

-MSH

-MSH

가

-MSH -MSH 가

2000 12

	-----	1
.	-----	2
.		
1.	-----	6
2.	-MSH -MSH -----	6
3.	-----	6
4.	-----	7
가.	-----	7
.	-----	7
.	-----	7
.	-----	7
(1)	-----	7
(2)	-----	8
5.	-----	8
.		
1.	-MSH 가 -----	9
2.	-MSH 가 -----	11

3. -MSH -MSH-ND, [Asn6]- -MSH-ND 가
-----13

4. -MSH -MSH-ND, [Asn6]- -MSH-ND 가
-----13

. -----15

. -----19

-----21

-----25

1.	-MSH 가	B7-2	----- 9
2.	-MSH 가	HLA-DR	-----10
3.	-MSH 가	HLA-DR	----- 11
4.	-MSH 가	B7-2	-----12
5.	-MSH	-MSH-ND 가	
	HLA-DR		-----14

-MSH -MSH 가

-MSH

. -MSH가

가

-MSH

가

-MSH -MSH

B7- 1,

B7-2, HLA-DR

-MSH

-MSH-ND

B7-2

HLA-DR

: -MSH, -MSH , , , B7- 1, B7-2,
HLA-DR,

-MSH -MSH 가

< >

.

Alpha-melanocyte stimulating hormone(-MSH)
proopiomelanocortin(POMC) 13 ^{1.}

adrenocorticotropin(ACTH) POMC

-MSH, -MSH -endorphin

POMC가

^{2.}

-MSH가

가 . -MSH

IL-1 , IL-6, TNF IFN-

^{3.}

-MSH IL-10

^{4.} -MSH

,

-MSH가 가

5.

-MSH가

-MSH

IL-10

,
6.

-MSH

POMC

POMC

melanocortin(MC)

5 가

가

7. MC-1

, , ,

8.

-MSH

가 .

-MSH

1

9.

-MSH

10.

B

-MSH

9.

B

-MSH가

B

10.

(dendritic cell)

가

가

.

,

CD34+

가 가

가

가

^{11,12}

GM-CSF

가 ¹⁴ IL-4 CD-14

¹⁵, TNF-

, GM-CSF

CD34+

¹⁶

IL-1

가

2

가 ¹⁷, IL-6

가

¹⁸

CD34+

CD34+

가

0.1%

5

10%

GM-CSF IL-4

IL-1

, TNF- , IL-6, PGE₂

-MSH

B7-1, B7-2 2

HLA-DR

-MSH

(agonist)

.

1. X-VIVO 15 (BioWhittaker, Walkersville, Maryland, USA) 1% 가 가 .
 . 800 U/Mℓ
 GM-CSF (Novartis, Frinley, UK), 1,000 U/Mℓ IL-4 (PBH, Hannover, Germany), 1,000 U/Mℓ IL-6 (PBH), 1 μg/Mℓ IL-1 (PBH), 10 μg/Mℓ TNF- (PBH), 1 μg/Mℓ PGE₂ (Sigma Chemical Co., St, Louis, MO, USA).

2. -MSH -MSH
 -MSH(Sigma Chemical Co.)
 -MSH-ND (, ,), [Asn⁶] -MSH-ND() 0.5%
 BSA/PBS .

3. B7-1 (Becton Dickinson, San Jose, USA), B7-2 (Ancell Co., Bayport, MN, USA), HLA-DR (Becton Dickinson) , FITC-conjugated anti-mouse immunoglobulin (Biosource International, Camarillo, CA, USA) . Isotype control mouse IgG₁(Biosource International) .

4. 가.

10Mℓ 5 U/Mℓ heparin, 2mM EDTA 가 20Mℓ

PBS 50Mℓ 15 Mℓ Ficoll/Hypaque

(density:1.0777 g/Mℓ)

20 (200 xg). 20

25Mℓ 20

(400 xg). 가 5mM

EDTA PBS 5 10

.

.

56 30

10

(1000 xg). 가

.

X-VIVO 15 , 6-well

plate well 1×10^7 40 (37 , 5% CO₂).

40 가 3

.

0.5 - 1 X 10⁶/well가 .

.

: 6-well tissue culture plate well

0.5- 1 × 10⁶ 3Mℓ 800 U/Mℓ

GM-CSF 1000 U/Mℓ IL-4 37 CO₂ .

