α-Melanocyte-Stimulating Hormone Inhibits Allergic Airway Inflammation

Ulrike Raap, Thomas Brzoska, Sirius Sohl, Günter Päth, Jörg Emmel, Udo Herz, Armin Braun, Thomas Luger and Harald Renz

*J Immunol* 2003; 171:353-359; doi: 10.4049/jimmunol.171.1.353

http://www.jimmunol.org/content/171/1/353

---

**References**  
This article cites 53 articles, 14 of which you can access for free at:
http://www.jimmunol.org/content/171/1/353.full#ref-list-1

**Subscription**  
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**  
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
\(\alpha\)-Melanocyte-Stimulating Hormone Inhibits Allergic Airway Inflammation\(^1\)

Ulrike Raap,\(^*\) Thomas Brzoska,\(^†\) Sirius Sohl,\(^*\) Günter Päth,\(^*\) Jörg Emmel,\(^*\) Udo Herz,\(^*\) Armin Braun,\(^*\) Thomas Luger,\(^†\) and Harald Renz\(^2*\)

\(\alpha\)-Melanocyte-stimulating hormone (\(\alpha\)-MSH) is a neuropeptide controlling melanogenesis in pigmentary cells. In addition, its potent immunomodulatory and immunosuppressive activity has been recently described in cutaneous inflammatory disorders. Whether \(\alpha\)-MSH is also produced in the lung and might play a role in the pathogenesis of inflammatory lung conditions, including allergic bronchial asthma, is unknown. Production and functional role of \(\alpha\)-MSH were investigated in a murine model of allergic airway inflammation. \(\alpha\)-MSH production was detected in bronchoalveolar lavage fluids. Although aerosol challenges stimulate \(\alpha\)-MSH production in nonsensitized mice, this rapid and marked stimulation was absent in allergic animals. Treatment of allergic mice with \(\alpha\)-MSH resulted in suppression of airway inflammation. These effects were mediated via IL-10 production, because IL-10 knockout mice were resistant to \(\alpha\)-MSH treatment. This study provides evidence for a novel function of \(\alpha\)-MSH linking neuroimmune functions in allergic airway inflammation. *The Journal of Immunology*, 2003, 170: 353–359.

\(\alpha\)-Melanocyte-stimulating hormone (MSH)\(^3\) is an endogenous neuroimmunomodulatory peptide derived from the precursor molecule proopiomelanocortin (POMC). Originally, POMC was discovered in the pituitary gland, but subsequently, POMC-derived peptides were also detected in lymphocytes, monocytes, Langerhans cells, and epithelial cells (1–5).

\(\alpha\)-MSH is well known for its role in the control of melanogenesis in pigmentary cells. However, recent studies demonstrated a potent and broad spectrum of activities as an antipyketic, antimicrobial, anti-inflammatory, and immunomodulatory peptide (6–8). The actions of \(\alpha\)-MSH are transmitted via specific melanocortin (MC) receptors (9–11). Five different subtypes of MC receptors have been identified, designated MC-1 to MC-5. Melanocortin receptor-1 (MC-1), which is specific for \(\alpha\)-MSH, is expressed on skin keratinocytes, dendritic cells, macrophages, endothelial cells, and epithelial cells (10, 12).

The skin is an extrapituitary site of \(\alpha\)-MSH generation and secretion. Elevated \(\alpha\)-MSH levels have been reported in several cutaneous inflammatory disorders, including psoriasis vulgaris and eczema (13, 14). The potent anti-inflammatory property of \(\alpha\)-MSH was shown in a murine model of delayed-type hypersensitivity and hapten-specific tolerance (15–17). In the latter model, \(\alpha\)-MSH-induced hapten-specific tolerance in both a preventive as well as therapeutic treatment regimen.

In addition to skin, the airways represent another primary site of the allergic response. To date, it remains unknown whether \(\alpha\)-MSH is also produced by cells residing in or invading lung and airways during allergic inflammation. Furthermore, MC receptor expression and subsequent effects of \(\alpha\)-MSH have not been studied in this compartment. These questions were addressed in the present study, which was conducted in a well-characterized model of allergic airway inflammation with Th2 cell influx and eosinophilia, as well as the presence of immediate-type hypersensitivity responses related to IgE/IgG1 Ab production (18, 19).

**Materials and Methods**

**Animals**

Female BALB/c and C57BL/6 mice (6–8 wk old) were obtained from Harlan Winkelmann (Borchen, Germany); homozygous C57BL/6-IL-10\(^{-/-}\) (6–8 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were housed in a pathogen-free facility with single ventilated cages and received OVA-free diet and water ad libitum.

