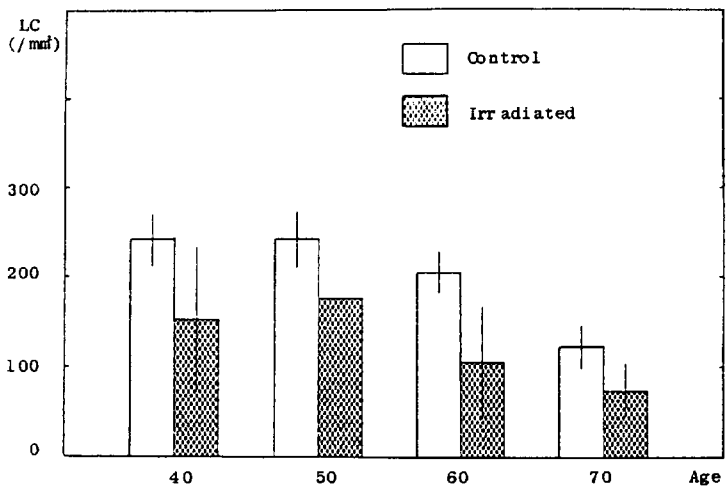

Fig. 4. Comparison in the densities of Langerhans cell between control and the irradiated cases within one month before autopsy.

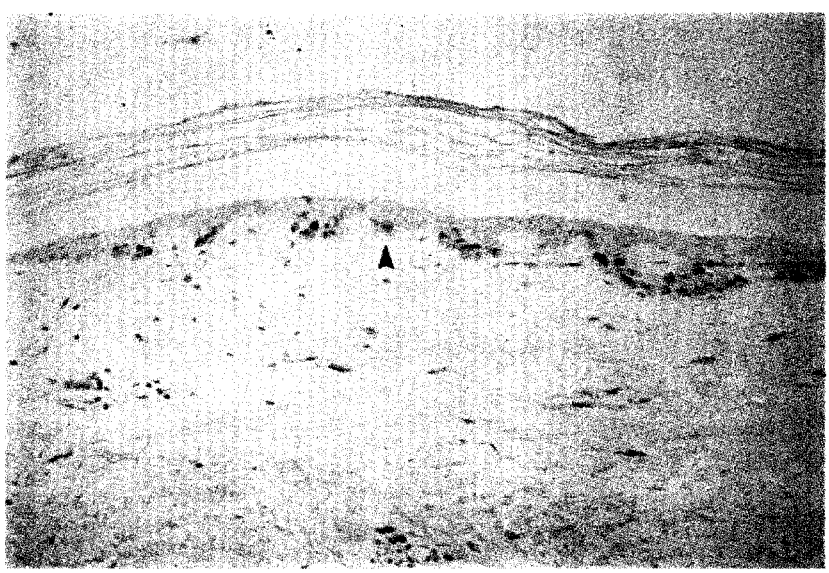

Fig. 5. 69 y.o. Male. The irradiated case.
The case irradiated 60 Gy one month before autopsy for germ cell tumor in the mediastinum. S-100 positive Langerhans cells (arrow head) are markedly reduced in number. (Immunostain, ×200)

Table 4.(a) The cases irradiated more than one month before autopsy, divided into (a) irradiated less than one year before and (b) irradiated more than one year before.

Age	Sex	Diagnosis	Total dose (Gy)	LC density (control)
11	M	Acute leukemia	Unknown	39.9 (309.0)
30	M	Esophageal ca.	60	696.8 (321.1)
35	M	Lung cancer	30	305.4 (321.1)
42	F	Breast cancer	60	253.4 (241.2)
45	F	Breast cancer	228	284.4 (241.2)
53	M	Lung cancer	50	366.8 (240.6)
64	M	Lung cancer	53	97.5 (203.4)
68	F	Breast cancer	260	36.6 (203.4)
69	M	Esophageal ca.	184	128.2 (203.4)
70	M	Lung cancer	42	76.9 (121.9)
72	M	Lung cancer	57	65.9 (121.9)
73	F	Lung cancer	60	389.2 (121.9)
77	M	Lung cancer	50	534.6 (121.9)
77	M	Esophageal ca.	24	234.1 (121.9)

Table 4.(b)

Age	Sex	Diagnosis	Total dose (Gy)	LC density (control)
13	F	Ependymoma	80	197.7 (309.0)
19	F	Medulloblastoma	29	1100 (309.0)
34	F	Thymoma	190	416.5 (321.1)
41	F	Breast cancer	60	187.5 (241.2)
43	F	Breast cancer	unknown	384.6 (241.2)
69	M	Esophageal ca.	91.5	101.2 (203.4)
75	F	Breast cancer	40	227.7 (121.9)

than age-matched controls (Table 4, Fig. 6).

Some cases showed manifest dermal fibrosis as chronic radiation-induced dermatitis (Fig. 7), though there was no definite correlation between LC density and morphological changes. Dyskeratosis, telangiectasia, or atypical fibroblast were not observed by light microscope in the irradiated cases.