2, 4, 6 , well 1 M ℓ 1600
U/M ℓ GM-CSF 1000 U/M ℓ IL-4 1 M ℓ 가 .
7 , well ,

0.5% BSA/PBS 6-well plates well 1
 $\times 10^6$ /M ℓ . 800 U/M ℓ GM-CSF, 500 U/M ℓ IL-4,
10 ng/M ℓ IL-1 , 10 ng/M ℓ TNF- , 1000 U/M ℓ IL-6, 1 μ g/M ℓ PGE2
가 .

CD83, B7- 1, B7- 2, HLA -DR 가, endocytosis

5.

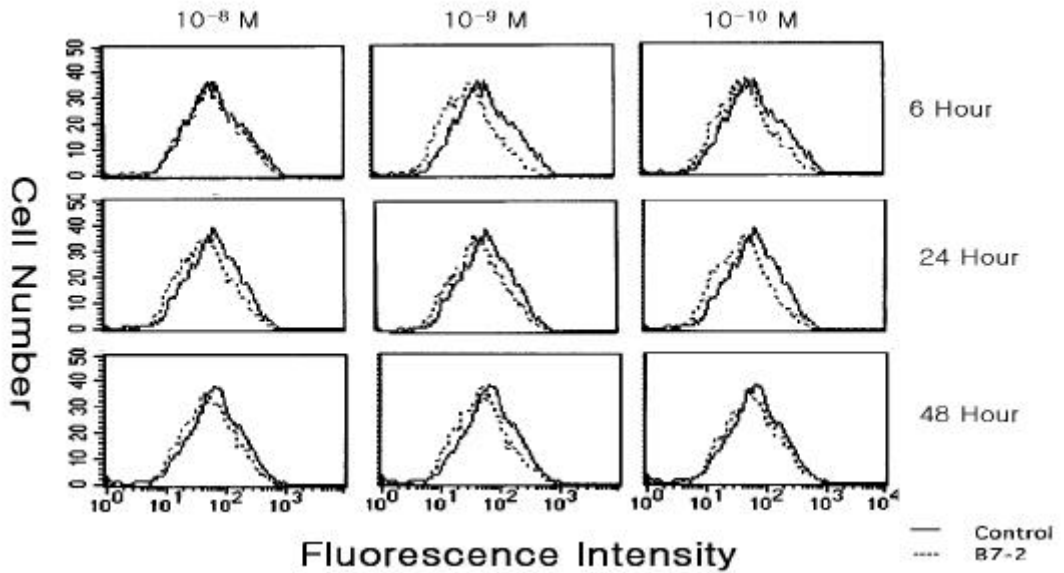
가 -MSH -MSH
0.5% BSA/PBS
가 30 4 .
0.5% BSA/PBS FITC-conjugated anti-mouse
immunoglobulin 가 30 4

FACScan (Becton Dickson, Mountain View, Ca, USA)

1. -MSH가

7 가 ($10^{-8}M$, $10^{-9}M$, $10^{-10}M$) -MSH B7-2 (mean immunofluorescence)

Concentration	6 Hour	24 Hour	48 Hour
$10^{-8}M$	107.8 (97.9%)	69.6 (75.1%)	70.0 (75.6%)
$10^{-9}M$	56.4 (52.3%)	60.8 (65.7%)	76.6 (79.0%)
$10^{-10}M$	71.6 (66.4%)	96.9 (86.2%)	83.5 (86.2%)



1. -MSH가

B7-2

가 -MSH ($10^{-8}M$, $10^{-9}M$, $10^{-10}M$) (D7) 6, 24, 48 B7-2 가

가 (1). 72

가 . 96

가

10⁻⁵M, 10⁻⁶M, 10⁻⁷M -MSH

B7-2 가

7 가 (10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M)