**Allergic sensitization, allergen challenge, and administration of \(\alpha\)-MSH**

Mice were sensitized by three i.p. injections of 10 µg OVA grade VI (Sigma-Aldrich, Deisenhofen, Germany) absorbed to 1.5 mg Al(OH)$_3$ (Inject Alum; Pierce, Rockford, IL) diluted in 200 µl PBS on days 1, 14, and 21. Nonsensitized animals (control) received adjuvant 1.5 mg Al(OH)$_3$ diluted in PBS alone. \(\alpha\)-MSH and \(\gamma\)-MSH (Bachem, Heidelberg, Germany) were stored in aliquots until use at \(-80^\circ\)C. Immediately before injection, the peptides were dissolved in PBS containing 0.1% BSA and kept on ice until application. A total of 1 mg/kg body weight of \(\alpha\)-MSH or \(\gamma\)-MSH was injected into the tail vein 30 min before sensitization or allergen aerosolization (\(\alpha\)-MSH/OVA and \(\gamma\)-MSH/OVA). \(\gamma\)-MSH is a POMC gene-derived peptide, with an amino acid sequence different from \(\alpha\)-MSH and no published immunomodulatory activity. All animals received two local allergen challenges performed by an exposure to 1% OVA diluted in PBS for 20 min on days 26 and 27. Experimental groups corresponding to this protocol are depicted in Fig. 1.

**Ig assays**

Blood was collected by incision of the tail vein. Total IgE and allergen-specific IgE, IgG1, and IgG2a Ab concentrations were measured in serum samples by ELISA, as previously described (18, 20).

---

\(^1\) Abbreviations used in this paper: MSH, melanocyte-stimulating hormone; BAL, bronchoalveolar lavage; \(^{125}\)I-\(\alpha\)-MSH, \(^{125}\)I-labeled \(\alpha\)-MSH; MC, melanocortin; NIL, untreated; POMC, proopiomelanocortin.

\(^*\)Department of Clinical Chemistry and Molecular Diagnostics, Philipps-University Marburg, Marburg, Germany; and \(^†\)Department of Dermatology, Ludwig-Boltzmann Institute, University Muenster, Muenster, Germany

Received for publication December 19, 2002. Accepted for publication April 17, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This study was supported by Deutsche Forschungsgemeinschaft Grant RE 737/7, Volkswagen Stiftung, and Kempke Stiftung 23/01.

Address correspondence and reprint requests to Dr. Harald Renz, Department of Clinical Chemistry and Molecular Diagnostics, Central Laboratory, Hospital of the Philipps University, Baldingerstr., D-35033 Marburg, Germany. E-mail address: renzh@med.uni-marburg.de

Copyright © 2003 by The American Association of Immunologists, Inc.
Bronchoalveolar lavage and lung histology

Bronchoalveolar lavage (BAL) fluid was taken 48 h after the last allergen challenge using a method previously described (21). BAL fluids were assessed for further analysis. BAL fluids were counted, an aliquot was taken for cytospin centrifugation (700 × g), and cytospins were stained with Diff-Quik (Dade Behring, Düdingen, Switzerland) for differential cell counting. From remaining BAL fluids, cells were removed and supernatants were aliquoted for further ELISA analysis. From cryostat sections, the lung was removed, embedded with Tissue-Tek (Sakura, Zoeterwute, The Netherlands), diluted in PBS 1/4, and cryoconserved. Sections (8 μm) were cut, dried for 30 min, and stained with 1% H&E (Merck, Darmstadt, Germany).

Cytokine assays

The cytokines IL-4, IL-5, IL-10, IL-13, and IFN-γ were measured in BAL fluids by ELISA, as described elsewhere (18). Kits for cytokine immunoassays were purchased from B&D (Heidelberg, Germany) for IL-4, IL-5, and IFN-γ, and from R&D Systems (Minneapolis, MN) for IL-10 and IL-13. Detection limits were 3.4 pg/ml for IL-4, 7.8 pg/ml for IL-5 and IL-10, and 31.25 pg/ml for IL-13 and IFN-γ, respectively.

