Alpha-Melanocyte Stimulating Hormone Modulates Contact Hypersensitivity Responsiveness in C57/BL6 Mice

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The neuropeptide alpha-melanocyte stimulating hormone (α-MSH) can act as an antagonist to interleukin 1 (IL-1) bioactivities such as inhibition of fever production, thymocyte proliferation, and inhibition of release of acute phase inflammatory molecules from the liver. In this report we have found that epicutaneous application of α-MSH suppresses both the sensitization and elicitation limbs of the cutaneous immune response (CIR) to potent contact sensitizers like dinitrofluorobenzene (DNFB) or oxazalone (OX) in mice. Further, the loss of contact hypersensitivity due to applications of α-MSH could be reconstituted by either intradermal or intravenous injections of epidermal thymocyte activating factor (ETAF)/interleukin-1. Topical application of α-MSH did not cause an alteration in Ia+ dendritic cells (i.e., Langerhans cells) but did produce a significant reduction in the expression of Thy1.2 marker on the Thyl + dendritic epidermal cells (Thy1+DEC). It has no effects on the phenotypic expression of asialo GM-1 on these same cells. These observations suggest that α-MSH, a peptide classically isolated from the pituitary but found in many other tissues and cells of the body, may represent an additional biologic modifier than can modulate suppression of the contact hypersensitivity responses to various haptens. However, the mechanisms by which α-MSH or potentially other peptides found in the skin produce these suppressive effects have not been elucidated. *J Invest Dermatol 93:511–517, 1989*

Alpha-melanocyte stimulating hormone (α-MSH) is a 13 amino acid peptide, one of the several proopiomelanocortin-derived molecules synthesized in the pituitary gland and, named originally for its ability to stimulate skin pigmentation in amphibians [1]. It is the classic melanotropic hormone. More recently, α-MSH has been shown to have other functions, i.e., modulation of immune inflammatory processes. Although α-MSH was initially isolated from the pars intermedia and is considered a neuropeptide, it has been isolated in a variety of other tissues and organs including the placenta [2,3].

Several investigators have documented that the pyrogenic properties of interleukin-1 (IL-1) can be abolished by minute quantities of α-MSH [4–6]. α-MSH is the most potent antipyrogenic substance known. In addition to its antipyrogenic effect, α-MSH blocks IL-1 induction of IL-2 receptor expression [7], polymorphonuclear (PMN) chemotaxis [8], and hepatic induction of acute phase reactants including C reactive protein and serum amyloid P [6,9]. However, in other studies α-MSH had no inhibitory effect in altering IL-1-induced prostaglandin release or thymocyte stimulation with mitogen [6]. Other studies have demonstrated that precursors to α-MSH (i.e., β-LPH and ACTH) can be found in extrapituitary cells, specifically thymocytes and splenocytes. The exact role of α-MSH and its precursor molecule in the modulation of the immune system is still poorly defined.

The epidermis is a source of IL-1 and/or ETAF (epidermal thymocyte activating factor). Recently it has been suggested that IL-6 may be part of the ETAF complex [10]. The epidermis contains mRNA coding for IL-1α and IL-1β, but also a unique mRNA with IL-1 activity referred to as κ-IL-1. ETAF/IL-1 can be isolated from the stratum corneum and is secreted by keratinocytes [11–13]. Melanocytes and Langerhans cells can also secrete IL-1 [14,15]. Whether ETAF from keratinocytes and IL-1 from melanocytes and Langerhans cells are identical or similar is not known. Langerhans cells and keratinocytes play an important role in both the sensitization and elicitation limbs of the cutaneous immune system following epicutaneous application of a variety of haptens [16–18].

The current investigations were carried out to determine whether

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Abbreviations:

ACTH: adenocorticotropic hormone
CHS: contact hypersensitivity
CIR: cutaneous immune response
DLN: draining lymph node
DNFB: 2,4-dinitro-1-fluorobenzene
ELR: epidermal lymphocyte experiments
ETAF: epidermal thymocyte activating factor
IL-1: interleukin 1
LC: Langerhans cells
α-MSH: alpha-melanocyte stimulating hormone
OX: 4-ethoxymethylene-2-phenyloxazol-5-one (oxazalone)
PBS: phosphate-buffered saline
PMN: polymorphonuclear
POMC: proopiomelanocortin
RBC: red blood cells
Thy1+ DEC: Thy-1 positive dendritic epidermal cell
T_h: T helper cells
T_s: T suppressor cells

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topical α-MSH could abrogate functional sensitization and elicitation of the cutaneous immune response to specific haptens. If so, we wished to determine whether exogenous administration of murine ETAF/IL-1 could reconstitute loss of contact hypersensitivity responsiveness.

MATERIALS AND METHODS

Animals Five to seven-wk-old male C57BL/6 (H-2^b) mice were purchased from the Charles Rivers Laboratory, Wilmington, MA, and were housed in the AAALAC approved University animal facilities, four per cage with free access to water and Purina mouse chow. Animals were maintained on a 12-h light:dark photoperiod. All experiments were performed twice and produced similar results.