-MSH HLA-DR -MSH

6 188.8 10⁻⁸M

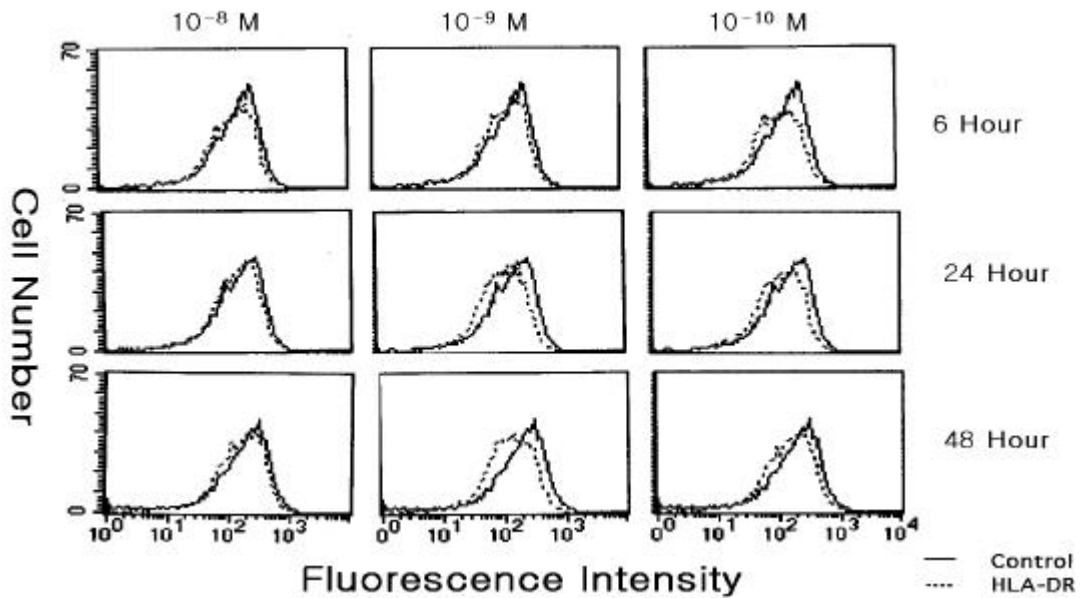
163.0(96.3%), 10⁻⁹M 167.8(88.9%), 10⁻¹⁰M 143.9(86.3%)

10⁻¹⁰M 가 . 24

180.1 10⁻⁸M 159.1(88.3%), 10⁻⁹M

129.3(71.8%), 10⁻¹⁰M 135.0(74.9%) 10⁻⁹M 가

. 48 225.0



2. -MSH가 HLA-DR

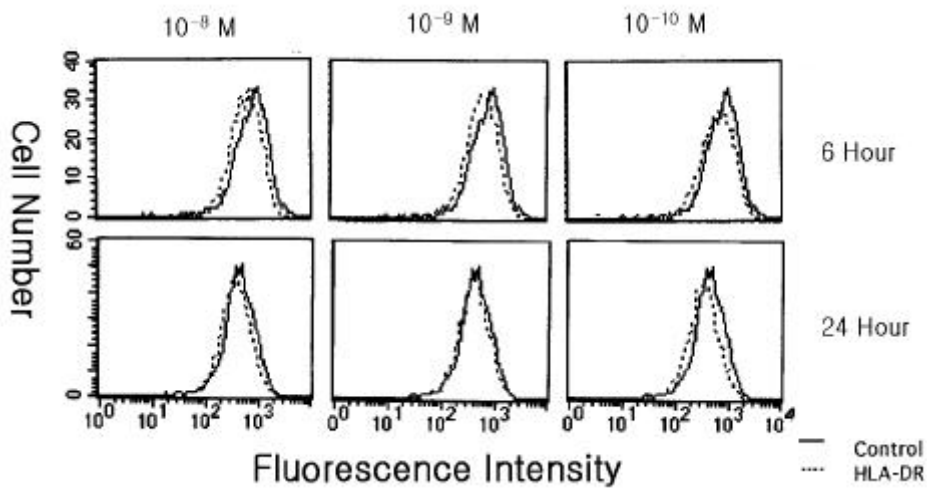
가 -MSH (10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M) (D7) 6 , 24 , 48

HLA-DR 가

$10^{-8}M$ 199.4(88.6%), $10^{-9}M$ 153.9(68.4%), $10^{-10}M$
 177.1(78.7%) $10^{-9}M$ 가 (2). 72
 -MSH $10^{-9}M$ 가
 가 . 96
 가 $10^{-5}M$, $10^{-6}M$,
 $10^{-7}M$ -MSH HLA-DR
 가
 B7-1 가

