Quantitative and Morphologic Changes of Epidermal Langerhans Cell after Photochemotherapy in Guinea Pigs

Soo Chan Kim and Yoon-Kee Park

We studied the number and morphologic alterations of ATPase-positive Langerhans cells (LCs) in guinea pig epidermal sheets and also performed electron microscopic observations on them following long-term systemic psoralen photochemotherapy (PUVA) using 8-methoxypsoralen (8-MOP) and trimethylpsoralen (TMP). We confirmed that the LCs are sensitive to PUVA treatment. The LC depleting effects of PUVA are dose-related and the restoration of LCs takes place by the 4th week following cessation of PUVA. Neither psoralens alone nor UVA alone showed any effects on LCs. There were no significant differences between 8-MOP and TMP when comparing the effects of 8-MOP plus UVA and TMP plus UVA on LC numbers and morphology. It seems that a moderate dose of PUVA causes an actual decrease in the number due to cell damage. Also, it appears likely that giant LCs appear during and following PUVA treatment as a compensatory process of the remaining cells.

Key Words: Photochemotherapy, langerhans cell

During the past few years, photochemotherapy using psoralen and long-wave ultraviolet light (PUVA) has shown considerable effectiveness in the treatment of psoriasis and several other skin diseases (Parrish, 1981). The psoralens used most widely in photochemotherapy are 8-methoxypsoralen (8-MOP) and trimethylpsoralen (TMP); 8-MOP is used primarily for the treatment of psoriasis, and TMP for the treatment of vitiligo (Park et al. 1985).

Langerhans cells (LCs) are dendritic cells found in the epidermis and have la antigens on their surface (Stingl et al. 1978). LCs are intimately involved in the immunological mechanisms of skin; for example, in antigen presentation (Toews et al. 1980; Streilein and Bergstresser, 1984) and as stimulators of T cell activation (Stingl et al. 1978).

Due to the fact that the skin is the only tissue that is naturally exposed to UV radiation, several investigators have studied the effect of UV on the LC population and on LC-dependent immune functions of the epidermis. LCs and their function seem to be particularly susceptible to the damage caused by UV radiation (Toews et al. 1980; Sauder et al. 1981; Stingl et al. 1981) and PUVA (Friedmann, 1981; Koulu and Jansen, 1982), but reports of the exact ways and the extent that their integrity is affected by UV are inconsistent. Some workers suggested that UV light selectively removes LC membrane markers (Aberer et al. 1981), others say that the entire LC population is lost from the epidermis (Noonan et al. 1984) or even that la-positive LCs increase in number after UVB exposure (Nordlund et al. 1981).

In order to clarify the exact mode of the LC depletion process following long-term systemic PUVA treatment and to compare the effects of 8-MOP and TMP on epidermal LC, we studied the number and morphologic alterations of ATPase-positive cells in guinea pig epidermal sheets after long-term PUVA treatment and also performed electron microscopic observations on them.

MATERIALS AND METHODS

Experimental animals

Female albino guinea pigs, weighing 400 to 500 gm, were used and divided into a control group and two PUVA treatment groups. Each group was sub-
divided as follows:

Group I: Control group, 9 subjects total
   a. UVA alone ........................................... 3
   b. 8-MOP alone ....................................... 3
   c. TMP alone .......................................... 3
   d. pretreatment group* ............................... 16
*Same as pretreatment animals of Group II
   and Group III

Group II: PUVA group with small dose of
           psoralens (1.5mg/kg), 8 subjects total
   a. 8-MOP+UVA ......................................... 4
   b. TMP + UVA .......................................... 4

Group III: PUVA group with large dose of
           psoralens (3 mg/kg), 8 subjects total
   a. 8-MOP+UVA ......................................... 4
   b. TMP+UVA ............................................. 4

**PUVA treatment**

An area on the back of the guinea pigs was prepared by depilating and subsequently clipping any
remaining hairs. One hour after the intraperitoneal
administration of psoralens, 1.5 mg/kg in group II and 3
mg/kg in group III respectively, the back skin was ex-
posed to 4J/cm² UVA. The psoralens were dissolved in
0.5% sodium carboxymethyl cellulose solution. The
Waldmann UV 600 emitting in the 315-400nm region
with a maximum at 360nm was used for irradiation.
This procedure was repeated twice a week for 5
weeks in group II and for 3 weeks in group III. PUVA
 treatments were discontinued at the 3rd week for
group III because the animals showed severe inflam-
mation on the exposed area.