DISCUSSION

Since the majority of malignant skin tumors are known to occur in sun-exposed regions, chronic exposure to ultraviolet light (UV) may induce skin tumors. Exposure to UV

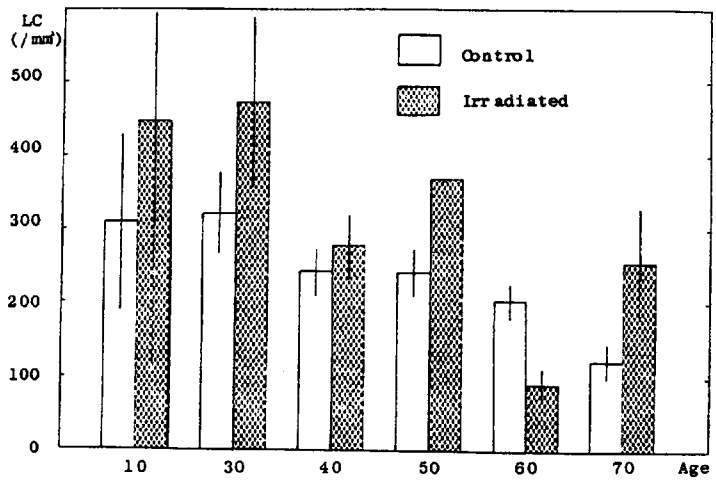

Fig. 6. Comparison in the densities of Langerhans cell between control and the irradiated cases more than one month before autopsy.

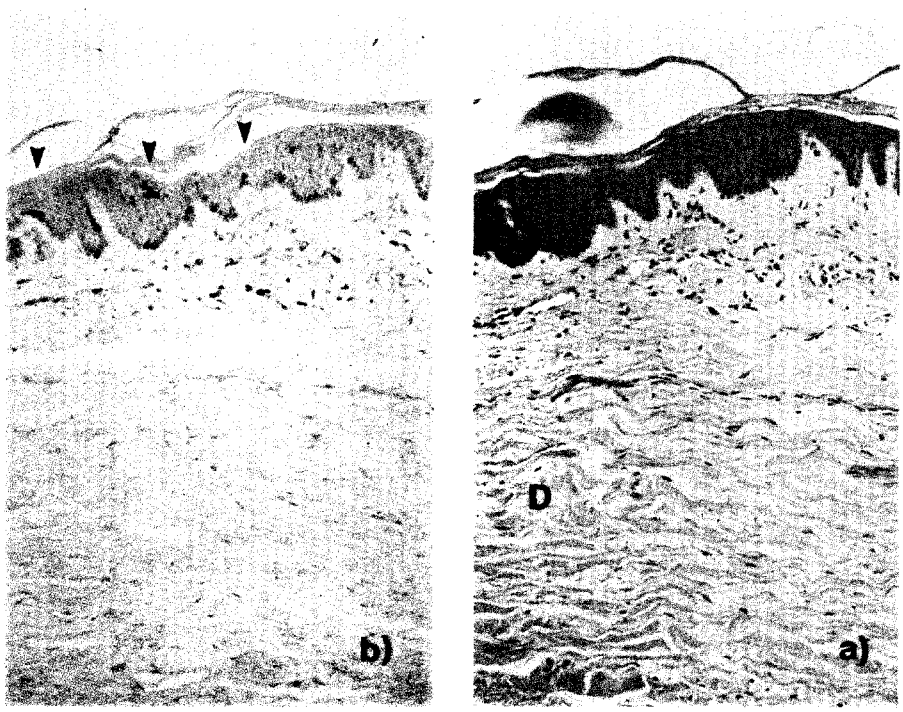

Fig. 7. 41 y.o. Female. The irradiated case.
The case irradiated 60 Gy in total five years before autopsy for breast cancer. Slight reduction in number of Langerhans cell (arrow head) in the epidermis and mild fibrosis in the dermis are observed in this case. E: epidermis, D: dermis. (a: H.E. ×100, b: Immunostain ×100)

radiation has been shown to induce immunological unresponsiveness to antigens and to impair the antigen-presenting capacity of the epidermis [11,12]. LCs may also play a vital role in immunosurveillance system, especially in presenting the development and growth of malignancy.

Though many skin tumors have been reportedly induced by ionizing irradiation[13–15], the effects of irradiation on LCs in human skin are poorly understood. The present study demonstrated that a detectable LC density was reduced in the acute phase, i.e. in the cases irradiated within one month of radiation therapy compared with in the non-irradiated cases. It is suggested that ionizing irradiation as well as UV irradiation may reduce number and impair function of LC in the acute phase, as previously reported in mice[16,17] and humans [18,19]. Furthermore it is reported that ionizing irradiation not only decreases the density of Ia^+ LC, but also reduces an interleukin-1-like cytokine termed epidermal cell-derived thymocyte activating factor (ETAF) production as acute effects of irradiation to mice[20].

In human skin, however, extensive long-term investigations have not been made on LC density after ionizing radiation exposure. It is observed in mice experiments had the irradiated epidermis shows focal reduction of LC number, which continued for 24 months[21]. In this study, by investigating autopsy cases that had received radiation therapy, no definite or consistent delayed effects of irradiation on LCs were demonstrated. In some irradiated cases LC densities decreased, compared with age-matched non-irradiated cases, and in other cases LC densities showed an increase. We speculate that one possible reducing factor in the delayed phase involves a circulatory disturbance due to radiation damage and that an increasing factor may the result from a reaction to newly encountered antigens in the radiation-damaged skin. Though long-term effects of irradiation of LCs themselves cannot be elucidated in this study, the microenvironment around LCs injured by ionizing irradiation may disturb smooth immunological responses by transporting antigens to regional lymph nodes and by presenting them to specific T cells.

The skin is not only a physical barrier but also is proposed to be an immunological barrier such as skin-associated lymphoid tissue (SALT) hypothesized by Streilein[22]. He has suggested the term "SALT" to described a putative integrated system of immune surveillance designed uniquely for the skin. One of the components of SALT, LC should be investigated further to elucidate acute and delayed effects of ionizing irradiation to the skin. This would help to clarify the alterations of immunological defence in radiation dermatitis and radiation oncogenesis in the human skin.

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