α-MSH RIA

α-MSH concentrations in BAL fluids were determined using a competitive RIA. α-MSH in samples competed with 125I-labeled α-MSH (125I-α-MSH) in binding to an antiserum, which was raised against an α-MSH-albumin conjugate. To increase the sensitivity of the assay, 125I-α-MSH was added delayed. Ab-bound 125I-α-MSH was separated from the free fraction using the double Ab polyethylene glycol precipitation technique. The radioactivity of the precipitate was measured. The antiserum used in this assay was directed to the C-terminal part of the α-MSH molecule and showed no cross-reactivity with adrenocorticotropic hormone. Briefly, 100 μl of samples was pipetted in 3-ml glass tubes, 200 μl anti-α-MSH serum was added, and the mixture was incubated for 24 h at 4°C. On the following day, 200 μl 125I-α-MSH was added and the mix was incubated for further 24 h at 4°C. On day 3, double Ab polyethylene glycol (500 μl) was added and the mixture was incubated for another 60 min. Finally, vials were centrifuged, supernatants were decanted, and the radioactivity was measured in the precipitates using a gamma counter (counting time, 3 min).

Flow cytometry and FACS analysis

BAL samples with a volume of 1.4 ± 0.2 ml were taken 48 h after the last allergen challenge. BAL fluids of five mice per group were pooled and pelleted (700 × g). Cells were prepared for further analysis (22). Cell samples were analyzed using a FACScan flow cytometer (BD Biosciences, San Jose, CA). The data were processed with CellQuest software (BD Biosciences). Before staining procedure, cells were incubated with 2% heat-inactivated mouse serum so as to avoid unspecific bindings. All incubations were performed in HBSS, supplemented with 2% BSA. MAC-3 surface expression was measured with a mAb clone M3/M84 (BD Pharmingen, Heidelberg, Germany), PE-conjugated, isotype control; rat IgG1 k PE. MC-1 surface expression was assessed using a polyclonal rabbit anti-mouse Ab custom made by Inventus Biotec (Muenster, Germany) directed against the aa sequences 2–18 of the extracellular domain. As a secondary Ab, a FITC-conjugated polyclonal goat anti-rabbit Ab was used (Dianova, Hamburg, Germany). Staining procedure was performed on ice for 15 min, and cells were washed twice with cold PBS. Total leukocytes were gated, and counting consisted of 10,000 events. Quadrants were defined by isotype/secondary Ab controls.

Determination of airway responsiveness

Measurements of airway responsiveness were performed 24 h after the last challenge (day 28) by head-out body plethysmography, as previously described (23). Air flow signals were recorded in response to methacholine (0, 12.5, 25, 50, 100, 200 mg/ml) and aerosolized with a nebulizer. The concentration of methacholine that caused a 50% reduction in expiratory flow (MCh50, milligrams per milliliter) was determined.

Data analysis

Results are presented as mean ± SEM or SD. The statistical significance between study groups was analyzed by Student’s t test. A p value of <0.05 was accepted as statistically significant.

Results

Production of α-MSH in the lung during allergic airway inflammation

The sensitization and airway allergen challenge protocol resulted in a rise of total and allergen-specific IgE, IgG1, and IgG2a Ab production (Fig. 2). A marked influx of inflammatory cells into the airways with predominance of eosinophils (Fig. 3) together with increased levels of IL-4, IL-5, and IL-13 cytokine production (Table I) was observed following airway allergen challenge. The inflammatory response was paralleled by decreased levels of IL-10 in BAL fluids (Fig. 4). The levels of α-MSH production were assessed in BAL at consecutive time points after airway allergen challenge. α-MSH was detected in all animals. Airway allergen challenge of nonsensitized mice triggered a rapid and marked rise in the concentration of methacholine that caused a 50% reduction in expiratory flow (MCh50, milligrams per milliliter) was determined.
Table I. α-MSH decreases IL-4 and IL-13 production in BAL fluid

<table>
<thead>
<tr>
<th>Cytokines (pg/ml)</th>
<th>Control</th>
<th>NIL/OVA</th>
<th>γ-MSH/OVA</th>
<th>α-MSH/OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>79 ± 16</td>
<td>95 ± 23</td>
<td>80 ± 16</td>
<td>51 ± 11**</td>
</tr>
<tr>
<td>IL-5</td>
<td>85 ± 16</td>
<td>169 ± 49***</td>
<td>183 ± 56***</td>
<td>178 ± 38***</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>240 ± 51</td>
<td>203 ± 79</td>
<td>215 ± 57</td>
<td>163 ± 72**</td>
</tr>
<tr>
<td>IL-13</td>
<td>81 ± 13</td>
<td>181 ± 52***</td>
<td>144 ± 58*</td>
<td>94 ± 44**</td>
</tr>
</tbody>
</table>

*Measurement of IL-4 (n = 8–10), IL-5 (n = 11–12), IL-13 (n = 5–8), and IFN-γ cytokine (n = 10) levels (pg/ml) in BAL fluids of controls, NIL/OVA, γ-MSH/OVA, and α-MSH/OVA. Study groups are described in Fig. 1. Presented are mean ± SD.