Identification of Ia+ and Thy1+ Dendritic Epidermal Cells Ia and Thy1 staining of epidermal cells was performed as described [19,20]. Briefly, ears were surgically excised from ether vapor killed mice. The ears were mechanically split into dorsal and ventral halves. Dorsal tissue was immersed in 0.020 M Na EDTA for 2 h at 37°C. After the incubation period, the epidermis was separated as an intact sheet. Staining of Ia+ bearing epidermal cells was performed by using a monoclonal anti-Ia^p (Accurate Chemical Company, Westbury, NY) antibody diluted 1:100 in phosphate-buffered saline (PBS). This antiserum can cross react with all standard haplotypes. The antibody was produced by growth of an hybridoma SP2/0 myeloma cells fused with Balb/C spleen cells. The immunoglobulin was isolated from ascitic tumors in PBS. Epidermal sheets were fixed in acetone before staining with the primary antibody and extensively washed in PBS before indirect immunoperoxidase staining. A Vecta-stain ABC immunoperoxidase staining kit (Vector Laboratories, Burlingame, CA) was used following incubation of the primary monoclonal antibody at 37°C for 1 h. Immunoperoxidase substrate was 3-amino-9 ethyl carbazole (Sigma Chemical Co., St. Louis, MO). Incubation for 10 min at 37°C with the secondary reagents was followed by several washes in PBS and final mounting in glycerol:PBS.

Thy1 + DEC were identified using double labeling indirect immunofluorescence. Epidermal sheets were fixed in acetone followed by rehydration by PBS. Sheets were simultaneously labeled with mouse anti-Thy1.2 (Accurate Chemical Company, Westbury, NY) diluted 1:100 or rabbit anti-asialo GM-1 diluted 1:20 (Wako Chemical Company, Dallas, TX) for 16 h at 4°C. The tissue specimens were washed three times in PBS for 60 min and incubated for an additional 60 min at 37°C with either goat antimouse rhodamine diluted 1:20 or goat anti-rabbit fluorescein (FITC) 1:20 in PBS. Tissues were then washed extensively in PBS and mounted as described [18,29]. Control experiments included epidermal sheets in which the primary antibodies were excluded and only secondary reagents were added to examine for cross-reactivity with antibody conjugated to rhodamine or fluorescein.

The number of Ia+ Langerhans cells or Thy1+ dendritic epidermal cells per square millimeter in four standardized peripheral areas of each epidermal sheet was determined. At least four animals were studied in each group. The reported Ia+ Langerhans cell and Thy1.2+ dendritic epidermal cell density for each sheet represents the mean of at least 16 counts (±SE).

Topical α-MSH Administration Mice were treated on days 1–5 by topical application of 50 μl of 10^{-5} M α-MSH (Sigma Chemical Company, St. Louis, MO) diluted in propylene glycol. Pilot experiments using concentrations ranging from 10^{-5}–10^{-8} M α-MSH were performed. α-MSH at 10^{-5} M applied topically was shown to be the most optimal concentration and therefore was used throughout the series of experiments. Mice were treated with α-MSH applied either to the dorsal surface of the ear (challenge site) or the shaved back (sensitization site). On day 6, pinnal epidermal specimens were obtained and the population density (cells/mm^2) of Ia+ Langerhans cells and Thy1+ dendritic epidermal cells in each experiment was determined both by indirect immunoperoxidase and indirect immunofluorescence staining, respectively.

Contact Sensitivity and Challenge: Suppression of the Sensitization and Elicitation Limbs of the CHS Response by α-MSH Groups of mice (four per group) were treated on the shaved back with 50 μl of 10^{-5} M α-MSH or diluent on days 1–5. One half hour following the last treatment, treated or diluent control mice were sensitized with 25 μl of 0.5% (0.5 g/dl) 2,4-dinitro-1-fluorobenzene (DNFB) (Sigma Chemical Company, St. Louis, MO) in a 4:1 acetone:olive oil mixture or with 25 μl of 10% 4-ethoxy-methylene 2-phenyloxazol-5-one (oxazalone) (Sigma Chemical Company, St. Louis, MO) applied to the shaved back epidermal site. On day 6, mice were sensitized again with the respective haptens, DNFB and/or OX, and rested for five days. On day 11, ears of treated and control mice were measured as baseline. The ears were challenged with 20 μl of 0.2% (0.2 g/dl) DNFB or 20 μl of 1% (1 g/dl) oxazalone. On day 12, the ear thickness was measured a second time. Percent suppression was calculated as:

$$\% \text{ suppression} = \left[\frac{1\text{-(experimental-negative control)}}{\text{positive control(diluent)-negative controls}}\right] \times 100\%.$$

The degree of ear swelling was determined as described [18]. Elicitation of the CHS response was carried out similarly except the animals were treated on the ears on days 1–5 with 50 μl of 10^{-5} M α-MSH or propylene glycol and then sensitized on the untreated shaved back with either DNFB or OX.

