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Anatomical mapping of epidermal Langerhans cell densities in adults

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SUMMARY

The densities of T6 antigen-bearing Langerhans cells in 112 biopsies of human skin from sixteen surgical out-patients and four cadavers were determined for eight anatomical regions. The regional mean densities ($\pm$ s.e.m.) of epidermal Langerhans cells per mm^2 were: head and neck, 489 ± 27; chest, 466 ± 22; back, 466 ± 11; upper extremities, 458 ± 25; lower extremities, 431 ± 30; buttocks, 411 ± 11; genitalia, 298 ± 45; soles, 58 ± 12. No statistically significant differences were found between any of these Langerhans cell densities except for that of the soles which was lower than those of all other regions ($P < 0{\cdot}002$). No significant differences were detected between the mean densities of patients and cadavers, Caucasians and Hispanics or males and females.

Although numerous studies of Langerhans cells have been performed little data is available regarding their quantitative distribution. In their study of guinea-pig epidermis, Wolff & Winkelmann (1967) observed no variation in Langerhans cell density in different anatomical regions (ears, lower back and abdomen) or in different strains, but recent studies have revealed regional differences in epidermal Langerhans cell densities of various rodent species (Bergstresser, Fletcher & Streilein, 1980).

Since Langerhans cells are important for the induction of delayed contact hypersensitivity, we have now studied the regional densities of human epidermal Langerhans cells to establish a baseline for other studies of the effects of diseases or physical and pharmacological agents on these cells.

METHODS

One hundred and twelve specimens of non-lesional skin were obtained from sixteen surgical out-patients and four cadavers. Preliminary time-course experiments revealed no significant

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difference between epidermal Langerhans cell densities of freshly excised newborn human foreskin and foreskin specimens stored at 4°C up to 24 hours. Therefore, specimens obtained within 24 hours post mortem from cadavers refrigerated almost immediately after death were included in this study.

All biopsies were 4 mm in diameter and, in the case of surgical out-patients, were excised following subcutaneous injection of 1% lignocaine with 1:1000 epinephrine.

Subjects

Cadavers. Eighteen to twenty-three selected sites were biopsied from each cadaver. We were unable to obtain specimens from the head, neck and hands because of legal restrictions.

Case 1. A 77-year-old Hispanic man with a history of diabetes mellitus developed an acute myocardial infarction with subsequent sudden death (Fig. 1A).

Case 2. A 59-year-old white woman with a history of atherosclerotic heart disease and congestive heart failure developed a lateral myocardial infarction with subsequent cardiogenic shock and death (Fig. 1B).

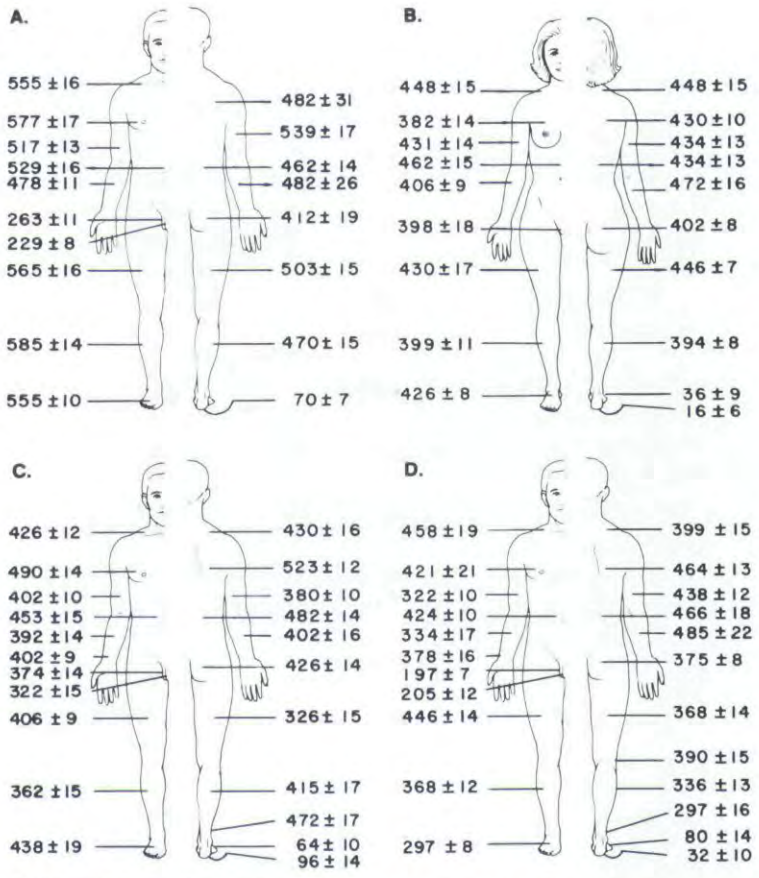

FIGURE 1. Epidermal Langerhans cell densities (mean ± s.e.m./mm^2) in biopsy specimens of selected anatomical sites of cadavers. A. Case 1, B. Case 2, C. Case 3 and D. Case 4.