†, Significant differences of α-MSH/OVA compared with NIL/OVA mice (†††, p < 0.001; ††, p < 0.01; †*, p < 0.05). **, Significant differences of NIL/OVA, γ-MSH/OVA, and α-MSH/OVA mice compared with controls (*, p < 0.05; ***, p < 0.01; †††, p < 0.001).
control group. In contrast, a dose of only 50 ± 9.8 mg/ml (NIL/OVA) and 49 ± 13.4 mg/ml (γ-MSH/OVA) was required to reach the same effect in allergic and γ-MSH-treated mice, indicating airway hyperresponsiveness ($p < 0.001$). α-MSH-treated allergic mice showed a tendency toward normalization with 56.6 ± 14 mg/ml (not significant compared with controls, NIL/OVA, and γ-MSH/OVA mice).

**Effect of α-MSH is IL-10 dependent**

IL-10 levels were significantly reduced in allergic mice. This effect was also observed in γ-MSH-treated mice. In α-MSH-treated allergic mice, however, this effect was reverted, as these mice showed higher IL-10 levels than NIL/OVA mice ($p < 0.001$) and controls ($p < 0.05$) (Fig. 4). To investigate whether the inhibitory effect of α-MSH on allergic airway inflammation is mediated via IL-10 production, IL-10 knockout animals were used. As IL-10 knockout mice are bred on a C57BL/6 background, the effects of α-MSH were also studied in this strain. Similar to sensitized BALB/c mice, α-MSH suppressed the influx of eosinophils (Fig. 8) in the airways, paralleled by a further increase of neutrophils in C57BL/6 animals. Allergen-specific Ab production, except for IgG1, was suppressed (Fig. 9). In IL-10−/− mice, treatment with α-MSH was without effect on Ab production and airway inflammation, indicating the dependency of α-MSH effects on IL-10.

**Discussion**

This is the first study demonstrating α-MSH production in the lung. In normal, nonsensitized mice, a nasal Ag challenge results in enhanced α-MSH production. This effect was absent in allergic mice, pointing to a negative regulatory loop of α-MSH production in the airways. This finding together with previous results obtained in murine models of delayed-type hypersensitivity led us to the hypothesis that α-MSH may also have anti-inflammatory functions in the lung. Therefore, sensitized mice were treated with α-MSH. The results demonstrate a strong anti-inflammatory effect of α-MSH in airways and lung that is IL-10 dependent.

Production of this neuropeptide has been reported following injury, UV light exposure, trauma, and infection (24). Increased production of α-MSH was measured in patients with psoriasis vulgaris and other cutaneous inflammatory conditions (13, 14). Monocytes and macrophages are major sources of this neuropeptide. Therefore, these cells may contribute to the α-MSH level observed in BAL fluids. α-MSH has immunomodulatory effects on several cell types. It inhibits the production of a number of pro-inflammatory cytokines, including IL-1β, IL-1α, IL-2, IL-6, IFN-γ, and TNF-α (25, 26). Because many of these cytokines are produced by macrophages, α-MSH seems to designate them as key target cells (1). In fact, the receptor with high affinity for α-MSH, MC-1, has been found on macrophages and other cell populations (10). However, to our knowledge, this is the first report demonstrating expression of this receptor on macrophages of the airways. MC-1 is expressed on at least a subset of resting cells and shows up-regulation on activated macrophages. The functional activities of human mast cells are affected (27, 28). For example, the release of histamine is altered under the influence of α-MSH (28, 29). B cells are further target cells of α-MSH action (10, 30). Modulation

**FIGURE 6.** α-MSH inhibits peribronchial inflammatory cell infiltrate. Histological images of the OVA triggered influx of inflammatory cells into the lung and airways. Staining with H&E. Lung sections from control (A), NIL/OVA (B, arrow points to mucus production), and α-MSH/OVA (C, arrow points to morphologically normal airway epithelium) mice. Presented is one of three similar experiments. Magnification ×50. D, High power field analysis of mucosal eosinophils (10 fields of vision; blinded investigators; mean ± SD; $p < 0.05$).

**FIGURE 7.** Macrophage activation and MC-1 surface expression. Assessment of activated (MAC-3 receptor-positive) and MC-1 receptor-positive macrophages in BAL fluid. Presented are dot plots of total leukocyte populations of five pooled BAL pellets of control, NIL/OVA, and α-MSH/OVA mice. Numbers in the upper right corners reflect the percentage of activated macrophages (MAC-3 positive) that were MC-1 receptor positive. Numbers in the lower right cases represent activated macrophages (MAC-3 positive) that were MC-1 receptor negative. Presented is one representative experiment of two.
of IgE synthesis and down-regulation of the expression of costimulatory molecules, including CD86 and CD40, have been described (1). In addition, inhibition of the expression of VCAM and E-selectin on endothelial cells was also shown (31). Moreover, in terms of intracellular signal transduction, α-MSH inhibits the activation of NF-κB (32).