Adoptive Transfer of Splenic Suppressor Cells Groups of mice were topically treated with 10^{-5} α-MSH or propylene glycol for five days on the shaved back. On days 5 and 6 the mice were sensitized with DNFB and five days later (days 10 and 11) were challenged on the ear to demonstrate the CHS response was impaired. On day 13, spleen cells were taken from treated and control mice and injected intravenously into syngeneic naive recipients.

Splenocytes from α-MSH-treated mice were prepared by teasing spleens with forceps or with a glass rod in HBSS followed by filtering through a nylon mesh (Tetko, Inc., Elmsford, NY). The cells were then washed three times in HBSS and the red blood cells (RBC) were lysed in NH_4Cl:Tris buffer. The unfractionated splenic lymphocytes were then layered onto Lympholite (density 1.087) (Accurate Chemical Company, Westbury, NY) and centrifuged for 30 min at 25°C. The interface containing the lymphocytes were then washed two additional times to remove any further cellular debris. Spleen cell suspensions were then injected intravenously via the tail vein of mice at a dose of 1×10^8 viable cells. Viability was consistently greater than 90% by trypan blue dye exclusion.

To determine whether T suppressor or T helper activity was induced by applications of α-MSH and/or DNFB, a functional adoptive transfer assay was used as described and used by other investigators [4,21]. Groups of recipient mice were challenged immediately, and 24 h later their ears were measured. Spleen or lymph node cells containing sensitized T helper cells (T_h) would induce these animals to give a positive response to elicitation challenge with the appropriate hapten applied immediately after adoptive transfer. Other recipient animals were sensitized and challenged five days later. Recipient animals receiving lymph node or splenic cells containing significant T suppressor activity would respond less vigorously than control animals. Such animals would functionally have received T suppressor (T_s) lymphocytes.

Adoptive Transfer of Draining Lymph Node Effector Cells Inguinal, axillary and brachial lymph nodes were surgically excised from α-MSH and control treated/DNFB contact sensitized mice. Single cell suspensions were prepared as described in the splenic cell preparation with the exception of the RBC lysing step. Groups of naive mice received at least 1×10^6 viable draining lymph node (DLN) cells intravenously into the tail vein. Groups of mice then were challenged on the ears immediately and ear swelling measured at 24 h later or were sensitized and challenged five days later. T helper or suppressor activity was assessed by the response as noted in the section on methods for adoptive transfer of spleen cells.

Reconstitution of CHS Responsiveness After ETAF Administration ETAF/IL-1 was prepared for in vivo reconstitution studies as follows: Murine ETAF was prepared by a modification of the described method [12]. Briefly, conditioned medium from the keratinocyte cell line PAM 212 was collected and the cell-free supernatant was purified by ultrafiltration and Sephadex G-100 chromatography. Active fractions were pooled and concentrated and chromatographed on a 41 × 250-mm Synchropak AX 300 ion exchange column (Synchrom Inc., Linden, IN). Active fractions then were pooled on a TSK 3000 column. The active fractions were pooled and concentrated yielding approximately 500 U/ml of ETAF/IL-1 activity.

Groups of mice were treated with 50 μl of 10^{-5} M α-MSH or diluent on the shaved back for five days. Thirty minutes after the final epicutaneous treatment, mice received 100 μl of ETAF or PBS diluent administered intradermally or intravenously via the tail vein. Thirty minutes later the mice were sensitized with 0.5% DNFB. Twenty-four hours later the mice received a second injection of ETAF/IL-1 or PBS intradermally or intravenously, followed 30 min later by a second application of 0.5% DNFB. Five days later the mice were challenged on the ears as described.

Statistics The statistical significance of differences between the mean ear thickness of the experimental control group was calculated with the Student's *t* test. Mean differences were considered significant when *p* was less than 0.05.

RESULTS

Effect of Topically Applied α-MSH on Epidermal Ia+ Langerhans Cells and Thy1+ Dendritic Epidermal Cells A 10^{-5}-M concentration of α-MSH applied to the dorsal surface ear skin of mice for five days produced no significant changes in the population density of identifiable Ia+ dendritic epidermal cells (presumably functionally active antigen presenting Langerhans cells) (Table I) ($t = 1.56$; $p < 0.12$) versus vehicle controls. To determine if topically applied α-MSH was affecting the phenotypic expression of the Thy1.2 differentiation marker and causing an alteration of these identifiable Thy1+ dendritic cells from the epidermis, other α-MSH-treated epidermal sheets and control specimens were simultaneously stained with Thy1.2 and asialo GM-1 membrane markers (Table I) [22–33]. In contrast to the Ia+ LC, there was a slight but significant 16% ($t = 2.10$; $p < 0.05$) decrease in Thy1.2 marker expression versus the diluent controls (Table I). There was no significant difference in the phenotypic expression of asialo GM-1 marker ($t = 0.406$; $p > 0.66$) (Table I). It should be noted that the diluent alone had no effects on Ia, Thy1 or asialo GM-1 expression (data not shown). We concluded from these experiments that epicutaneous application of α-MSH could selectively alter the Thy1.2 phenotypic cell marker expression, but not asialo GM-1. This suggests that α-MSH had no effect on the density of the cells within the epidermis.