Case 3. An 86-year-old white man with a history of chronic obstructive pulmonary disease expired following acute respiratory failure (Fig. 1C).

Case 4. A 32-year-old white man with a history of intravenous drug abuse and alcoholism succumbed to progressive hepatic failure (Fig. 1D).

Surgical out-patients. Eight Hispanic and eight Caucasian surgical out-patients, 26 to 61 years of age, with no history of significant medical illnesses and receiving no medications (Fig. 2) were included in this study. Ten patients were female and six were male.

Antibody

Fluorescein isothiocyanate-conjugated mouse monoclonal anti-human T6 antigen IgG1 (Ortho Pharmaceutical Corp., Raritan, N.J.) was used throughout. This has been shown to react with 70% of human thymocytes and with epidermal Langerhans cells (Fithian *et al.*, 1981; Chu *et al.*, 1982) but not with peripheral mononuclear cells (Kung *et al.*, 1979). A 1:25 dilution of the antibody in sterile minimum essential medium with Earle's salts and 5% bovine serum albumin was employed, detecting the same number of epidermal Langerhans cells as undiluted antibody.

Preparation of epidermal sheets

Fat-trimmed tissue specimens were immersed in 1 N sodium bromide for 30 min at 37°C. The epidermis was subsequently teased off the dermis with fine forceps and washed in phosphate-buffered saline prior to immunofluorescent staining.

Immunofluorescent detection of epidermal Langerhans cells

Epidermal sheets were incubated in fluoresceinated mouse monoclonal anti-human T6 antigen IgG1 for 1 h at 37°C in a humidified incubator and subsequently washed five times with cold phosphate-buffered saline. The specimens were mounted in glycerol gelatin between glass

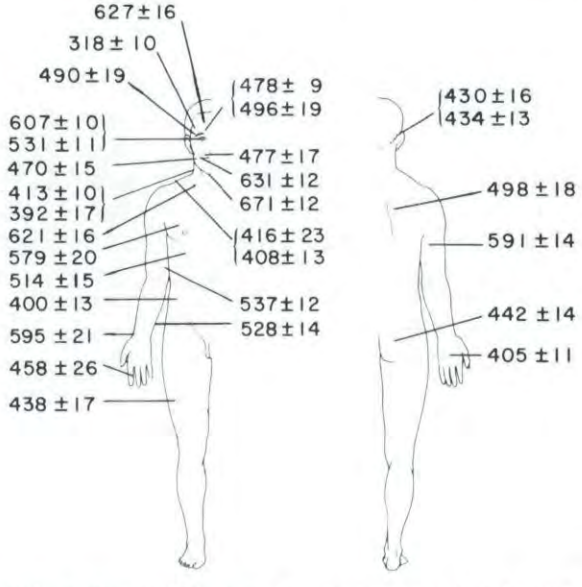

FIGURE 2. Epidermal Langerhans cell densities (mean ± s.e.m./mm^2) in biopsy specimens of selected anatomical sites of sixteen surgical out-patients (composite).

coverslips and fluorescing dendritic cells were enumerated under epifluorescent microscopy. Between ten and twenty randomly chosen, non-overlapping fields (0·0248 mm^2 usually containing approximately 12 T6+ cells) were examined, and the mean number of Langerhans cells ± s.e.m. per mm^2 calculated.

In preliminary experimentation to determine if incubation with 1 N sodium bromide affects subsequent detection of T6 antigen-bearing cells, a biopsy of breast skin was bisected and the 4-mm halves incubated at 37°C for 30 min in either phosphate-buffered saline or sodium bromide and stained for T6+ cells as described above. The mean number ± s.d. of fluorescing T6+ cells in twenty 400× fields of 6-micron thick cryostat sections of the saline and sodium bromide treated halves were not significantly different, being 7·65 ± 1·18 and 7·90 ± 1·16, respectively.

Statistical analyses. Student's t test comparisons were performed and only P values less than 0·002 were considered significant due to multiple inter-regional comparisons.

RESULTS

The mean number of epidermal Langerhans cells (± s.e.m.) per mm^2 in specimens from different sites is shown (Figs 1 and 2). Since the greatest number of cadaver biopsy specimens were from the trunk (back and chest), this mean density in this area was compared with that from the corresponding area of live patients.

No significant differences were found in mean truncal Langerhans cell densities between Hispanic patients (502 ± 42) and the Hispanic cadaver (521 ± 48), nor between Caucasian patients (446 ± 34) and Caucasian cadavers (447 ± 5).