**ATPase staining**

For light microscopic observation of LC, biopsies
were performed weekly on the backs of each group
before and during PUVA treatment. In order to study
the recovery phase of the experiment, additional biop-
sies were taken for 4 weeks following cessation of
treatment.

Epidermal sheets were prepared and stained with
adenosine triphosphatase (ATPase) according to the
report of Jutlin and Shelley (1977). That is, the epider-
mis was separated from the dermis by incubation in
EDTA and fixed in a cacodylate-formaldehyde solu-
tion. Then it was incubated in ATP-lead nitrate solu-
tion for 20 min and immersed in an ammonium
sulfide solution. The specimen was mounted in
glycerine jelly.

**Electron microscopy**

Samples for ultrastructural study were taken from
each group prior to treatment and 2, 4 and 5 weeks
after the initial treatment. Additional samples were
also taken 4 weeks after the cessation of PUVA treat-
ment. Small pieces of skin samples were fixed in a mix-
ed solution of 2.5% glutaraldehyde and 1%
paraformaldehyde and post-fixed with 1% osmium
tetroxide. After dehydration in an ethanol series, the
specimens were embedded in Epon and cut into
several ultrathin sections. They were stained with
uranyl acetate and lead citrate and examined with an
Hitachi H-500 electron microscope. LCs were iden-
tified by their general morphology and their specific
Birbeck granules.

**Cell evaluation**

The ATPase-positive cells, which were easily iden-
tified as dark brown dendritic cells, were counted by
means of a reticle fitted into the eyepiece of the
microscope and calibrated at a magnification of 400.
For each specimen, cells were counted in 7 fields, in
which interfollicular areas were selected. The cell
populations were expressed as the average number of
cells per mm². To assess the cell size and mor-
phology, we drew shapes of 10 cells in each specimen
using a camera lucida, then obtained the real cell size
(μm²) and circumference (μm) using the IBM PC
Digitizer DT-3100.

**Statistical analysis**

The statistical analysis was processed using the
Statistical Package for Social Sciences (SPSS).

**RESULTS**

**Changes in LC numbers**

**Control group:** The mean number of ATPase-
positive LC in the control group is summarized in
Table 1. The mean number of LC in the pretreatment
group (pretreatment animals of both Group II and
Group III) was 1,305 ± 165/mm². Neither psoralens
alone nor UVA alone influenced the density of LCs
(Table 1, Fig. 1).

**Group II:** In the 8-MOP plus UVA group, the
number of LCs decreased from a pretreatment value
of 1,168 ± 41 cells/mm² to 738 ± 411 cells/mm², i.e.
63% of the pretreatment value 5 weeks after initial
treatment, and then the number of LCs gradually
returned to normal after cessation of treatment. In
the TMP plus UVA group, the number of LCs decreas-
ed from a pretreatment value of 1,220 ± 70 cells/mm²
Table 1. The number of ATPase-positive epidermal Langerhans cells in subgroups of the control group

