The polycyclic aromatic hydrocarbons in the environment have been known to act as chemical carcinogens, and 7,12-dimethylbenz[a]anthracene (DMBA) has been used as a complete carcinogen in chemical carcinogenesis experiment. The topical application of this compound to the skin resulted in inflammation in the administered tissue site, followed by healing and cancer under yet-unidentified mechanisms [7, 9].

A wide variety of cytokines have been involved in the inflammatory process. Among them, interleukin-6 (IL-6), a multi-functioning cytokine, that harbors functions for the antibody production and cellular proliferation, is produced by a variety type of cells, such as antibody-producing cells, macrophages, fibroblasts as well as other skin tissue cells [6]. IL-6 is rapidly released by inflammatory stimuli, resulting in the induction of biosynthesis of acute-phase proteins that are involved in the defense in the inflammatory process. In the present study, we have studied whether IL-6 is involved in the DMBA-induced inflammation in the skin by the use of IL-6 null mice. Administering recombinant human IL-6 (rhIL-6) to IL-6 null mice, we also studied its putative effect on the DMBA-induced skin inflammation.

DMBA and other reagents were purchased from Sigma (St. Louis, MO, U.S.A.) and Wako Pure Chemical Industries (Osaka, Japan), respectively. Recombinant human IL-6 was kindly provided by Dr. Hideki Suzuki at Pharmaceutical Research Laboratories, Ajinomoto Co. Inc. (Kawasaki, Japan). IL-6 null mice produced by homologous recombination and their wild-type control mice (B6J/129Sv) were originally obtained from Jackson Laboratory (Bar Harbor, ME, U.S.A.) and bred at the vivarium of National Institute for Environmental Studies (NIES). The mice were maintained under the following conditions: a 12-hr light-dark cycle, a temperature of 23 ± 1°C, and humidity of 50 ± 10%. The mice were housed in a plastic cage, given food and water ad libitum, and received humane care according to the guidelines of the NIES. The female mice (20-week-old) were shaved on the back and abdomen. Two days later, DMBA was administered on the back at a dose of 500 µg/100 µl of acetone. To infuse constant amounts of rhIL-6 to IL-6 null mice, we used Alzet osmotic pump (Alzet model, 1007D, 0.5 µl/hr for 7 days) with a flow rate of 10µg/day or 25 µg/day. The pump was filled with rhIL-6 dissolved in 2% normal mouse serum in phosphate-buffered saline and was placed under the abdominal skin at 21 hr before the DMBA application according to the method of Suzuki et al. [8].

Vehicle-treated animals received 2% mouse serum in PBS. Gross observation was performed every 12 hr. Five days post-administration of DMBA, we found by gross observation ulcer formation in the skin of IL-6 null mice only, in contrast to no gross alterations in the skin of wild-type mice. We killed all the animals under ethyl ether anesthesia by cardiac puncture and collected blood and skin tissue from the back, and the skin tissue was fixed in 10% phosphate-buffered formalin and fixed in paraffin. The sections (5-µm in thickness) were deparaffinized and dehydrated through xylene and a series of graded concentrations of alcohol, and stained with hematoxylin and eosin.

Pathological alterations in the skin were examined for each mouse to classify hyperplasia, erosion and ulcer. The morphometric analysis was carried out by the use of image analysis software (Leica Qwin), and used for diagnosing each mouse by the lesion type. When each mouse had more than two different types of lesion such as hyperplasia, erosion, and ulcer, we adopted for diagnosis the lesion type which occupied the largest area in the total measured area.

NOTE

Interleukin-6 Protects Skin Lesion Caused by 7,12-Dimethylbenz[a]anthracene

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ABSTRACT. Interleukin-6 (IL-6) regulates essential physiological functions such as acute phase reaction, immune response and hemato-poiesis. We here studied possible protective effects of IL-6 on skin damage caused by 7,12-dimethylbenz[a]anthracene (DMBA) by using IL-6 null mice. The mice were topically applied with a single dose of DMBA (500 µg/mouse) on the dorsal skin. Osmotic pumps filled with recombinant human IL-6 (rhIL-6) were implanted subcutaneously on the ventral side of the mice. Control mice received PBS instead of rhIL-6 by the pump. Severe skin damage was observed in IL-6-null mice, whereas only epidermal hyperplasia was observed in the wild-type mice. Recombinant hIL-6 treatment to DMBA-treated IL-6-null mice suppressed the occurrence of the skin damage, indicating.

KEY WORDS: 7,12-dimethylbenz[a]anthracene, interleukin-6, recombinant human IL-6.
The DMBA-administered IL-6-null mice were found to have ulcer all around the DMBA-applied skin area (Fig. 1B, Table 1). In contrast, all four DMBA-treated wild-type mice were observed to have a lesser degree of inflammation, characterized as hyperplasia in the epidermis (Fig. 1E), but the two out of the four animals had erosion (Table 1) with a small size of ulcer (Fig. 1F).

