Effect of Aging on Epidermal Dendritic Cell Populations in C57BL/6J Mice

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The density and function of epidermal dendritic cell populations were investigated in aged C57BL/6J mice. The densities of both Langerhans cells (LC) and Thy-1+ dendritic epidermal cells were found to decrease with age. Epidermal cell suspensions from aged mice showed impaired immunologic function as assessed in vitro by the skin-lymphocyte reaction assay and by measuring the ability of epidermal cell suspensions to stimulate the proliferation of sensitized T cells in the presence of the sensitizing antigen. However, the capacity of LC to transport antigen from the skin to the draining lymph nodes was found in vivo to be comparable to that of young mice. Results of transplantation of bone marrow cells from young and old donors into irradiated recipients indicated that the decreased Langerhans cell density found in old mice may result from a deficiency in Langerhans cell bone marrow progenitors. J Invest Dermatol 94:247-253, 1990

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esence is known to lead to severe changes in the skin cellular components [1] and is also accompanied by defects in certain immunologic parameters [reviewed in Refs 2-4], including delayed allograft rejection [5], reduced proliferative response to phytohemagglutinin [6], depressed humoral immunity [7], diminished responsiveness in the mixed-lymphocyte reaction [8], lowered interleukin-2 production [9], and reduced production and response to interleukin-1 [10]. Contradictory results [reviewed in Ref 11] on the effects of aging on antigen-presenting cell function have been reported, including impaired function of adherent cells in supporting interleukin-2 production [9], impaired ability of macrophages to process antigens [12], increased phagocytic activity of macrophages [13,14], increased activity of macrophage lysosomal enzymes [15], and intact function of splenic dendritic cells [16].

The etiology of the various changes observed in immunologic function with age is not well-defined. These changes might be related to intrinsic degenerative changes in bone marrow stem cells [17] because old recipients are immunologically rejuvenated when given young marrow and an infant thymus [18]. However, when young and old mice are parabiosed, the immune responses of the young mice decrease, whereas the immune function of old mice is not affected [19,20]. These findings suggest that suppressive bone marrow-derived factors contribute to immunologic deficiencies in aged mice [20]. In the same studies, stem cells from aged mice were shown to function equally as well as marrow stem cells from young animals [20]. Hirokawa et al showed that the age of the bone marrow recipient microenvironment as well as the age of the bone marrow cell donor affect the capacity of prothymocytes to populate the thymus [21]. Although the effects of aging on lymphocyte and macrophage populations have been extensively studied, the association between senescence and epidermal dendritic cell populations has received less attention. Recently, the function of Langerhans cells (LC) and Thy-1+ dendritic epidermal cells (Thy-1+ DEC) in the initiation of immunologic responses has been an area of great interest [22-24]. The present report investigates the effect of aging on density and function of dendritic cell populations in the murine epidermis.

MATERIALS AND METHODS

Mice C57BL/6J and C3H/HeJ mice were obtained from the Animal Resources colony of The Jackson Laboratory. Aged C57BL/6J mice were obtained from the aging colonies maintained by Dr. D. Harrison at the Jackson Laboratory. All mice were kept at three to four per cage on sterilized pine bedding, fed a standard diet of Wayne Lab Blox, and received chlorinated tap water ad libitum.

Epidermis Separation Mice were killed by CO2 asphyxiation prior to excision of skin from the rear footpads. Skin samples were incubated for 2 h at 37°C in a 5% CO2/95% air atmosphere in an 8.2 g/L solution of Na2EDTA · 2H2O in a buffer containing 116 mM NaCl, 2.6 mM KCl, 8 mM Na2HPO4, and 1.4 mM KH2PO4, adjusted to pH 7.3 with 0.1M HCl. The epidermis was then mechanically separated from the dermis and washed in PBS prior to subsequent procedures [25].
Monoclonal Antibodies and Polyclonal Antisera  The following antibody and antiserum combinations were used: LC were identified by incubating epidermal sheets with anti-Ia ( IgM, clone 25.5.16) followed by affinity-purified (AP) FITC-conjugated sheep anti-mouse Ig [Southern Biotechnology Associates Inc. (SBA)] and by incubation with anti-NLDC-145 [26] (rat IgG2a), followed by AP goat anti-rat Ig (SBA). Thy-1.2 DEC were identified by incubation with anti-Thy-1.2. (mouse IgM, dilution:1/50, clone HO-13-4) followed by AP FITC-conjugated rabbit anti-mouse Ig (SBA).