<table>
<thead>
<tr>
<th>Week</th>
<th>UVA</th>
<th>B-MOP</th>
<th>TMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1,232±158</td>
<td>1,248±115</td>
<td>1,269±107</td>
</tr>
<tr>
<td>1</td>
<td>1,365±196</td>
<td>1,360±292</td>
<td>1,472±80</td>
</tr>
<tr>
<td>2</td>
<td>1,440±85</td>
<td>1,403±24</td>
<td>1,552±158</td>
</tr>
<tr>
<td>3</td>
<td>1,376±242</td>
<td>1,381±245</td>
<td>1,323±103</td>
</tr>
<tr>
<td>4</td>
<td>1,323±259</td>
<td>1,333±112</td>
<td>1,600±178</td>
</tr>
<tr>
<td>5*</td>
<td>1,280±42</td>
<td>1,280±111</td>
<td>1,509±51</td>
</tr>
<tr>
<td>6</td>
<td>1,317±185</td>
<td>1,415±131</td>
<td>1,175±613</td>
</tr>
<tr>
<td>7</td>
<td>1,376±105</td>
<td>1,428±184</td>
<td>1,493±199</td>
</tr>
<tr>
<td>8</td>
<td>1,280±224</td>
<td>1,440±153</td>
<td>1,536±105</td>
</tr>
<tr>
<td>9</td>
<td>1,179±92</td>
<td>1,269±138</td>
<td>1,413±56</td>
</tr>
</tbody>
</table>

Values are means±S.D. (cells/mm²)
UVA: ultraviolet A
B-MOP: 8-methoxypsoralen
TMP: trimethylpsoralen
*Treatment was discontinued at 5th week

Fig. 1. Time course changes in ATPase-positive epidermal Langerhans cell numbers in subgroups of the control group

Fig. 2. Time course changes in ATPase-positive epidermal Langerhans cell numbers in animals given small doses of phototoxic drugs

Table 2. The number of ATPase-positive epidermal Langerhans cells in animals given small doses of phototoxic drugs

<table>
<thead>
<tr>
<th>Week</th>
<th>B-MOP+UVA</th>
<th>TMP+UVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1,168±41</td>
<td>1,220±70</td>
</tr>
<tr>
<td>1</td>
<td>1,332±164</td>
<td>1,308±236</td>
</tr>
<tr>
<td>2</td>
<td>1,228±68</td>
<td>1,295±38</td>
</tr>
<tr>
<td>3</td>
<td>1,099±116</td>
<td>1,235±156</td>
</tr>
<tr>
<td>4</td>
<td>896±490</td>
<td>852±261t</td>
</tr>
<tr>
<td>5*</td>
<td>738±411</td>
<td>784±142t</td>
</tr>
<tr>
<td>6</td>
<td>904±367</td>
<td>784±376t</td>
</tr>
<tr>
<td>7</td>
<td>916±246</td>
<td>600±611</td>
</tr>
<tr>
<td>8</td>
<td>1,045±161</td>
<td>912±200t</td>
</tr>
<tr>
<td>9</td>
<td>1,067±130</td>
<td>1,244±180</td>
</tr>
</tbody>
</table>

Values are means±S.D. (cells/mm²)
*P<0.05 as compared with 0 week
B-MOP: 8-methoxypsoralen
UVA: ultraviolet A
TMP: trimethylpsoralen
*Treatment was discontinued at 5th week

There were no statistically significant differences between B-MOP and TMP in their effects on the changes in the number of LC.

Changes in LC size

Control group: The mean value of the ATPase-positive LC size in the control group is summarized in Table 4. The mean LC size in the pretreatment group was 221.5 ± 14.9 µm². Neither psoralens alone
Table 3. The number of ATPase-positive epidermal Langerhans cells in animals given large doses of phototoxic drugs

<table>
<thead>
<tr>
<th>Week</th>
<th>B-MOP+UVA</th>
<th>TMP+UVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1,461±215</td>
<td>1,411±126</td>
</tr>
<tr>
<td>1</td>
<td>680±321†</td>
<td>644±403†</td>
</tr>
<tr>
<td>2</td>
<td>188± 89†</td>
<td>219± 24†</td>
</tr>
<tr>
<td>3*</td>
<td>48± 42†</td>
<td>239±133†</td>
</tr>
<tr>
<td>4</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>5</td>
<td>432±121†</td>
<td>440± 11†</td>
</tr>
<tr>
<td>6</td>
<td>1,008±132†</td>
<td>986±104†</td>
</tr>
<tr>
<td>7</td>
<td>1,020±102†</td>
<td>908± 28†</td>
</tr>
</tbody>
</table>

Values are means±S.D.(μm²)
N.D.: not done
†p<0.05 as compared with 0 week
8-MOP: 8-methoxypsoralen
UVA: ultraviolet A
TMP: trimethylpsoralen
*Treatment was discontinued at 3rd week

Fig. 3. Time course changes in ATPase-positive epidermal Langerhans cell numbers in animals given large doses of phototoxic drugs

nor UVA alone influenced the size of the ATPase-positive LCs (Table 4, Fig. 4).

