

-12

CD4+ T

-12

CD4+ T

2002 12



가

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8.	IL-12		CD4+ T	
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		4	(E)	.....25

-12

CD4+ T

interleukin (IL)-4 IL-13 type 2  
 가 CD4+ T  
 Th2 IL-12 R<sub>2</sub> Naive CD4+ T Th1  
 IL-12 가 IL-  
 12R<sub>2</sub> Th2 가  
 IL-12  
 Th2 가 CD4+ T IL-12 가  
 가  
 가 CD4+ T anti-IL-4 (200 ng/ml)  
 IL-12 (2 ng/ml) 가 RPMI-1640  
 IL-12R<sub>2</sub>  
 CD4+ T Modified  
 silver 가 1500 ,  
 IL-12 가  
 40 kDa pI 가 6.0-10 ,



20 kDa pI 가 6.5-7.0

IL-12

40 kDa pI 가 5.6,

29 kDa, pI 가 5.7

IL-12 가

CD4+ T IL-12

CD4+ T IL-12

가

---

: , , CD4+ T , IL-12

< >

•

1,2

3,4

5,6

가

1,6,7

1,6

IgE

, interleukin (IL)-4, IL-13

type 2

T 가 가

8

interferon (IFN)-

T

9,10

type 2

가

1,7 IL-4

(polymorphism), signal transducers and

activators of transcription (STAT)-6

1,11

<sup>1</sup> CD2 , CD2 IL-12  
 T helper (Th)1 Th1 Th2  
<sup>12,13</sup>  
 T <sup>14,15</sup>  
 IL-4 IL-13 Th1  
 IFN- IL-12  
 가 chemokine  
 IL-12 가 T Th1  
<sup>1,6,15,16</sup> Th2  
 가 IL-12  
<sup>17-19</sup>  
 CD4+ T Th1 Th2 ,  
 Th <sup>20,21</sup> Naive CD4+ T  
 Th1  
 IL-12 IFN- type 1 , IL-12 NK  
 T

<sup>22</sup>

<sup>23</sup> IL-12

IL-12R<sub>1</sub> IL-12R<sub>2</sub> <sup>23</sup> naive T cells

Th1

Th2

IL-12 <sup>17,24,25</sup>

STAT-4 T-box (T-bet) Th2

GATA-3 down-regulation, suppressor of cytokine signaling (SOCS)

<sup>26</sup> IL-12 T

가 <sup>27</sup>

(proteomics)

가

(posttranslational modification),

<sup>28,29</sup>

가 <sup>30,31</sup>

<sup>30,32</sup>

<sup>33,34</sup>

<sup>35</sup>

<sup>36</sup>

<sup>37</sup>

1970

MS) 38,39 (mass spectrometry:  
40

pH gradient (IPG) 42 41 immobilized

matrix assisted laser desorption  
ionization/time of flight (MALDI-TOF) electrospray ionization (ESI) MS

가 가

가 43  
가

30,31,

Th1 Th2  
oligonucleotide microarrays mRNA

44

45

46-49 Th

1984 murine T, B

mitogen

50 2000 Jurkat T

가 . T

Th1/Th2

(primary) T 가 .<sup>51</sup> T

SWISS-PROT

NCBI protein 가 <sup>52</sup>

가 T

Th2 IL-12R<sub>2</sub>

가 CD4+ T IL-

12

CD4+ T IL-12

1.