Immunofluorescence Microscopy  Epidermal sheets were fixed for 20 min at 23°C in acetone. Specimens were washed three times in PBS containing 5% fetal bovine sera (FBS) for 30 min at 4°C before incubation with the appropriate monoclonal antibody in a total volume of 0.2 ml. The specimens were then incubated at 4°C for 16 h and washed three times at 23°C for 2 h. The sheets were then incubated for 100 min in the appropriate secondary FITC-conjugated antisera. The specimens were finally washed three times on a rocker platform for 2 h at 23°C, mounted in a 9:1 solution of glycerol/PBS, and examined with a Leitz Orthoplan fluorescence microscope equipped for epi-illumination. Labeled cells were counted with the aid of an eyepiece piece (for Ia and NLDC-145 staining, 0.046 mm² = one field, for Thy-1.1 staining, 0.75 mm² = one field, twenty fields chosen at random counted for each specimen) [27]. Controls consisted of specimens incubated with an irrelevant primary antibody or medium followed by incubation with the appropriate fluoresceinated antisera.

ATPase Staining  Epidermal sheets were washed 3 times in 0.2M pH 7.3 Tris maleate buffer (containing 6.85% sucrose) at 4°C for 20 min and then fixed for 20 min at 4°C in 4% cacodylate-buffered formaldehyde. The sheets were then washed three times in Tris maleate buffer (6.85% sucrose) for 30 min at 4°C and stained with a solution containing 10 mg of ATP, 5 ml of 2% PbNO₃, 5 ml of 5% MgSO₄, and 92 ml of Tris maleate buffer (8.52% sucrose) at 37°C for 20 min. The preparations were then washed again 3 times in Tris maleate buffer at 23°C and put into a 1% solution of ammonium sulfide for 5 min. They were finally washed twice with distilled water, mounted in a 9:1 solution of glycerol/PBS, and counted with the aid of an eyepiece grid (0.046 mm² = one field, 20 fields chosen at random counted for each specimen) [28].

Sensitization Procedures  In order to sensitize the mice to rhodamine B isothiocyanate (FITC), they were painted on their shaved abdomen or thorax with 5 mg of FITC (Sigma) in 0.4 ml of acetone:dibutyl phthalate (50:50, v/v). Twenty four hours later, their inguinal, axillary, and subcapsular lymph nodes were removed.

Cell Suspensions  Animals were killed by cervical dislocation. In order to obtain epidermal cell suspensions, ears were carefully shaved and washed in PBS. The ears were then transferred to a solution of 95% ethanol for 2 min before washing and separation into ventral and dorsal parts. The cartilage was then removed, and the skin samples were put in a sterile solution of trypsin (Sigma, type XI) 0.3% in GNK buffer (0.8% NaCl, 0.04% KCl, 0.1% glucose adjusted to pH 7.6 with 7.5% NaHCO₃) for 30 min in 5% CO₂/95% air at 37°C. RPMI 1640 (Gibco) containing 0.01% DNase I (Sigma), 20 mM glutamine, 15% FBS, and antibiotics [50 mg/ml cefotaxime (Roussel), 50 mg/ml amikacin (Bristol) and 50 mg/ml gentamycin (Gibco)] was then added to the skin samples to neutralize trypsin activity, and the dermis was separated mechanically from the epidermis with the aid of two forceps and discarded. The cell suspension was gently pipetted for 5 min, filtered through a 70 micron mesh, and washed three times in medium [29]. For obtaining lymphoid cell suspensions, spleen or lymph nodes (popliteal and inguinal, for ovalbumin-injected mice, or axillary, subcapsual and inguinal for FITC-sensitized mice) were removed aseptically and passed through sterile nylon mesh. The cell suspensions were then filtered and washed twice. Two ml (per spleen) of buffered ammonium chloride were added to the splenocytes to lyse red cells and the cells resuspended for 5 min incubation at 37°C and finally washed twice before use. Cell viability was assessed by trypan blue exclusion and was always greater than 95%.