Group II: Both the 8-MOP plus UVA group and the TMP plus UVA group showed a slight but insignificant decrease in cells size by the 4th week of PUVA treatment and an increase in cell size by the 5th week. Four weeks after cessation of treatment the cell size had returned to normal. However, these changes were not statistically significant (Table 5, Fig. 5).

Group III: Changes in cell size during PUVA treatment were statistically insignificant, but the cell size increased by 139-159% of the pretreatment value 3 weeks after cessation of treatment, and subsequently returned to normal the next week (Table 6, Fig. 6).

There were no significant differences in their effects on LC size between 8-MOP and TMP.

Changes in LC circumference

Control group: The mean value of the ATPase-positive LC circumference in the control group is summarized in Table 7. The mean LC circumference in the pretreatment group was 115.5 ± 17.7 μm. Neither psoralens alone nor UVA alone had any effect on the circumference of the ATPase-positive LCs (Table 7, Fig. 7).

Group II: The changing pattern of LC circumference following PUVA was similar to that of LC size. Both the 8-MOP plus UVA group and the TMP plus UVA...
group showed a slight decrease in LC circumference in the 4th week following PUVA. After cessation of treatment, both groups tended to increase their cell circumferences. Four weeks after cessation of treatment, the values returned to normal in the TMP plus UVA group, whereas they were still elevated in the 8-MOP plus UVA group. However, these changes were not statistically significant (Table 8, Fig. 8).

**Group III**: The LC circumferences decreased to 44-55% of the pretreatment value within 2 weeks of initial treatment. After cessation of treatment the circumference gradually increased, returning to normal values by the 4th week (Table 9, Fig. 9).

### Table 5. The size of ATPase-positive epidermal Langerhans cells in animals given small doses of phototoxic drugs

<table>
<thead>
<tr>
<th>Week</th>
<th>8-MOP + UVA</th>
<th>TMP + UVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>196.8±19.1</td>
<td>234.0±11.1</td>
</tr>
<tr>
<td>1</td>
<td>209.8±18.0</td>
<td>220.7±40.5</td>
</tr>
<tr>
<td>2</td>
<td>208.5±16.3</td>
<td>199.5±17.7</td>
</tr>
<tr>
<td>3</td>
<td>219.0±24.8</td>
<td>186.3±13.1</td>
</tr>
<tr>
<td>4</td>
<td>173.8±32.2</td>
<td>181.5±44.1</td>
</tr>
<tr>
<td>5</td>
<td>234.3±75.5</td>
<td>235.8±50.5</td>
</tr>
<tr>
<td>6</td>
<td>235.0±58.3</td>
<td>312.3±113.7</td>
</tr>
<tr>
<td>7</td>
<td>264.8±44.9</td>
<td>370.3±153.4</td>
</tr>
<tr>
<td>8</td>
<td>255.7±27.0</td>
<td>277.5±81.9</td>
</tr>
<tr>
<td>9</td>
<td>252.3±47.2</td>
<td>235.0±24.8</td>
</tr>
</tbody>
</table>

Values are mean±S.D. (µm²)

8-MOP: 8-methoxypsoralen
UVA: ultraviolet A
TMP: trimethylpsoralen
* Treatment was discontinued at 5th week