Hanifin Rajka 10  
20 ml  
Heparin Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden)  
700 × g 15

2. CD4+ T

가 CD4+ isolation kit (Miltenyi  
Biotech, Auburn, CA, USA) negative selection CD4+ T  
5% bovine serum albumin (BSA) (Sigma, St.  
Louis, MO, USA), 2 mM ethylenediaminetetraacetic acid (EDTA) (Sigma)  
phosphate-buffered saline (PBS), pH 7.4 3 hemocytometer  
CD4+ T hapten-antibody cocktail (anti-CD8, anti-CD16, anti-CD19,  
anti-CD36, anti-CD56) anti-hapten microbeads (Miltenyi Biotech, Auburn, CA, USA)  
MidiMACS system (Miltenyi Biotech)

30  $\mu\text{m}$  nylon mesh

PBS/0.5% BSA, 2 mM EDTA

$10^7$  80  $\mu\text{l}$

$10^7$

20  $\mu\text{l}$

hapten-antibody cocktail

가

10 4

2

$10^7$

20  $\mu\text{l}$

anti-hapten microbeads

가

15

4

2

MidiMACS

LS

5 ml

500  $\mu\text{l}$

10 ml

CD4+ T

FACStar (Becton Dickinson, San

Lose, CA, USA)

CD4-FITC (Becton Dickinson)

mouse IgG<sub>1</sub>

### 3. CD4+ T

가

CD4+ T

10 ng/ml

phorbol 12-myristate 13-acetate (PMA) (Sigma), 100 ng/ml ionomycin (Sigma), 2 ng/ml

recombinant human IL-12p70 (Pharmingen, Franklin Lakes, NJ, USA) 200 ng/ml

neutralizing anti-IL-4 antibody (Pharmingen) 가

RPMI 1640 (10% FBS,

2mM L-glutamine, 50  $\mu\text{M}$  2-mercaptoethanol (2-ME) , 100 U/ml penicillin, 100  $\mu\text{g/ml}$



streptomycin sulfate, 5 µg/ml Fursion)

3 FACStar R-phycoerythrin (R-PE)-conjugated rat anti-human IL-12R<sub>2</sub> antibody (Pharmingen)

#### 4. CD4+ T

CD4+ T IL-12  
pH 8.0, 1 M Tris, 0.3% sodium dodecyl sulfate (SDS), 3% dithiothreitol, 1 mM phenyl methyl sulphonyl fluoride (PMSF) 가  
30 µl 95 5 가 7 M urea, 2 M thiourea, 2 mM tributyl phosphine (TBP), 4% 3-[(3-cholamidopropyl)dimenthylammonio-]-1-propane-sulfonate (CHAPS), 0.5% carrier ampholytes, 40 mM Tris, 0.002% bromophenol blue dye 가 multiple chaotrope 400 µl sonicator  
endonuclease (Sigma) 150 U/ml 25  
20 , 12,000 rpm 20  
18 cm pH 3-10 non-linear (NL) immobilized pH gradient (IPG) (Amersham Phamarcia Biotech, Piscataway, NJ, USA) 24 . IPG-phor (Amersham Phamarcia Biotech, San Francisco, CA, USA) 100,000 vhr (isoelectric focusing: IEF) . 3.6  
g urea, 2% SDS, 5 M Tris 2 ml, 50% glycerol 4 ml, 25% acrylamide 1 ml, 200 mM TBP 250 µl 가 25 (equilibration) 10-16%

gradient polyacrylamide gel (1.875 M Tris buffer, 40% acrylamide stock solution, )

agarose embedding 3 mA/gel 2 prerun 15  
mA/gel .

#### modified silver

가<sup>53</sup> , acetic acid 25 ml, methanol 100 ml, 125 ml 15  
, methanol 75 ml, 5% sodium thiosulfate 10 ml, sodium acetate 17 g,

165 ml 30 . 10 3 , 2.5% silver nitrate  
25 ml 225 ml 1 .

sodium carbonate 6.25 g, formaldehyde 100  $\mu$ l, 250 ml ,  
EDTA 3.65 g, 250 ml , 250 ml 5 3

## 5.

IL-12 CD4+ T

GS-800 Calibrated Imaging Densitometer (Bio-Rad, M $\ddot{u}$ nich,  
Germany) PDQuest (Bio-Rad)

Sensitivity, min peak, size scale edit spot tool

가

matchset IL-12 replicate



acid (CHCA) 가 50% ACN/0.1% trifluoroacetic acid (TFA) MALDI  
plate loading  
Peptident (<http://www.expasy.org/tools/peptident.html>) MS-Fit  
(<http://prospector.ucsf.edu/ucsfhtml4.0/msfit.htm>)

**1. IL-12 CD4+ T IL-12R<sub>2</sub>**

가 CD4+ T IL-12  
 IL-12R<sub>2</sub> FACStar . 72  
 (A) 10 ng/ml PMA, 100 ng/ml

ionomycin, 2 ng/ml recombinant IL-12p70, 200 ng/ml neutralizing anti-IL-4 antibody

가 (B) IL-12R<sub>2</sub> 가 ( 1).