T cells were enriched by incubating splenocytes or lymph node cells in petri dishes for 2 h in an incubator at 37°C and 5% CO₂. The non-adherent cell population was then gently decanted and passed through nylon wool columns according to the method of Julius et al [30]. The nylon wool non-adherent cells were incubated for an additional hour in order to eliminate residual macrophages. The non-adherent cell population was decanted and washed twice before use. The T-cell enriched cell suspension was shown by indirect immunofluorescence and FACS analysis to be > 95% Thy-1.2. Dendritic cells in lymph node cell suspensions were enriched by the method of Knight et al [31]. Cells were resuspended in RPMI containing 10% FBS, 10⁻⁵M 2-mercaptoethanol, and 100 μg/ml of gentamycin at a concentration of 5 × 10⁶ cells/ml. The cell suspensions in 8 ml volume were layered onto 2 ml of 14.5% metrizamide (Nygaard, analytical grade) in medium and centrifuged for 10 min at 600 g. Cells at the interface were recovered and washed twice before subsequent use. IA⁺ cell subpopulations were depleted by incubating cells with an anti-IAb monoclonal antibody (kindly provided by Dr. Eytan Yefenof, Hebrew University, Jerusalem, Israel) at 4°C for 30 min, washed, and reincubated for 45 min at 37°C in the presence of fresh guinea pig serum, which was used as a source of complement. The serum was absorbed before the experiment on monocytes to remove non-specific cytotoxic activity.

Bone marrow cells were obtained from young and old C57BL/6j mice. Mice were killed by cervical dislocation, and each femur was disarticulated and stripped of muscle. Small needle bone marrow were made at each end, and the narrow cavity was flushed with medium. Bone marrow cells were washed, counted, and resuspended in medium at 25 × 10⁶ cells per ml.

Immunofluorescence Microscopy on Cell Suspensions  Cell suspensions were incubated for 30 min on ice with the appropriate dilution of monoclonal antibody in PBS supplemented with 5% FBS and 0.1% sodium azide at 10⁶ cells/100 μl. The cells were washed twice and resuspended at the same concentration in the appropriate dilution of the secondary labeled antiserum for 30 min on ice. The cells were finally washed and counted by using an Orthoplan fluorescence microscope equipped for epi-illumination (E. Leitz, Inc.).

Bone Marrow Transplantation  Recipients were administered total body irradiation from a 137Cs source (Shepard Mark I) at a rate of 240 rads per min for a total dose of 1000 rads. Following irradiation, mice were injected into the lateral tail vein with 5 × 10⁶ cells.

Mixed Skin Lymphocyte Reaction (SLR)  All tests were performed in RPMI 1640 medium supplemented with 5% FBS (Hyclone Laboratories, Inc.), 20 mM glutamine (Sigma), 10 mM HEPES (Sigma), 2.5 × 10⁻⁵M 2-mercaptoethanol (Sigma), and antibiotics. Quadruplicate mixed cultures of 1.5 × 10⁶ irradiated epidermal cells (derived from old or young C57BL/6j mice) and 1.5 × 10⁶ allogeneic [derived from C3H/He] (H-2b) mice nylon wool enriched T cells were established in 96-well plates (Nunc) containing 0.2 ml of culture medium at 37°C and 5% CO₂ as described in Ref. 32. Five days later, 1 μCi of tritiated thymidine (Amersham) was added to each well, and the cultures were further incubated for 18 h. Cells were harvested with the aid of a multiple sample automatic harvester (MASH II, Biomed. Res. Inst.), and the radioactivity incorporated into cellular DNA was measured by using a scintillation counter (Beckman).