### Table 6. The size of ATPase-positive epidermal Langerhans cells in animals given large doses of phototoxic drugs

<table>
<thead>
<tr>
<th>Week</th>
<th>8-MOP + UVA</th>
<th>TMP + UVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>199.0±22.6</td>
<td>188.5±11.0</td>
</tr>
<tr>
<td>1</td>
<td>195.8±24.1</td>
<td>206.3±39.6</td>
</tr>
<tr>
<td>2</td>
<td>139.8±9.6</td>
<td>169.0±36.3</td>
</tr>
<tr>
<td>3</td>
<td>161.3±33.5</td>
<td>237.5±129.9</td>
</tr>
<tr>
<td>4</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>5</td>
<td>150.7±6.1</td>
<td>152.0±5.7</td>
</tr>
<tr>
<td>6</td>
<td>275.7±33.5</td>
<td>298.8±26.4</td>
</tr>
<tr>
<td>7</td>
<td>199.0±52.1</td>
<td>241.5±66.4</td>
</tr>
</tbody>
</table>

Values are mean±S.D. (µm²)
t < 0.05 as compared with 0 week
N.D.: not done

8-MOP: 8-methoxypsoralen
UVA: ultraviolet A
TMP: trimethylpsoralen
* Treatment was discontinued at 3rd week

**Fig. 5.** Time course changes in ATPase-positive epidermal Langerhans cell size in animals given small doses of phototoxic drugs

**Fig. 6.** Time course changes in ATPase-positive epidermal Langerhans cell numbers in animals given large doses of phototoxic drugs

There were no significant differences between 8-MOP and TMP in their effects on LC circumference.

### Morphologic changes

The morphologic changes in LCs following PUVA administration were more prominent in the group which had received the larger dose. Whereas the LCs of group II showed minimal changes during PUVA treatment, most of the remaining cells in group III were observed to be round, lacking dendrites. As LC density diminished, a few large, bizarre LCs appeared; this was more striking 2-3 weeks after cessation of
Table 7. The circumference of ATPase-positive epidermal Langerhans cells in subgroups of the control group

<table>
<thead>
<tr>
<th>Week</th>
<th>UVA</th>
<th>B-MOP</th>
<th>TMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>114.7±0 7.8</td>
<td>115.3±8.7</td>
<td>96.0±8.8</td>
</tr>
<tr>
<td>1</td>
<td>125.3±3.2</td>
<td>129.3±8.2</td>
<td>104.7±22.8</td>
</tr>
<tr>
<td>2</td>
<td>99.0±10.0</td>
<td>103.0±8.2</td>
<td>97.3±13.8</td>
</tr>
<tr>
<td>3</td>
<td>103.0±7.2</td>
<td>136.7±26.8</td>
<td>117.0±14.1</td>
</tr>
<tr>
<td>4</td>
<td>119.7±25.0</td>
<td>121.3±22.7</td>
<td>124.0±26.2</td>
</tr>
<tr>
<td>5*</td>
<td>101.3±10.4</td>
<td>117.0±6.2</td>
<td>105.0±13.8</td>
</tr>
<tr>
<td>6</td>
<td>126.3±9.1</td>
<td>126.7±17.0</td>
<td>116.3±34.7</td>
</tr>
<tr>
<td>7</td>
<td>123.7±21.4</td>
<td>143.3±17.6</td>
<td>119.7±35.0</td>
</tr>
<tr>
<td>8</td>
<td>110.0±15.1</td>
<td>138.3±18.0</td>
<td>111.0±6.6</td>
</tr>
<tr>
<td>9</td>
<td>123.7±15.1</td>
<td>122.7±14.5</td>
<td>109.7±6.7</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. (μm)
UVA: ultraviolet A
B-MOP: 8-methoxypsoralen
TMP: trimethylpsoralen
*Treatment was discontinued at 5th week

Fig. 7. Time course changes in ATPase-positive epidermal Langerhans cell circumference in subgroups of the control group

PUVA. Their size was approximately 2-3 times larger than normal with elongated and bizzare dendrites. However, by the 4th week post PUVA, such giant cells began to decrease in size. Therefore it seemed that the appearance of giant LCs was closely related to the reduction in the number of LC (Fig. 10, 11, 12). A schema of morphological changes of LCs following PUVA is presented in Fig. 13.

Electron microscopic findings

The LC are easily identified in the pretreatment specimens by their general morphology and Birbeck granules (Fig. 14). Most LCs showed only one or two Birbeck granules in the cytoplasm (Fig. 15, 16).