72 가 가 96, 120

가 IL-12R<sub>2</sub>

( 2).

CD4 microbead

positive selection hapten-antibody cocktail

negative selection CD4-FITC

CD4+ T 93%

IL-12 가 72

**2. CD4+ T**

PDQuest (Bio-Rad)

CD4+ T . Silver nitrate

1500 가 ( 3, 4).  
 ( 5)  
 40 kDa pI 가 6.0-10 ( 6) 20  
 kDa pI 가 6.5-7.0 ( 7). B (  
 6) 10 2 , C ( 7)  
 2  
 . 10 2 IL-12  
 ( ), 2 3  
 2 IL-12  
 .  
 4 D, E IL-12

### 3. IL-12

### CD4+ T

IL-12 matchset  
 replicate group  
 PDQuest (Bio-Rad) IL-12  
 .  
 PDQuest analysis set tool IL-12

가

parameter 10  
 29 kDa pI 가 5.7 IL-12 가  
 10 6 가 ( 8).  
 40 kDa pI 가 5.6 10 6 IL-12  
 가 가 ( 9).

#### 4. CD4+ T MALDI-MS

Modified silver 가

가 Silver

MALDI-MS 가

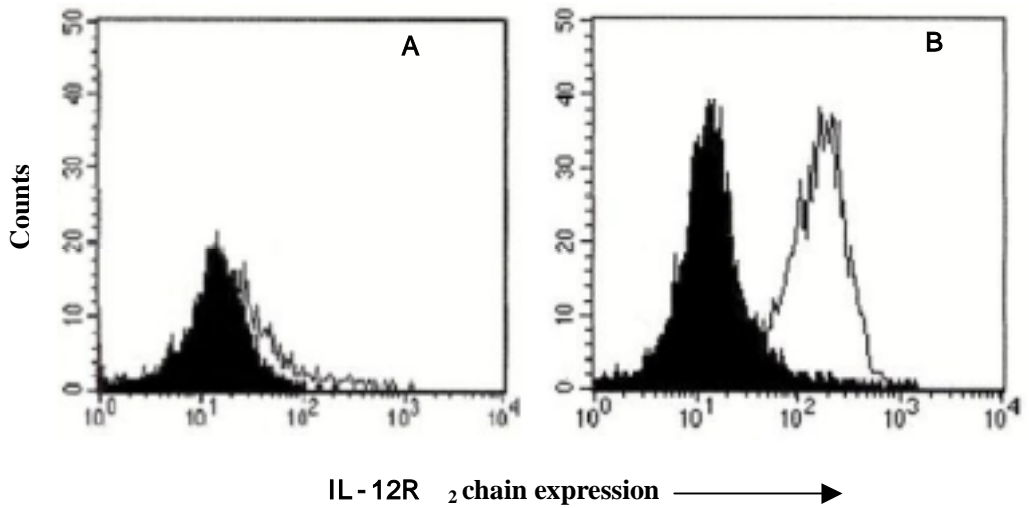
400 µg

80 µg silver 가

가

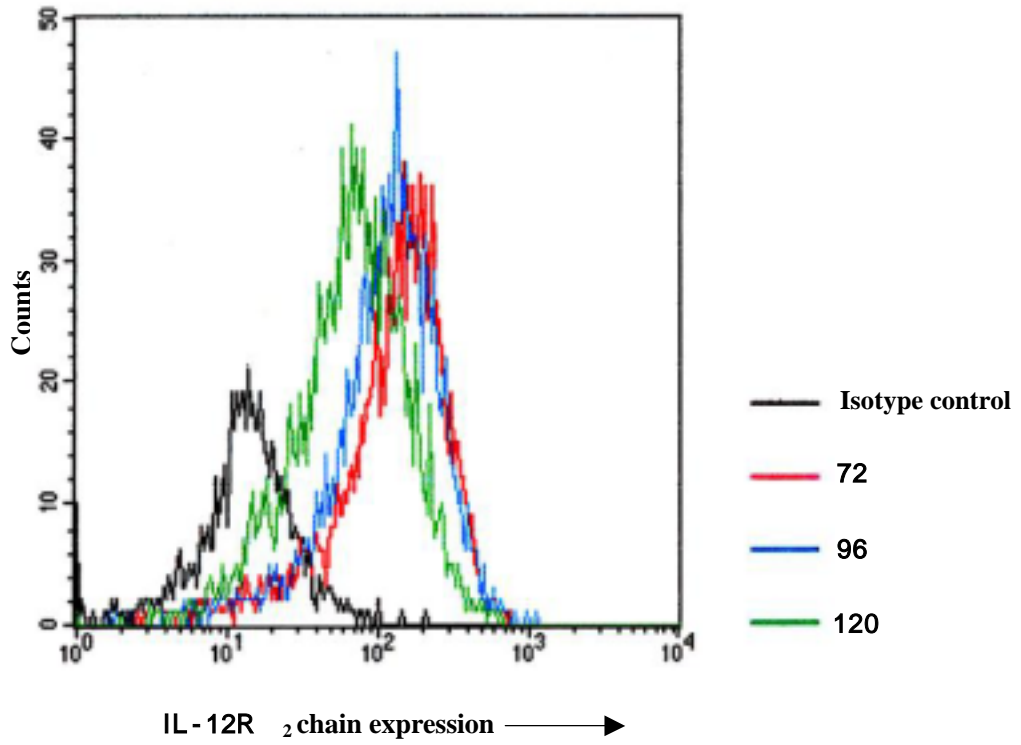
MALDI-MS 가

가



1. IL-12 CD4+ T IL-12R<sub>2</sub> FACS (A) PMA, ionomycin, recombinant human IL-12p70, neutralizing anti-IL-4 antibody 가 72 (B) R-phycoerythrin (R-PE)-conjugated rat anti-human IL-12R<sub>2</sub> antibody FACStar IL-12 IL-12R<sub>2</sub> 가 . (solid histograms: isotype control antibody, outlined histograms: IL-12R<sub>2</sub> antibody)





2. IL-12

CD4+ T

IL-12R<sub>2</sub> IL-12 72, 96, 120

CD4+ T

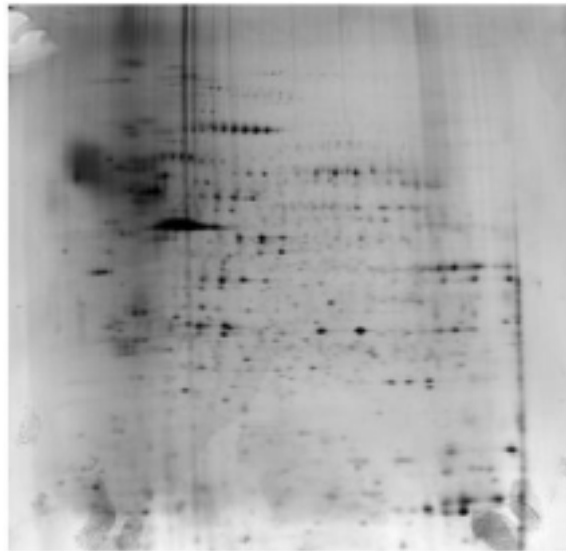
IL-12R<sub>2</sub> FACS 72

가 가 96, 120

가 IL-12R<sub>2</sub>

A.