Antigen-Presentation Assay  Eight-week-old C57BL/6j mice were immunized twice at 2-week intervals with 50 μg of ovalbumin (OVA) (Sigma) emulsified in 0.05 ml of complete Freund's adjuvant (CFA) (Sigma) in both hind footpads. Two to 3 weeks later, the mice were killed by cervical dislocation and their popliteal lymph nodes were aseptically removed. Cultures were performed in flat bottomed microtiter plates (Costar) with 2 × 10⁶ purified T cells derived from the lymph nodes of immunized mice in the presence or absence of various numbers of epidermal cells (from 6-week-old
or 20-month-old C57BL/6J mice) and 200 µg/ml of ovalbumin (Sigma) as described in Ref 33. Culture medium was RPMI 1640 medium supplemented with 10% horse serum (Emcy), 2 mM glutamine (Sigma), 10 mM HEPES (Sigma), 2.5 × 10⁻⁵ M 2-mercaptoethanol (Sigma), and antibiotics. After 4 d of culture, 1 µCi of tritiated thymidine was added to each well for 18 h, cells were harvested, and radioactivity incorporated into cellular DNA determined as described for the SLR assay.

Statistics Experimental group mean and standard deviation were calculated for each experiment. Comparisons of group means were performed by using the Student's t-test. Differences between values were considered statistically significant at p < 0.05.

RESULTS

LC Density in the Epidermis of Aging Mice LC density in the epidermis of aging mice was determined by immunofluorescence microscopy with an anti-Lc and with anti-NLDC-145 monoclonal antibodies as described in [27]. The results shown in Fig 1 demonstrate a progressive decrease with age in LC density in C57BL/6J mice. While density of Lc LC in one-month-old mice was approximately 1300 cells/mm², there were fewer than 500 Lc LC/mm² as assessed by staining with anti-Lc antibody at 20 months of age (p < 0.001). Decrease in NLDC-145⁺ LC density with age was found to be less marked but nevertheless significant: epidermis from one-month-old mice was found to contain 1113 LC/mm², whereas epidermis from 22-month-old mice contained 733 LC/mm² (p < 0.008). Staining of epidermal sheets for ATPase showed a similar decrease in LC numbers. At the age of 1 month, C57BL/6J mice had 1190 ± 59 ATPase-positive cells per mm². At the age of 4 months, this number dropped to 765 ± 65 cells/mm², whereas at the age of 20 months, only 406 ± 48 ATPase-positive cells per mm² were found in epidermal sheets from C57BL/6J mice (p < 0.001 for each time point, four animals tested for each time point).

Skin Lymphocyte Reaction (SLR) and Antigen-Presenting Cell (APC) Function in the Skin of Old Mice Two in vitro assays were used to assess the functional capacity of LC in epidermal cell suspensions. The SLR assay measures the capacity of LC to induce the proliferation of allogeneic T cells [32]. The APC assay estimates the capacity of LC to present antigen to sensitized T cells by measuring the proliferation of the T cells in the presence of the antigen and LC [33]. When epidermal cells derived from young (8-week-old) C57BL/6J mice were cultured in the presence of allogeneic cells, a strong proliferative response of the allogeneic T cells was observed (Table I). A marked decrease in proliferation was observed in three successive experiments when epidermal cells derived from 20-month-old C57BL/6J mice were used as stimulator cells (Table I). Pretreatment of the epidermal cell suspension with anti-Lc monoclonal antibody and complement, in contrast to treatment with complement alone, abolished the proliferative response (not shown).