Group II samples, taken 4 weeks after PUVA treatment, showed degenerative changes in both LCs and keratinocytes, though these changes were much more prominent in LCs. Many LCs showed degenerative changes such as a decrease in the number of intracytoplasmic organelles and many intracytoplasmic vacuoles (Fig. 17).

The specimens from Group III, taken 3 weeks after PUVA treatment, showed severe degenerative changes including many intracytoplasmic vacuoles and pyknotic nuclei in the keratinocytes (Fig. 18). However, we could not identify any LCs in these samples.
In the samples taken 4 weeks after cessation of PUVA, LCs with intact morphology were encountered (Fig. 19).

Table 9. The circumference of epidermal Langerhans cells in animals given large doses of phototoxic drugs

<table>
<thead>
<tr>
<th>Week</th>
<th>8-MOP + UVA</th>
<th>TMP + UVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>118.0±17.6</td>
<td>106.5±8.2</td>
</tr>
<tr>
<td>1</td>
<td>81.8±21.2†</td>
<td>83.8±7.1</td>
</tr>
<tr>
<td>2</td>
<td>51.8±6.3†</td>
<td>59.0±10.8</td>
</tr>
<tr>
<td>3*</td>
<td>56.0±7.2†</td>
<td>112.8±99.6</td>
</tr>
<tr>
<td>4</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>5</td>
<td>76.0±4.4</td>
<td>73.5±6.4</td>
</tr>
<tr>
<td>6</td>
<td>157.7±17.6</td>
<td>163.0±18.8</td>
</tr>
<tr>
<td>7</td>
<td>112.0±35.3</td>
<td>144.3±35.0</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. (μm)
†p<0.05 as compared with 0 week
N.D.: not done
8-MOP: 8-methoxypsoralen
UVA: ultraviolet A
TMP: trimethylpsoralen
*Treatment was discontinued at 3rd week

**DISCUSSION**

Recently, much interest has been focused on the epidermal LC since it became evident that this cell plays an important role in immunological reactions of the skin (Stingl et al. 1980). PUVA treatment and

![Fig. 9. Time course changes in ATPase-positive epidermal Langerhans cell circumference in animals given small doses of phototoxic drugs](image)

![Fig. 10. ATPase-positive Langerhans cells in an epidermal sheet from a pretreatment guinea pig (ATPase stain, × 400)](image)
Fig. 11. ATPase-positive, non-dendritic, round cells in a PUVA treated epidermal sheet from a group III animal, 3 weeks after PUVA treatment (ATPase stain, × 400)

Fig. 12. Giant Langerhans cells in a PUVA treated epidermal sheet from group III, 2 weeks after cessation of PUVA treatment (ATPase stain, × 400)

Fig. 13. Schema of morphological changes in epidermal Langerhans cells following PUVA treatment
Fig. 14. Electronmicrograph of a Langerhans cell prior to treatment with a pale cytoplasm devoid of tonofilaments. A dendrite (d) is shown (× 5,000).

Fig. 15. Langerhans cell before treatment with a racket-shaped Birbeck granule (arrow) (× 34,000). Inset: High magnification of a Birbeck granule (× 88,000)
Fig. 16. Langerhans cell before PUVA treatment with a rod-shaped Birbeck granule (arrow) and mitochondria (m) (× 28,500)

Fig. 17. Langerhans cell from group II with many intracytoplasmic vacuoles (V) 4 weeks after treatment (× 4,200)
Fig. 18. Keratinocytes from group III exhibiting severe degenerative changes 3 weeks after PUVA treatment (× 4,200)

Fig. 19. A Langerhans cells from group III shows no degenerative changes 4 weeks after cessation of PUVA. Arrow indicate intracytoplasmic granule (× 10,000). Inset: High magnification of Birbeck granules (× 28,200)
UVB radiation have been shown to deplete the epidermis of LC in both experimental animals and humans (Aberer et al. 1981; Okamoto and Hori, 1981).