2. -MSH가

9 가 ($10^{-8}M$, $10^{-9}M$, $10^{-10}M$)
 -MSH HLA-DR . -MSH
 6 836.8 $10^{-8}M$



3. -MSH가

HLA-DR

가 -MSH ($10^{-8}M$, $10^{-9}M$, $10^{-10}M$) (D9) 6 , 24 , 48
 HLA-DR 가

3. -MSH -MSH-ND, [Asn⁶]- -MSH-ND가

7 가 (10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M)

-MSH-ND [Asn⁶]- -MSH-ND 6 B7-2, HLA-DR

가 . -MSH-ND [Asn⁶]-

-MSH-ND 24 , 48 B7-2, HLA-DR

가 10⁻⁵M,

10⁻⁶M, 10⁻⁷M -MSH-ND [Asn⁶]- -MSH-ND

B7-2, HLA-DR 가

.

B7-1 가 .

4. -MSH -MSH-ND, [Asn⁶]- -MSH-ND가

9 가 (10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M)

-MSH-ND B7-2 HLA-DR

. -MSH-ND 6 B7-2 597.4

10⁻⁸M 535.5(89.6%), 10⁻⁹M 531.7(89.0%), 10⁻¹⁰M

505.5(84.6%) . HLA-DR

434.9 10⁻⁸M 249.3(57.3%), 10⁻⁹M 415.3(95.5%),

10⁻¹⁰M 336.3(77.3%) 10⁻⁸M 가

(5). -MSH-ND 24 , 48

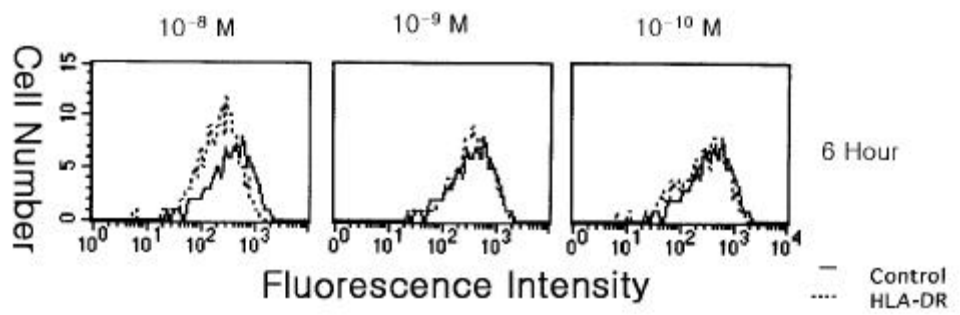
B7-2, HLA-DR .

10⁻⁵M, 10⁻⁶M, 10⁻⁷M -MSH-ND

B7-2, HLA-DR 가

.

[Asn⁶]- -MSH-ND 6 , 24 , 48 B7-2,
 HLA-DR . 10⁻⁵M,
 10⁻⁶M, 10⁻⁷M [Asn⁶]- -MSH-ND
 B7-2, HLA-DR 가 .
 B7-1 가 .



5. -MSH -MSH-ND가 HLA-DR
 가 -MSH-ND (10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M) (D9) 6 , 24 ,
 48 HLA-DR 가 .

(neuropeptide)

(immunocompetent)

calcitonin-gene related peptide(CGRP)

¹⁹. pituitary adenylate cyclase activation polypeptide(PACAP), gastrin releasing peptide(GRP)

²⁰. -MSH

가

8,9,10,21

1983 Thody

-MSH

²².

-MSH 가 가

-MSH

pituitary gland가

-MSH

가

²³. POMC mRNA

POMC

-MSH

²⁴.

POMC

mRNA가

-MSH

ACTH, -endorphin

POMC

가

²⁵.

Th1+

POMC

mRNA

-MSH

²⁶.

-MSH가

가 .

-MSH POMC . -MSH

IL-1 , IL-1 , IL-6, TNF-
⁵ . -MSH IL-1
, leukocytosis, , acute phase protein
²⁷ . -MSH
IL-10 . -MSH
. . .