The capacity of epidermal cells from aged mice to present antigen to sensitized T cells was also examined. Eight-week-old C57BL/6J mice were immunized with OVA in CFA and T cells purified from their lymph nodes. Epidermal cell suspensions from old (20-month-old) and young (6-week-old) animals were cultured with the sensitized T cells. The experiment was repeated three times with similar results. Epidermal cells derived from 6-week-old animals induced strong proliferation of T cells in the presence of ovalbumin (Table II). Pretreatment of the epidermal cell suspension with anti-Lc monoclonal antibody and complement, in contrast to pretreatment with complement alone, abolished the proliferative response (not shown). When 1–2 × 10⁵ epidermal cells derived from old (20 months) mice were cultured in the presence of sensitized T cells and OVA, the proliferative response induced was significantly lower (p < 0.01) than that induced by epidermal cells from young (6 weeks) mice (Table II). Thus epidermal cell suspensions derived from old mice are deficient in their capacity to present antigen to T cells. In both young and old mice, the response of T cells cultured alone (653 ± 84 cpm) or in the presence of the antigen only (1438 ± 169 cpm) was significantly lower than that of T cells cultured in the presence of epidermal cells. This could reflect a syngeneic SLR reaction as previously reported [32].

NLDC-145⁺ Cell Isolation From the Lymph Nodes of Old Mice LC function by migrating along the lymphatics as veiled cells and present antigen to T cells in the paracortical area of peripheral lymph nodes as interdigitating cells [34,35]. All three types of these dendritic cells stain with the NLDC-145 antibody [26]. Four-week-old and 20-month-old C57BL/6J mice were sensitized with RITC as previously described by Macatonia et al [36]. Twenty-four hours later, their draining lymph nodes were removed and dendritic cells were isolated on metrizamide gradients. Subsequently, the cells were incubated with anti-NLDC-145 monoclonal antibody, which was revealed with FITC-conjugated goat anti-rat antiseraum. Most of the NLDC-145⁺ cells from old mice did not differ from those of young mice in their ability to transport antigen from the periphery to the local draining lymph nodes. Thus in both young and old mice, most of NLDC-145⁺ cells recovered from the metrizamide gradient were observed to contain rhodamine when examined by immunofluorescence microscopy (Table III). In addition, similar or slightly higher numbers of low density NLDC-145⁺ cells were recovered from the lymph nodes of old mice as compared with young mice (Table III). These findings indicate that transfer of antigens from the epidermis to the draining lymph nodes is not impaired in aged mice.

Thy-1⁺ DEC Density in the Epidermis of Old Mice The density of Thy-1⁺ DEC in the skin of old mice was assessed by staining epidermal sheets with a monoclonal anti-Thy-1.2 antibody. A pro-

### Table I. Effect of Aging on the Stimulatory Capacity of Epidermal Cells for Allogeneic Mixed Skin Leukocyte Reactivity

<table>
<thead>
<tr>
<th>Exp no.</th>
<th>Stimulators</th>
<th>H-TdR Uptake (cpm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8-week-old C57BL/6J</td>
<td>64773 ± 6784</td>
</tr>
<tr>
<td>2</td>
<td>20-month-old C57BL/6J</td>
<td>33225 ± 2603</td>
</tr>
<tr>
<td>3</td>
<td>20-month-old C57BL/6J</td>
<td>28438 ± 2467</td>
</tr>
<tr>
<td>4</td>
<td>5-week-old C57BL/6J</td>
<td>19962 ± 987</td>
</tr>
<tr>
<td>5</td>
<td>20-month-old C57BL/6J</td>
<td>43986 ± 1391</td>
</tr>
</tbody>
</table>

* Mixed epidermal-lymphocyte cultures were established as described in Materials and Methods. Responders were cells in each case non-adherent, nylon wool column purified spleen T-cell suspensions, derived from 6–8-week-old C57BL/6J mice. The cpm values were obtained by determining the mean cpm from quadruplicate stimulated cultures of T cells minus the mean cpm of T cells cultured in the absence of epidermal cells. Each result represents the mean ± standard deviation from the mean. 