Suppression of Sensitization CHS Reactivity by α-MSH Groups of mice that were treated with α-MSH (Fig 1) on the dorsal skin demonstrated 64% suppression in ear thickness in animals treated with DNFB and 99% depression in ear swelling following OX treated vs control mice ($t = 2.54$; $p < 0.05$; $t = 2.80$; $p < 0.01$, respectively). Negative control animals did not show any significant difference in the ear swelling (challenged only) and served as an indicator for irritant effects of the DNFB hapten. Other negative control mice challenged only with OX demonstrated similar CHS responsiveness as the DNFB negative control mice (i.e., both negative control groups demonstrated only 13% increase in ear thickness) after DNFB or OX treatment (data not shown). Further, pilot experiments demonstrated that the diluent (propylene glycol), when applied alone, produced no effects on either the sensitization or elicitation limbs of the CHS response (data not shown), or the density (cells/mm²) of Ia+ and Thy1.2+ dendritic cells. The results suggest that the epicutaneous application of α-MSH can abrogate sensitization to two different antigens/haptens resulting in the absence of an elicitation response at a distal challenge site.

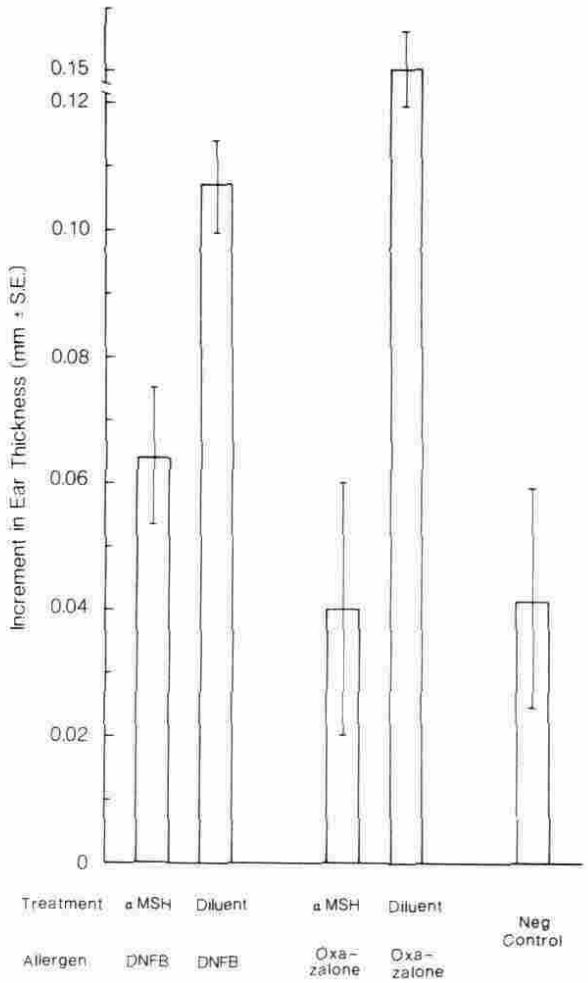

Figure 1. CHS responsiveness to DNFB and OX in C57BL/6 mice treated on shaved dorsal epidermis for five days with 50 μl of 10^{-5} M α-MSH or propylene glycol, followed by sensitization by DNFB or OX on the treated shaved dorsal area. Five days later the animals were challenged on the pinnal epidermis with either DNFB or OX. Diluent ears were significantly thicker ($p < 0.001$) than treated ears in both DNFB and OX treatment groups. The treated ears were not significantly thicker than negative control mice (challenge only) for both DNFB and OX approximately 13% increase in ear thickness. Data represents the mean increase in ear thickness from two combined experiments (four mice/trial ± SEM).

Table I. Identification of Ia+, Thy1.2+, and Asialo GM-1+ Phenotypic Markers on Ia+ Dendritic Cells and Thy1+ DEC Cells/mm² ± SEM on Pinnal Epidermis of C57BL/6 Mice Treated With α-MSH or Diluent

Treatment	Dose Applied	Site	Ia	Thy1.2	Asialo GM-1
α-MSH	10^{-5} M	Ear	408 ± 19	340 ± 15[a]	465 ± 18
Propylene glycol (diluent)		Ear	446 ± 19	404 ± 14	434 ± 16

[a] $p < 0.01$ compared with diluent (propylene glycol) control animals.

Data represents the mean numerical density (cells/mm²) of identifiable Ia+, Thy1+, and asialo GM-1+ phenotypic markers from dendritic epidermal cells from two combined experiments, four mice/trial ± SEM

Suppression of the Efferent CHS Inflammatory Response After Application of α-MSH Elicitation of CHS responsiveness was assessed in animals whose ears were treated on days 1–5 with 50 μl of 10^{-5} M α-MSH or propylene glycol. Animals were then