, -MSH
. Luger -MSH 가
, , MHC class
MHC class ICAM-1
⁹ . -MSH lipopolysaccharide(LPS)
CD80(B7-1) CD86(B7-2)
⁸ . -MSH가
B7-2 HLA-DR .
가

. B7-2
HLA-DR 가

-MSH B7-2
MC-1 . Becher
GM-CSF IL-4 -MSH가 MC-1
가 5 MC-1

가 8 28 .
 GM-CSF IL-4 7, 8, 9
 7 cytokine
 cocktail 가 9 , 10 , 11 .
 -MSH 가
 MC-1
 가 .
 -MSH-ND B7-2
 HLA-DR [Asn⁶]- -MSH-ND B7-2
 HLA-DR . -MSH
 -MSH-ND [Asn⁶]- -MSH-ND
 Ahx-Asp-His-D-Phe-Arg-Trp-Lys, Ahx-Asp-Asn-D-Phe-Arg-Trp-Lys
 가 7 . Li hMC1R,
 rMC3R hMC4R cDNA CHO (stable
 transfection) MC
 radioligand-binding assay cAMP-generating assay
 -MSH .
 -MSH-ND [Asn⁶]- -MSH-ND MC-1
 -MSH-ND가 [Asn⁶]- -MSH-ND
 29 . MC
 MC-1 28 .
 [Asn⁶]- -MSH-ND MC-1 가
 -MSH-ND가 MC-1
 . -MSH -MSH
 -MSH

$10^{-8}M$ $10^{-10}M$. . -MSH -MSH
 가 . Joosten neonatal
 rat corticospinal neuron in vitro neurite
 -MSH 가
 30
 -MSH -MSH $10^{-8}M$ $10^{-10}M$
 -MSH -MSH-ND
 -MSH
 -MSH가
 가
 T -MSH
 가 가

.

-MSH

-MSH -MSH-ND, [Asn⁶]- -MSH ,

B7- 1, B7-2, HLA-DR

.

1. 10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M -MSH 6 , 24 , 48

B7- 2, HLA-DR

B7- 1 가 .

10⁻⁵M, 10⁻⁶M, 10⁻⁷M B7- 1, B7-2, HLA-DR

가 .

2. 10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M -MSH 6 , 24 , 48

HLA-DR

B7- 1, B7-2 가 .

10⁻⁵M, 10⁻⁶M, 10⁻⁷M B7- 1, B7-2, HLA-DR

가 .

3. 10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M -MSH -MSH-ND [Asn⁶]-

-MSH-ND 6 , 24 , 48

B7- 1, B7-2, HLA-DR 가 .

10⁻⁵M, 10⁻⁶M, 10⁻⁷M

B7- 1, B7-2, HLA-DR 가 .

4. 10⁻⁸M, 10⁻⁹M, 10⁻¹⁰M -MSH -MSH-ND [Asn⁶]-

-MSH-ND 6 , 24 , 48

-MSH-ND 6 B7-2, HLA-DR

24 , 48 가 .

[Asn⁶]- -MSH-ND

가 .

가

가 .

B7- 1, B7-2, HLA-DR

$10^{-5}M$, $10^{-6}M$, $10^{-7}M$

B7- 1, B7-2, HLA-DR

-MSH

B7-2 HLA-DR

-MSH-ND

,

.

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Abstract

The changes of expression in surface molecule of human monocyte-derived dendritic cells with α -MSH and its agonist

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(Directed by Associate Professor Mn-Geol Lee)

There is an accumulating evidence that α -MSH, besides being a pigmentary hormone also plays a crucial role in the regulation of immune and inflammatory reactions. α -MSH might exert its immunomodulatory effects by downregulating accessory molecules on antigen-presenting cells. Dendritic cells (DC) are a system of highly efficient antigen-presenting cells that initiate the primary immune response. Recently, new two-step culture system for immature / mature DC generation from human peripheral blood-derived monocytes was established.

In this study, we generated monocyte-derived immature / mature DC according to the two-step culture system and observed the changes of surface molecule of human monocyte-derived dendritic cells with α -MSH and its agonist by flow cytometry. The following results were obtained:

1. α -MSH suppressed the expression of B7-2 and HLA-DR in immature DCs.
2. α -MSH suppressed the expression of HLA-DR in mature DCs.
3. α -MSH-ND(α -MSH agonist) also suppressed the expression of B7-2 and HLA-DR in mature DCs.

From the result, it is suggested that α -MSH and its agonist α -MSH-ND are capable of downregulating immune response through suppressing accessory molecules on antigen presenting cells.

Key Words : α -MSH, α -MSH agonist, monocyte, dendritic cells, B7-1, B7-2, HLA-DR, flow cytometry