In our study, we also observed that LCs are remarkably sensitive to PUVA. A small dose of PUVA had only a slight effect but a large dose and repeated treatments caused marked depletion of LC numbers and altered the morphology of those that remained. However, the density and morphology of LC were almost restored to normal 4 weeks post PUVA.

There is controversy in the literature as to the fate of LC following UV exposure, i.e., whether a fraction of the LC dies or emigrates from the epidermis in response to the injurious effect of UV light, or whether the cells merely lose their staining reactions while remaining in the epidermis. Toews et al. (1980) originally interpreted the loss of ATPase-positive cells as indication that the cells had been destroyed or had emigrated out of the epidermis. However, a study by Aberer et al. (1981) suggested that although there is a reduction in the number of ATPase-positive cells after UV irradiation of the skin, many of these cells are still present in the epidermis and can be detected by electron microscopy. They concluded that UV irradiation depletes only LC surface markers. Sauder et al. (1981) also demonstrated that antigen presentation by LCs was affected by UV treatment, while it had no significant effect on the expression of Fc receptors or la antigens by LCs. Therefore, the apparent depletion of ATPase-positive cells by UVB or PUVA treatment does not necessarily mean the destruction of LCs, but may represent functional changes of the cell. In contrast, Nordlund et al. (1981) suggested that the continued decrease of LCs in the epidermis for 3 weeks following cessation of PUVA and the additional several weeks needed for the cells to regain their normal density indicate that PUVA kills LCs rather than merely alters their surface la antigens. By electron microscopic morphometric techniques, Ree (1982) confirmed that there is an actual depletion of LC following PUVA. Obata and Tagami (1985) reported that electron microscopy and immunoelectron microscopy showed that the majority of the LCs disappeared due to actual cell damage, while some LCs simply lost their la marker without any structural alterations following UVB exposure or PUVA. Iacobelli et al. (1985) suggested that only high dosages of UVB are able to physically damage the LC membrane, whereas lower dosages produce only a disturbance of the spatial distribution of the ATPase loci of the LC membrane, followed by their aggregation. It is possible that only the surface marker is affected by lower UV radiation doses or earlier stages after irradiation. Our electron microscopic findings demonstrated that moderate and repeated dose of PUVA can cause actual damage to LC, and the effects of PUVA are dose-related. Restoration of ATPase-positive LCs after PUVA treatment seems to be achieved partly through the influx of new LCs originating in the bone marrow and partly by the reappearance of surface markers on residual cells. In addition to this mechanism, LCs seems to undergo mitosis and contribute to the repopulation during the early recovery phase (Myauchi and Hashimoto, 1987).

In addition to inducing LC depletion, PUVA exposure also induced morphological changes in the cells. It is of interest that giant LCs appeared during and after PUVA treatment. The earliest change was a loss of the dendritic processes following PUVA. As LC density diminished, a few large, bizarre LCs were found. These giant LCs had elongated dendritic processes and their size was 2–3 times larger than normal. These cells appeared more frequently during the recovery phase following cessation of PUVA. Four weeks after cessation of PUVA these giant cells decreased to normal size. Such giant LCs were previously demonstrated with ATPase staining in patients with psoriasis treated with PUVA (Juhiin and Shelley, 1979). Obata and Tagami (1985) also demonstrated the la-positive giant LC in mice epidermal sheets after UVB irradiation or PUVA treatment. It seems likely that the appearance of giant LCs is a compensatory process of the remaining LCs following PUVA. However, their nature and function remain obscure.

Current data demonstrated that exposure to a sufficiently high cumulative dose of PUVA substantially increases the risk of squamous cell carcinoma of the skin (Stern, 1986). Development of UV-induced tumors is associated with the appearance of suppressor T lymphocytes, and UV-induced changes in LC number and morphology may be related to the induction of antigen-specific suppressor T lymphocytes (Noonan et al. 1981; Granstein et al. 1984). Therefore, very high doses of PUVA over a long-term period must be avoided in order to minimize the risk of UV-induced skin cancer.

REFERENCES


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