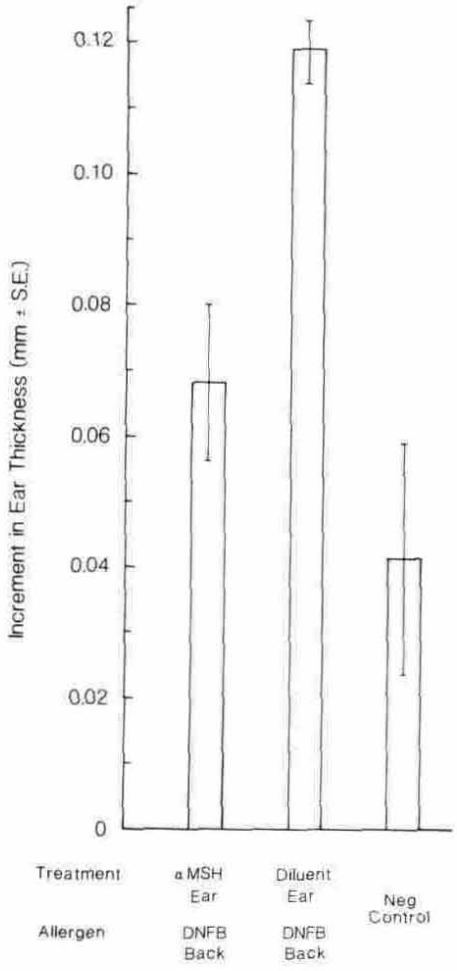

Figure 2. Elicitation of the CHS response to DNFB of C57BL/6 mice treated on the dorsal surface of the ear with 50 μl of 10^{-5} M α-MSH or diluent, and sensitized on untreated dorsal epidermis. Postchallenge ear thickness of diluent was significantly greater than the α-MSH treated ears ($p < 0.001$).

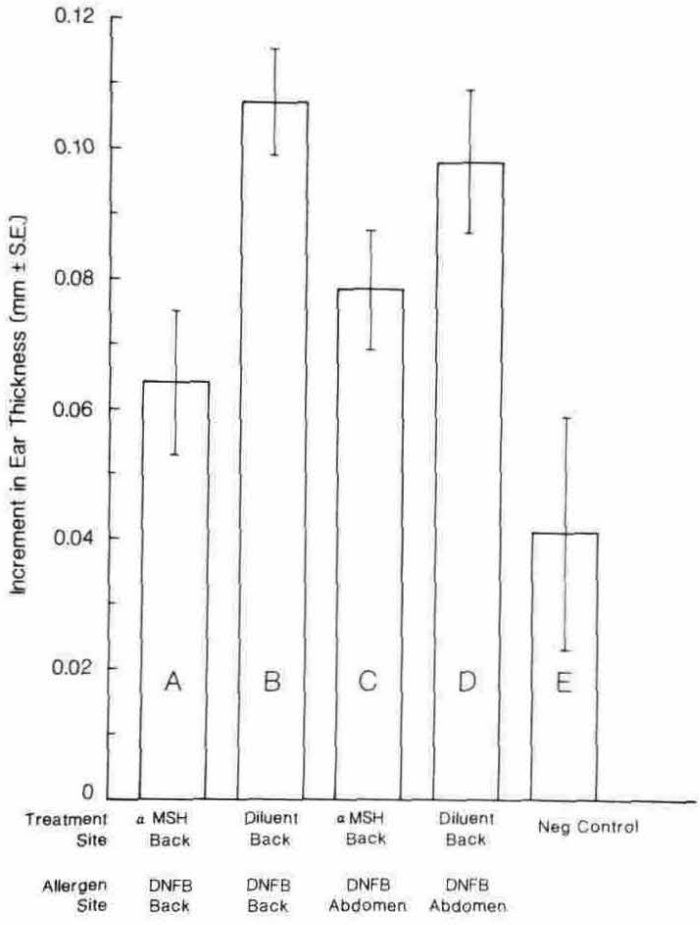

Figure 3. Local effect of epicutaneously applied α-MSH on CHS responsiveness to DNFB. Groups of mice were treated with 50 μl of 10^{-5} α-MSH or propylene glycol on the shaved dorsal surface for five days. The mice were then sensitized with 0.5% DNFB on the treated back *(bars A and B)* or abdomen *(bars C and D)*. Five days later the ears were measured and challenged with 0.2% DNFB. Mice treated with α-MSH *(bar A)* or diluent *(bar B)* and then sensitized on the treated dorsal epidermis demonstrated significant decrease in ear thickness versus the diluent controls *(bar B)* ($p < 0.001$). However, mice treated with α-MSH or propylene glycol *(bars C and D)* on the dorsal epidermis and sensitized on the untreated abdomen were not significantly different ($p > 0.87$). Negative controls *(panel E)* received challenge dose of DNFB only.

sensitized with DNFB on the untreated shaved back and challenged on day 11 as described above. Twenty-four hours post-DNFB challenge, the α-MSH-treated ears demonstrated 61% suppression in ear swelling versus control mice ($t = 2.23$; $p < 0.05$) (Fig 2).

To determine if the effects of α-MSH treatment could be due to a local effect or to systemic reactivity, the following experiments were performed. Initial experiments were carried out in which C57BL/6 mice were treated with α-MSH on the shaved dorsal surface for five days followed by sensitization with DNFB on the untreated shaved abdomen and challenged on day 11 on the ears as was performed in the earlier experiments. Although mice treated with α-MSH on the back and DNFB on the abdomen responded less vigorously than controls (column B and C, Fig 3), the difference was not significant statistically ($t = -1.60$; $p > 0.87$). It would appear, at least from these data, that topically applied α-MSH, at the dose applied under these specific experimental conditions in vivo, may act locally. Subsequent experiments suggested α-MSH had systemic/splenic effects as well.