In the model of allergic bronchial asthma, α-MSH suppressed allergen-specific IgE, IgG1, and IgG2a Ab production. Based on the known effects of α-MSH on B cells, it is likely that B cells are among the targets of α-MSH action. The local anti-inflammatory properties of α-MSH are characterized by a marked suppression in airway eosinophilia. This was accompanied by an increase in the influx of neutrophils. However, the suppression of airway eosinophilia was not related to decreases in IL-5 production, although IL-5 is known as a potent cytokine for eosinophil development, recruitment, activation, and survival (33, 34). The reduction of eosinophils may be related to the down-regulation of certain endothelial adhesion molecules including VCAM. The expression of VCAM, in turn, is under the close control of cytokines, including IL-4, IL-13, and TNF-α (35). The production of all these cytokines is apparently modulated by α-MSH. Why treatment with α-MSH resulted in an increased influx of neutrophils into the airways remains currently unclear. However, because this effect was also observed with γ-MSH, a peptide also derived from POMC that has to date no effects on the immune system, the observed effects might be considered as unspecific. From our data, we have no indication that the influx in neutrophils was associated by a Th1 response. There are no increases in IFN-γ, and IgG2a Abs were even significantly reduced by α-MSH. Furthermore, the influx in neutrophils was rather small as compared with a recent study investigating Th1 responses in the lung (36).

Similar to the effects observed in the model of delayed-type hypersensitivity, α-MSH exerts its anti-inflammatory potency also in the lung via the induction of IL-10. IL-10 is a pleiotropic cytokine with potent immunostimulatory effects on a variety of cell types. One major effect that has recently been extensively investigated is the suppression of effector functions on macrophages, monocytes, NK cells, and Th cell subsets (37). IL-10 is mainly produced by T cell subsets of the Th2 phenotype, but also by other cells, including B cells, macrophages, monocytes, keratinocytes, and mast cells (37). Increased production of IL-10 has been observed during antiallergic specific immunotherapy and corresponds to the success of this mode of therapy (38). Further analysis indicates that this effect is related to the induction of regulatory T cells by IL-10 (39). Most recently, the in vitro induction of CD25+ CD4+ regulatory T cells by α-MSH has been described. In that system of experimental autoimmune uveoretinitis, α-MSH appeared to convert a population of effector T cells polarized to mediate hypersensitivity into a population of T cells with no mediated immunoregulation (40). These regulatory T cells are characterized by the production of IL-10 among other cytokines (41). It is also well known that IL-10 prevents IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes (42).

The association between airway inflammation and airway hyperresponsiveness is still a matter of debate. There are a number of papers indicating a link between eosinophilia and/or IL-5 production and increased airway responsiveness (43–47). In contrast, there is also a substantial body of literature pointing toward a dissociation between airway inflammation and airway hyperresponsiveness (18, 48–50). Even more recently, data from clinical trials using anti-IL-5 mAbs revealed unchanged lung function in the absence of eosinophils (51). We have measured airway responsiveness to methacholine. In addition, there are several other pathways controlling airway smooth muscle contractility. They include...
serotonin, neurokinin, and substance P-dependent mechanisms. Whether α-MSH has any effect on these other pathways still remains unknown. In addition to a qualitative relationship between cosinophil, airway inflammation, and airway hyperresponsiveness, there might also be a quantitative relationship between these events. For example, the number of (remaining) eosinophils might be still sufficient to maintain a level of airway hyperresponsiveness.

Elevated levels of α-MSH were detected in BAL fluids of OVA-challenged, but nonsensitized mice. It is well known that repeated Ag exposure via this route induces tolerance. Furthermore, it has been shown more recently that tolerance induced by similar protocols is mediated to a large degree by IL-10 (52, 53). It might be therefore, possible that the importance of endogenous α-MSH lies in its ability to induce airway tolerance. This would make triggering the α-MSH/α-MSH receptor pathway an interesting therapeutic target.

Acknowledgment

For their excellent technical assistance, we thank Brigitte Auffarth, Helga Fischer, Steffen Heuser, Britta Kosche, Verena Kräling, Delia Miklay, Jürgen Kurz, and Caroline Schwalm.

References