The specimens obtained from cadavers and live patients were assigned to one of eight anatomical regions, and the mean densities of epidermal Langerhans cells of each region calculated (Table 1). Epidermis from the soles contained significantly fewer Langerhans cells (58 ± 12, $P < 0·002$) than any other region. Although the genitalia contained fewer Langerhans cells (298 ± 45) than the head and neck, chest, upper extremities, lower extremities and buttocks, the differences were not statistically significant.

The mean number of Langerhans cells ± s.e.m. per mm^2 was 507 ± 34 within the epidermis from truncal regions of Hispanic individuals and 446 ± 15 within the corresponding region of Caucasian subjects. These densities are not statistically different. In addition, race- and sex-matched cadavers of 32 and 86 years of age, respectively, did not significantly differ in their mean truncal (439 ± 28; 455 ± 48, respectively) or in their mean total body (322 ± 50; 366 ± 47) Langerhans cell densities. Males and females did not differ significantly in their mean truncal Langerhans cell densities.

DISCUSSION

Variations in regional densities of rodent epidermal Langerhans cells as detected by ATPase activity have been reported by Bergstresser *et al.* (1980). They found significantly lower densities within the hamster cheek pouch when compared with specimens from the ear, back, foot pad and buccal mucosa. The density of epidermal Langerhans cells of the cornea of the hamster (MHA and CB inbred strains), mouse (C57 BL/6J, A/Jax and BALB/c nu/nu) and guinea-pig (albino and red outbred strains) and of the tail of the mouse were also found to be less than within the other aforementioned anatomical sites. This and other investigations suggest

TABLE 1. Epidermal Langerhans cell densities in eight anatomical regions

Anatomical region[a]	Number of subjects	Number of biopsies	Mean number of Langerhans cells[b] ± s.e.m./mm²
Head and neck	13	15	489 ± 27
Chest	9	18	466 ± 22
Back	5	12	466 ± 11
Upper extremities	7	24	458 ± 25
Lower extremities[c]	5	5	431 ± 30
Buttocks	5	5	411 ± 11
Genitalia[d]	4	7	298 ± 45
Soles	4	7	58 ± 12

[a]Four-mm diameter skin biopsies were obtained from sixteen surgical out-patients and four cadavers.

[b]T6 antigen-bearing Langerhans cells were counted under epifluorescent microscopy and an average Langerhans cell density for each region for each subject was used to calculate the mean density for each region.

[c]Excluding the soles.

[d]Shaft of penis, scrotum and labia majora.

that a local paucity of Langerhans cells reduces or modifies the processing and presentation of antigens coming in contact with these sites (Bergstresser *et al.*, 1980; Toews, Bergstresser & Streilein, 1980; Streilein, Toews & Bergstresser, 1980). This contention is supported by the hyporesponsiveness of transplanted tail grafts of C57 BL/6 mice sensitized with dinitrofluorobenzene, suggesting that Langerhans cell density determines the induction of contact hypersensitivity (Toews *et al.*, 1980; Streilein *et al.*, 1980). Increased numbers of Langerhans cells have been reported in lichen planus, a disease in which enhanced cell-mediated hypersensitivity has been implicated (Bhan *et al.*, 1981; Ragaz & Ackerman, 1981). Conversely, we have recently reported lower Langerhans cell densities in epidermis of anergic patients with sarcoidosis than in control epidermis (Fox *et al.*, 1983).

Friedmann (1981) stained suction blister tops from the anterior forearms of six volunteers and reported the mean (± s.e.m.) density of Langerhans cells to be 730 (60)/mm². This value is greater than that calculated for the upper extremity, 458 (25), in this study in which T6 antigenicity was the Langerhans cell marker. Unlike T6 antigens which, within epidermis, are specific for both Langerhans and indeterminate cells, ATPase activity is present to some degree in all epidermal cells, and might stain 'passenger leukocytes' as well as Langerhans cells. We examined the twenty-four upper extremity biopsies at higher magnification than that used by Friedmann, in order to count only Langerhans cells and to exclude their dendritic processes. The lower specificity, greater subjectivity, limited number of specimens tested, and the possible inclusion of dendrites in Friedmann's study may have resulted in an overestimation of Langerhans cells. On the other hand, the possibility exists that although immunofluorescent detection of T6 antigenicity is specific for epidermal Langerhans cells, it may not detect all such cells.

Our data reveal significantly lower densities within the epidermis of the soles than in any other region tested. These lower densities were not due to an inability to detect Langerhans cells

through thick stratum corneum, since the numbers of these cells were also markedly diminished in cross-sectional specimens (unpublished data). It is clinically recognized that allergic contact dermatitis of the foot usually involves the dorsum more commonly than the sole. Although this finding has generally been attributed to the possibility of reduced antigen penetration due to a thickened stratum corneum, our findings may suggest reduced ability to present antigen by Langerhans cells.

Epidermal Langerhans cell densities calculated for corresponding regions from Caucasian and Hispanic individuals did not differ significantly. Further investigations employing greater numbers of subjects are necessary to substantiate these findings and to determine any racial differences.

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