† p < 0.05

Figure 1. LC density in the skin of C57BL/6J mice as a function of age as determined by immunofluorescence microscopy with anti-Lc antibody and with anti-NLDC-145 antibody. Each point represents the mean ± SD of two to eight mice.
Table II. Ability of Epidermal Cells from Aging Mice to Stimulate Antigen-Specific T-cell Proliferation

<table>
<thead>
<tr>
<th>Age</th>
<th>Antigen</th>
<th>Epidermal cell No. x 10^6</th>
<th>^H-TdR Uptake</th>
<th>Δ CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 weeks</td>
<td>OVA</td>
<td>0.5</td>
<td>14,503 ± 1,984</td>
<td>7,894 ± 2,109</td>
</tr>
<tr>
<td>20 months</td>
<td>OVA</td>
<td>0.5</td>
<td>11,597 ± 2,319</td>
<td>6,953 ± 1,324</td>
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<tr>
<td>6 weeks</td>
<td>OVA</td>
<td>1.0</td>
<td>25,875 ± 2,345</td>
<td>16,789 ± 1,751</td>
</tr>
<tr>
<td>20 months</td>
<td>OVA</td>
<td>1.0</td>
<td>8,670 ± 1,673</td>
<td>6,372 ± 1,669</td>
</tr>
<tr>
<td>6 weeks</td>
<td>OVA</td>
<td>2.0</td>
<td>28,303 ± 2,945</td>
<td>11,543 ± 2,590</td>
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<tr>
<td>20 months</td>
<td>OVA</td>
<td>2.0</td>
<td>9,594 ± 2,145</td>
<td>3,212 ± 679</td>
</tr>
<tr>
<td>6 weeks</td>
<td>OVA</td>
<td>4.0</td>
<td>7,031 ± 1,398</td>
<td>11,489 ± 2,134</td>
</tr>
<tr>
<td>20 months</td>
<td>OVA</td>
<td>4.0</td>
<td>7,524 ± 2,134</td>
<td>3,497 ± 1,257</td>
</tr>
</tbody>
</table>

* T cells were purified from lymph nodes of five C57BL/6 mice at 8 weeks of age following immunization with ovalbumin. The cells were cultured with various numbers of epidermal cells (pooled from four animals each) from 6-week-old or 20-month-old C57BL/6 mice in the presence (OVA) or in the absence (—) of ovalbumin as described in Materials and Methods. Results represent the mean ± SD of quadruplicate cultures.

a Age of C57BL/6 mice used as a source of epidermal cells.

Δ CPM represent the difference between the mean CPM from cultures in the presence of OVA and the mean CPM determined in the absence of OVA.

Table III. Isolation and Staining of Low-Density Lymph Node Cells with Anti-NLDC-145 Antibody

<table>
<thead>
<tr>
<th>Exp no.</th>
<th>Age (months)</th>
<th>Total lymph node cells</th>
<th>Low density lymph node cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>no. cells x 10^6</td>
<td>no. cells x 10^6</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>64</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>40</td>
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<td>2</td>
<td>2</td>
<td>38</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>32</td>
<td>46</td>
</tr>
</tbody>
</table>

* Two inguinal, axillary, and subcapsular lymph nodes from each mouse were removed; cells were pooled from three to four animals.

b Lymph node cells from RITC-sensitized old and young mice were enriched on metrizamide gradients and recovered (low density lymph node cells) as described in Materials and Methods. RITC+ = % of fluorescent cells out of 200 cells examined for red fluorescence; FITC+ = % of fluorescent cells out of 200 cells examined for green fluorescence; NLDC+/RITC+ = cells staining positive for NLDC-145 (revealed with a FITC conjugated anti-rat antisemur) that were observed to contain rhodamine.

gressive decrease in Thy-1+DEC density was found with age in the epidermis of C57BL/6J mice. At the age of one month, epidermis from C57BL/6J mice contained more than 100 Thy-1+ DEC/mm², whereas at the age of 11 months, this density drops to half this number (p < 0.002). At the age of 22 months, a threefold decrease in Thy-1+DEC density was found in comparison with one-month-old young mice (p < 0.001) (Fig 2).