Effect of Splenic Suppressor and Helper Cell Activity After α-MSH Topical Treatment on DNFB/CHS Responsiveness To determine if α-MSH could induce suppression via splenic suppressor cell activity, passive cell transfer studies were performed. Results are depicted in Figure 4. There was a significant percent suppression (45%; $t = 2.35$, $p < 0.05$) in ear swelling in immunologically naive mice, which received splenocytes from α-MSH/DNFB (treatment α-MSH-cell transfer spleen)-treated mice that were then sensitized and challenged with DNFB. α-MSH or another as yet to be identified biomolecule may be inducing the observed systemic suppressor activity [34].

Role of DLN Cells in CHS Responsiveness After α-MSH Treatment In the above experiments, we noted suppression following passive transfer of splenocytes to naive mice after topical α-MSH and DNFB sensitization treatment. Separate experiments were designed to examine whether donor lymph node effector cells were capable of sensitizing naive mice after topical α-MSH treatment and DNFB allergen sensitization. Donor mice were treated with 10^{-5} M α-MSH or propylene glycol for five days followed by DNFB sensitization and challenged five days later. Draining lymph node cells were isolated from MSH-treated mice and control mice. These cells were injected intravenously at 1×10^6 viable cells via the tail vein into naive recipients. Groups of mice were either immediately challenged and ears measured 24 h later or immediately sensitized and challenged five days later. The extent of ear swelling was measured 24 h postchallenge (Fig 4). Our results demonstrate that there was no significant difference in the elicitation of CHS response in recipient mice receiving draining lymph node cells from

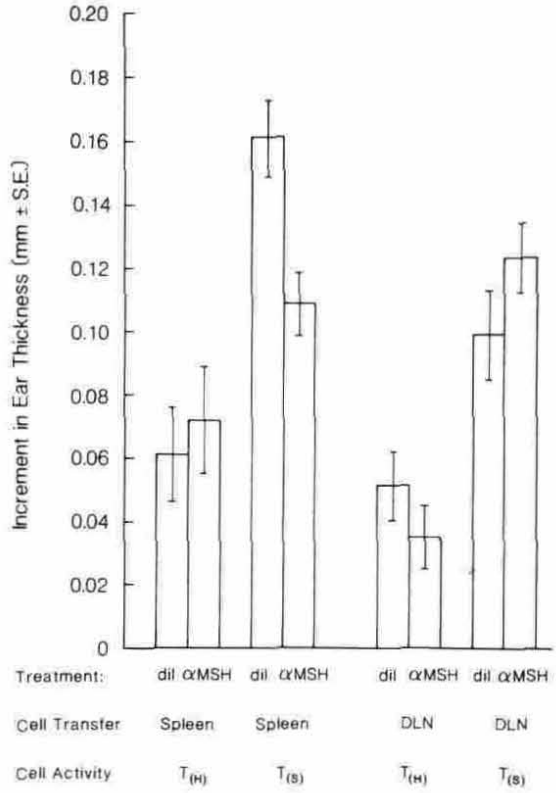

Figure 4. Adoptive transfer of splenocytes and draining lymph node cells from topically treated α-MSH or diluent treated DNFB sensitized mice to immunologically naive recipient mice. Groups of recipient mice were either immediately challenged or sensitized with DNFB and effector (Th-helper) or suppressor (T_s) cell activity assessed at either 24 h or five days later after a challenge dose of 0.2% DNFB to pinnal epidermis. Adoptive transfer of spleen cells (T_s) from α-MSH/DNFB sensitized donors into naive recipients resulted in a significant amount of suppressor activity ($p < 0.05$) in treated animals versus propylene glycol control mice (treatment dil/α-MSH – cell transfer spleen). Splenocytes from α-MSH treated and sensitized mice transferred into naive mice followed by immediate challenge resulted in no discernible changes in responsiveness between α-MSH and diluent controls. In separate experiments we observed that the capacity to elicit the CHS response to DNFB using DLN cells was not significantly different in mice challenged immediately or sensitized and challenged five days later. Data shown are results of two experiments (four mice/trial ± SEM) with splenic cells and two separate experiments on DLN cells.

either treated or diluent control mice. This suggests, albeit by no means absolute, that applications of DNFB after treatment of the skin with α-MSH resulted in an immunologically null event after passive transfer of draining lymph node cells. However, the above experiments suggest that α-MSH may activate both local and splenic suppressor activities possibly by several different mechanisms.