Pretreatment of rhIL-6 suppressed the occurrence of ulceration in DMBA-administered IL-6-null mice at a dose of 10 and 25 µg/day in a dose-dependent fashion, and erosion was found to be a cardinal pathological sign to DMBA insult (Fig. 1C, Table 1). DMBA-treated IL-6 null mice having rhIL-6 pretreatment showed a lesser degree of DMBA-induced lesion than those without receiving rhIL-6 pretreatment.

To confirm the persistent infusion of rhIL-6, we determined serum rhIL-6 concentrations by ELISA (Amersham Pharmacia Biotech UK, Buckinghamshire, UK). The concentration of most of the rhIL-6 infused IL-6 null mice exceeded 400 pg/ml, the upper detection limit of ELISA kit, on day 5 after DMBA administration while no IL-6 was detected in IL-6 null mice that were not administered with rhIL-6.

In another set of the present experiment we found that the skin ulcer that occurred in the DMBA-treated wild-type mice was observed in a relatively restricted area in comparison to the extensive lesion in DMBA-treated IL-6 null mice. In brief, when IL-6 null mice were topically applied with DMBA (1,000 µg/mouse), the entire area of the administered skin showed ulcer by day 7 post-administration and

![Fig. 1. Typical inflammatory histopathological features of the skin of IL-6 null mice (A-C) and wild-type mice (D-F) topically administered DMBA at 500 µg per mouse. (A) Acetone administered IL-6 null mouse skin. (B) In IL-6 null mice, epidermis hyperplasia was observed after severe ulceration and considerable numbers of infiltrating cells were observed in the dermis and hypodermal tissue. (C) In IL-6 null mice with infusion of rhIL-6 (25 µg/day), the structure of epidermis remained and no ulceration but erosion was found. (D) Acetone administered wild-type mouse skin. (E) In DMBA-administered wild-type mouse, hyperplasia of the epidermis (F) or erosion with a small-size ulcer was observed. Hematoxyline and eosin staining. Bar=100 µm.](image-url)
IL-6 PROTECTS DMBA-CAUSED SKIN LESION

11 out of 20 mice (55%) died of hyposthenia (Zhang et al., unpublished data). On the other hand, DMBA-treated wild-type mice showed erosion and ulcer in limited area of the skin, without severe degenerative changes or lethality. In addition, the regeneration of skin lesion in wild-type mice treated with 1,000 µg DMBA/mouse occurred much faster than that in IL-6 null mice. These results suggest that IL-6 was involved in the protection against inflammation caused not only by a low dose of DMBA but also by a high dose of DMBA.

It has been reported that IL-6 null mice were not endowed with the full capacity of antibody production against viral infection and of induction of acute-phase proteins against turpentine oil and lipopolysacharide. Nishimura and coworkers [4, 5] reported irradiation of ultraviolet ray B exerted more intense lesion to the skin of IL-6 null mice than that to the wild-type mice. These results suggest that IL-6 null mice harbor a reduced level of immune function and self-defense system than that of the wild-type mice [2].

It was reported that it took more time for partially-hepatectomized IL-6 mice to regenerate the liver tissue than partially-hepatectomized wild-type mice and that IL-6 plays a yet-identified role in liver regeneration via the regulation of cell cycle [1]. Furthermore, liver fibrosis caused by treatment of carbon tetrachloride was found severer in IL-6 null mice than in wild-type mice [3]. Taken together with these observations, it is thought that IL-6 plays a very important role in the tissue regeneration not only in the liver but also in the skin, and the present study showed that IL-6 is one of the crucial factors that are responsible for the sensitivity of inflammatory responses.

REFERENCES


**Table 1.** Incidence of skin damage in IL-6-Null mice and wild-type mice after topical treatment of DMBA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-6-null</th>
<th>Wild-type</th>
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<tbody>
<tr>
<td></td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5/5</td>
<td>–</td>
</tr>
<tr>
<td>DMBA (10 µg/day)</td>
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<td>–</td>
</tr>
<tr>
<td>+DMBA (500 µg/mouse)</td>
<td>–</td>
<td>1/5</td>
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<tr>
<td>rhIL-6 (10 µg/day)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>+rhIL-6 (500 µg/mouse)</td>
<td>Not Performed</td>
<td>Not Performed</td>
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</table>

a) Skin damage was rated according to the following criteria: –, Normal; +, Hyperplasia; ++, Erosion; +++ Ulcer.

b) When there were more than two types of lesion in the skin of a mouse, we adopted for diagnosis the lesion type which occupied the largest area in the total measured area.