Effect of Bone Marrow Transfer from Old Mice to Young Recipients on Epidermal LC Density Significant decreases in LC and Thy-1+DEC densities were found in old C57BL/6J mice in comparison with young mice. These decreases could be related to changes in bone marrow-derived progenitors or, alternatively, to extrinsic (microenvironmental) causes. In order to distinguish between these two possibilities, two sets of experiments were performed. Two groups of 2-month-old C57BL/6J mice were lethally irradiated and injected with bone marrow derived from 2- and 20-month-old C57BL/6J donors. Three and 4 months following irradiation, the recipients of young bone marrow had significantly elevated LC numbers (as assessed by staining the epidermal sheets with an anti-Iaα antibody) as compared with recipients of old bone marrow (p < 0.03 and p < 0.05, respectively) (Fig 3A).

The decrease in numbers of LC and Thy-1+DEC in recipients of bone marrow from old donors could have resulted from an increased rate of disappearance of host LC or a slower rate of repopulation by donor LC precursors compared with recipients of young bone marrow. In order to distinguish between donor and host-derived LC, one-month-old C57BL/6J mice were lethally irradiated and injected with bone marrow derived from 2- and 24-month-old (CBA × C57BL/6) F1, mice. Six weeks after irradiation, there was no difference in the density of Iaα-positive LC, but a significant difference in numbers of Iaα-positive LC (p < 0.02) was observed. At 14 weeks after bone marrow transfer, the recipients of old marrow displayed a lower density of donor-derived Iaα-positive cells (p < 0.02) (Fig 3B). Thus the difference in LC density observed after irradiation in recipients of old vs young marrow was due to a different capacity of donor-derived LC to repopulate the skin.

Figure 2. Thy-1+DEC density in the skin of C57BL/6J mice as a function of age as determined by immunofluorescence microscopy with anti-Thy-1.2 antibody. Each point represents the mean ± SD of Thy-1+DEC from three to five mice.
be demonstrated in the epidermis, including serum thymic factor (STF) [47], thymosin b4 [48], and thympoietin [49]. Decreased levels of thymic hormones with age have been correlated with the senescence of immune function [50]. In addition, epidermal cells have been shown to be able to promote the differentiation of T cells [51]. Thus, it is understandable that any damage caused to the skin-associated lymphoid tissues (SALT) [52] could lead to severe dysfunction of the immune system, as has been reported in old mice. Langerhans cell deficiency could participate in such a process.

Our results and previous reports [37–40,42] indicate that low LC and Thy-1+DEC densities characterize the epidermis of old animals. LC are thought to play an important role in the control of infectious and proliferative disorders of the skin [53–56]. Thus, decrease in LC density could contribute to the higher incidence of skin infections and neoplasms in old individuals. Moreover, LC are known to be responsible for the initiation of contact hypersensitivity in the skin [57]. Conflicting reports have been published on the effect of aging on delayed-type hypersensitivity (DTH) in the skin. The DTH response has been shown to remain normal or severely decreased with age depending on the species or animal strain studied [58–63]. Shultz and Bailey demonstrated a decrease in DTH response of C57BL/6j mice with age [62]. Although decreased T-cell function partially accounts for the diminished DTH response with age, the defective antigen-presenting cell function of LC in old mice may contribute to this deficiency [41].

Recently, Komatsubara et al [16] reported that in old mice, dendritic cell function is not impaired with age. In contrast, our present results demonstrated a decreased capacity of epidermal cell suspensions from old mice to stimulate the proliferation of allogeneic T cells and to present antigen to sensitized T cells. However, the two reports differ in their technical approach to the testing of dendritic cell functions. Although our experiments were carried out with non-enriched epidermal cell suspensions, Komatsubara et al used highly enriched preparations of splenic dendritic cells. Thus, it is possible that LC function is not impaired on a per cell basis and that the low LC density in the skin of old mice is solely responsible for our observation that epidermal cells prepared from old mice function poorly as antigen-presenting cells in comparison with such cells derived from young mice.