Reconstitution of CHS Response After Administration of ETAF/IL-1 to α-MSH-Treated Mice To determine if suppression of CHS responsiveness was related to alterations in ETAF/IL-1 bioactivity, reconstitution experiments were performed. α-MSH-treated mice received either intradermal or intravenous injections of ETAF/IL-1 before sensitization with DNFB. The results are shown in Figure 5. Mice treated with α-MSH followed by intradermal or intravenous injections of ETAF demonstrate significantly greater increase in thickness versus mice treated with α-MSH/DNFB only ($t = 2.73, p < 0.01$; $t = 2.92, p < 0.01$, respectively). We were able to conclude from this experiment that intradermal and intravenous systemic administration of the ETAF/IL-1 molecule could restore CHS responsiveness to DNFB in C57BL/6 mice. Mice treated topically with propylene glycol followed by injections of ETAF reacted less vigorously to DNFB sensitization and challenge than the diluent control. No further experiments were undertaken at this point to determine if this was related to the dose or other variables.

DISCUSSION

Alpha-melanocyte stimulating hormone (α-MSH) is the best known and classic melanotropic agent. While originally described in the pars intermedia of the pituitary gland, α-MSH can be found in other organs of the body including the skin [3]. Further, it is known that immune cells from the spleen and thymus can serve as an extra pituitary source for precursors of α-MSH, i.e., β-LPH and ACTH, that in turn can modulate immune responsiveness locally or systemically without pituitary influences [35]. Preliminary data from our laboratory including immune localization by EM and immunoblots on PAGE-separated proteins suggest that the Thy1+ dendritic epidermal cell, a cell of T-cell lineage, may produce α-MSH or other MSH peptides or promolecules that have an immunomodulatory effect on the skin [36]. MSH is derived from a much larger peptide, proopiomelanocortin (POMC).

In the last four years α-MSH has been found to be a potent inhibitor of IL-1 bioactivity [4–6]. α-MSH in nanogram quantities can apparently block many activities of the IL-1 molecule. Other investigators have documented both in vitro and in vivo the inhibiting capacity of α-MSH on IL-1 immune activities [4–6]. Although

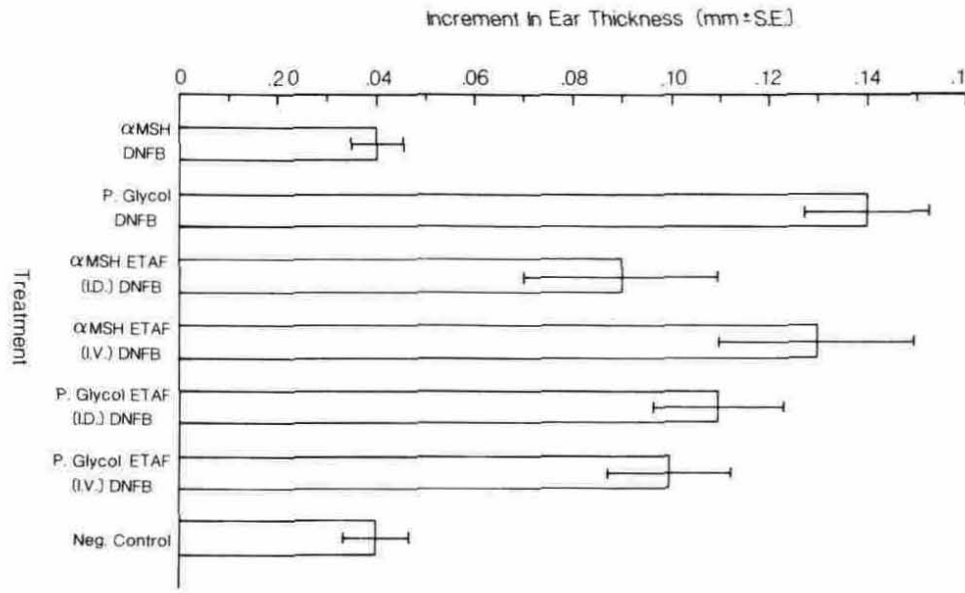

Figure 5. Suppression of CHS responsiveness in α-MSH topically treated mice could be reconstituted with either intradermal or intravenous injections of keratinocyte derived ETAF/IL-1. Groups of mice topically treated with 50 μl of 10^{-5} M α-MSH or diluent for five days. Thirty minutes after treatment, groups of mice received 0.1 ml of ETAF (500 U/ml) either intradermally into the topically treated site or intravenously via the tail vein. Thirty minutes after ETAF/IL-1 administration, mice were sensitized to 25 μl of 0.5% DNFB on the treated shaved dorsal epidermis. The ETAF/IL-1 sensitization procedure was repeated again on day 6. Five days later, on day 11, the ears were measured and challenged with 20 μl of 0.2% DNFB. Twenty-four hours later the ears were measured again. Mice treated with α-MSH followed by ETAF/IL-1 treatment resulted in reconstitution of the CHS response with values similar to that obtained with diluent controls.

α-MSH can "down regulate" generation of acute phase reactant proteins from the liver, thymocyte proliferation, and induction of neutropenia, there has been a paucity of studies on the role of topically applied α-MSH regarding its effect on the epidermal immune system, versus systemic intravenous administration of α-MSH.