Normal numbers of NLDC-145+ cells were isolated from the lymph nodes of old mice, most of these carrying the antigen used for sensitization. Various reasons could account for this result. First, it is possible that a compensatory mechanism causes the mobilization of a greater number of LC at the site of sensitization than in young mice, allowing fewer LC to perform the function of the high number of LC found in the young mouse epidermis. Such compensatory mechanisms are thought to account for the enhanced activity of macrophages observed in old mice [13,64]. Second, it is possible that fewer LC are actually transporting the antigen from the skin to the peripheral lymph nodes where they could transfer it to resident NLDC-145+ dendritic cells. Age might not affect the number and/or function of these resident cells. Such a discrepancy between the effect of age on cells of the same lineage, depending on their location, has been reported for humoral responses as well as for T-cell responses to mitogens and alloantigens [65,66]. Although decreased numbers of LC were present in aged mice, LC function might not be impaired on a per cell basis. This situation is similar to observations of other cell subsets where age-associated decreases in precursor frequency were not accompanied by a decline in the function of individual cells [67]. Finally, it is possible that the observed decrease in LC density was due to decreased expression of certain cell surface differentiation markers. Thus, the decreased ability of epidermal cell suspensions to function in the SLR and APC assays might be due to a failure to express cell surface Ia molecules. It is possible that a lack of Ia expression might not interfere with the uptake of antigens and that LC acquire cell surface Ia and other functional markers as they migrate to the lymph node, as has been demonstrated in vitro [32] and is thought to occur in vivo [36]. Such decreased Ia expression in aged BALB/c mice is thought to be due to impaired cytokine regulation [41].

**DISCUSSION**

Previous studies have shown decreased numbers of Langerhans cells in aged humans and experimental animals. Gilchrist et al [37] and Thiers et al [38] demonstrated that ATase-positive LC density decreased with age in humans. Hazlett et al [39] reported only a slight decrease in ATase-positive cell density in the limbal epithelium of aged mice. Beliso et al [40,41] demonstrated a twofold decrease in the LC density in 24-month-old mice by immunohistochemistry for the Ia antigen and Ly-5 antigens. Finally, a recent report by Gu et al [42] showed a twofold decrease in the Ia+ cell population of the murine epidermis in old vs younger mice. They also showed a more moderate decrease in the Thy-1+DEC density with age. However, mice in this previous study were not examined beyond the age of 1 year.

In the present report, it was shown that old mice have decreased densities of LC and Thy-1+DEC in comparison with young controls. The epidermis is considered to play an essential role in the control and initiation of immune-mediated reactions. Interleukin 1 [43,44], GM-CSF [45], and interleukin 3-like [46] activities can be detected in the epidermis, suggesting that the skin participates actively in the induction of cellular immune responses. Various thymic hormones, involved in T-cell development and maturation, can
Our bone marrow transfer studies indicated that the change in LC density due to aging is associated with a decreased rate or capacity of LC progenitors from old mice to repopulate the epidermis of irradiated recipients. This decreased capacity could reflect two different deficiencies associated with aging. The diminished ability of stem cells to repopulate the epidermis of the recipient mice could result from an intrinsic impairment in stem cell function [17]. However, if the defect is intrinsic to the primitive stem cell, it is very specific as old marrow cells repopulate most immune functions normally in young recipients [20]. Furthermore, old C57BL/6J bone marrow injected into young recipients results in the production of circulating lymphocytes and erythrocytes at least as well as young marrow [68]. It is possible that the bone marrow of old mice might contain a suppressor cell population [20] that could lower the capacity of LC progenitors to repopulate the epidermis. Alternatively, as has been suggested by the studies of Belsto et al. [41], the observed decrease in LC numbers may be a consequence of the loss of LC antigens. Thus, the deficient function of LC precursors in aged mice might be due to their lack of response to regulatory cytokines. We have preliminary results indicating that when young and old mice are parabiosed for a period of 4 months, the old partner shows numbers of Langerhans cells typical of young skin. Thus, loss of LC with age seems to be associated with a precursor defect rather than an environmental defect.

REFERENCES


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