In this study, we have shown that epicutaneous application of the molecule α-MSH on the epidermis can suppress both the sensitization (induction) and elicitation limbs of the cutaneous immune response. Dawson and co-workers [37] have shown that α-MSH rapidly penetrates the epidermis of murine and human skin. The effect of α-MSH appears to demonstrate both local as well as systemic reactivity. However, the exact biochemical and immunological mechanisms for these observations remain unknown.

In the current studies, not only could we suppress the sensitization and elicitation limbs of the cutaneous immune response, but we could reconstitute the in vivo activity after injections of ETAF/IL-1 both intradermally as well as intravenously. Although injections of ETAF intradermally and intravenously did not demonstrate 100% reconstitution of CHS to control (diluent) levels, they were significantly greater than the α-MSH treatment groups. It is quite possible that the dosage and kinetics of ETAF/IL-1 injected intravenously and/or intradermally may have different bioactivities that could explain our observed discrepancies concerning absence of 100% reconstitution of the CHS response. Further, small contaminants to ETAF/IL-1 (i.e., IL-6 and GM-CSF) might have some effect. Preliminary experiments suggest this is not the case (data not shown). We have no explanations for the apparent suppressive effects of intravenous doses of ETAF (Fig 5).

The fact that mice were sensitized twice after α-MSH treatment which still resulted in cutaneous immune suppression suggests that several factors may be involved in the observed phenomena. It should be noted that α-MSH has a short half life when injected intravenously. Topical treatment of α-MSH may alter a variety of epidermal cell functions and/or secretion of lymphokines, cytokines, or other epidermal biomolecules (i.e., prostaglandins, leukotrienes, ACTH, or epidermal growth factor), which may prove to be necessary for contact sensitization [34]. Our studies are at least consistent with some of the observations of Cannon et al [5], who documented in 1986 that α-MSH could inhibit IL-1-dependent thymocyte stimulation. Caution is necessary in comparing our results and those of Cannon because the experimental conditions and organ systems used were different.

Daynes et al [6] demonstrated in 1987 that intravenous administration of either α-MSH or a synthetic analogue was unable to abrogate IL-1 to stimulate production of PGE_2 or PHA stimulation of murine thymocytes. We also used a synthetic analog of α-MSH (Nle,D-Phe α-MSH) and found no alterations in function of the cutaneous immune response or epidermal immune cells with this analogue (data not shown). This observed discrepancy may be due to several mechanisms working together with ETAF/IL-1 acting on various epidermal cells, which may lead to alterations in the contact hypersensitivity response. For example, ETAF and a novel IL-1 termed κ-IL-1 [11,38] comprise at least three peptides, IL-1α and IL-1β, all of which are produced by epidermal cells and may separately, synergistically, or additively alter CHS reactivity, depending on the route of α-MSH administration. However, in other studies we have investigated the agonist/antagonist effects of α-MSH and ETAF in more detail. We observed that the inhibitory effects, at least in the melanocyte system, could not be attributed to simple receptor competition. Moreover the inhibitory effects of ETAF could not be reproduced by IL-1α, IL-1β, CSF-GM, or IL-6 alone or in any combination [41]. Sufficient quantities of κIL-1 are not available for study. Although we have not completed identical experiments on Langerhans cells or lymphocytes, we are devoting significant amounts of our attention at this time to elucidating the agonist/antagonist effects of ETAF and MSH.

When C57BL/6 mice were topically treated with α-MSH, there was no loss of identifiable dendritic Ia+ Langerhans cells. However, there was a 16% decrease in Thy1.2 markers on Thy1+ dendritic epidermal cells, although the number of asialo GM-1 markers, the marker found predominantly on N-lymphocytes (NK cells), remained constant. α-MSH may have many effects on phenotypic marker expression on some subpopulations of Thy1+ cells by perhaps down regulating Thy1.2 marker expression itself or serving as a transducer signal to another biologically relevant molecule that is necessary for CHS reactivity. From concurrent studies, we would like to suggest that the Thy1+ DEC may serve as a reservoir for a variety of proopiomelanocortin neuropeptides (i.e., α-MSH, β-lipotropin, ACTH) which in turn can down regulate the epidermal immune system [36]. This may be of particular importance in light of the fact that the exact function of the Thy1.2 differentiation marker remains an immunologic enigma [39]. The Thy1.2 marker may function in trafficking the Thy1+ DEC to the epidermis, which in turn may serve as an extra thymic environment for this particular dendritic cell. In vitro epidermal lymphocyte experiments (ELR) are currently underway with enriched FACS sorted epidermal cells in the presence of α-MSH and other neuropeptides to begin to ascertain information on these questions using a more precise experimental system.

The results of our data have possible significance for understanding the regulation of epidermal immune functions. We have identified by immunfluorescence α-MSH in the skin (data not shown), as well as β-lipotropin [36]. Recent preliminary work from our lab with appropriate cDNA probes confirmed the presence of mRNA for POMC in epidermal cells [40]. We suggest that the release of α-MSH from the appropriate cell may function as a biological modifier to both increase melanocyte function as well as to modulate the intensity of an immune/inflammatory activity. This and the results of other work suggest that biological modifiers like α-MSH may be useful therapeutically in the care of patients with many or all types of epidermal inflammation.

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