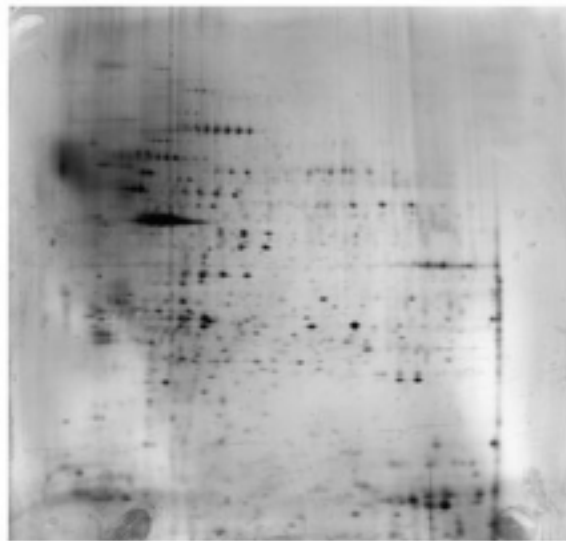
MW



pl 3 ← non-linear IPG-strip → 10

B.  
IL-12

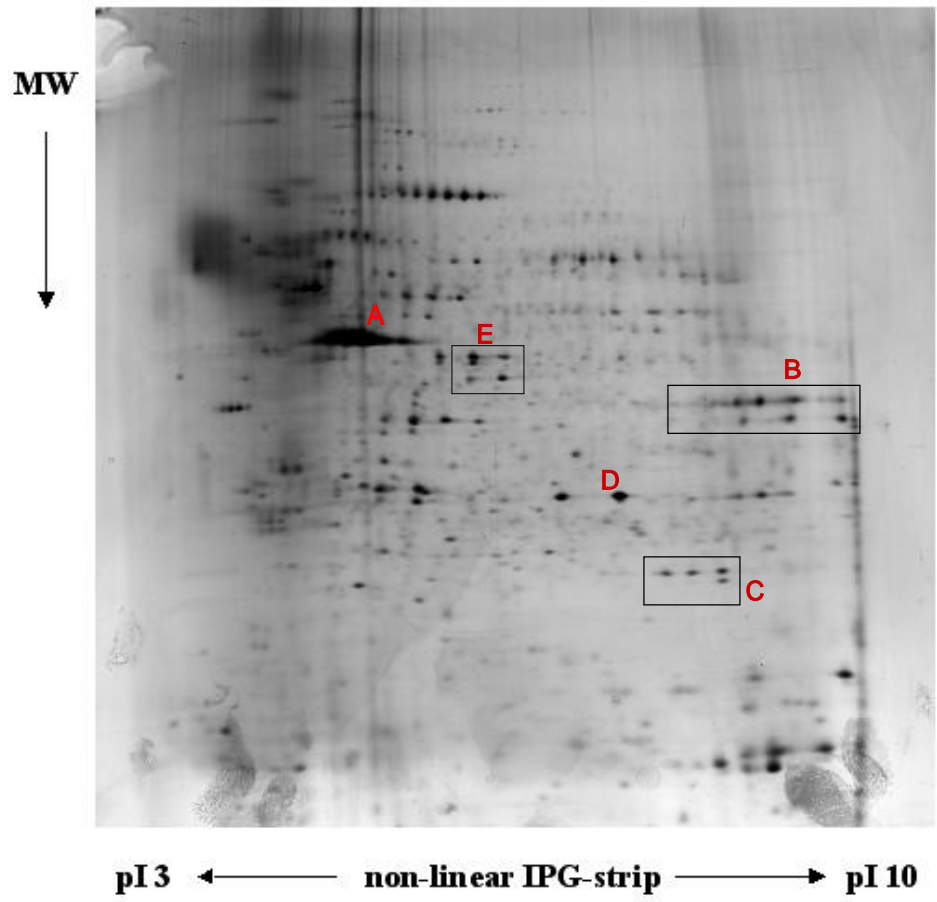
MW



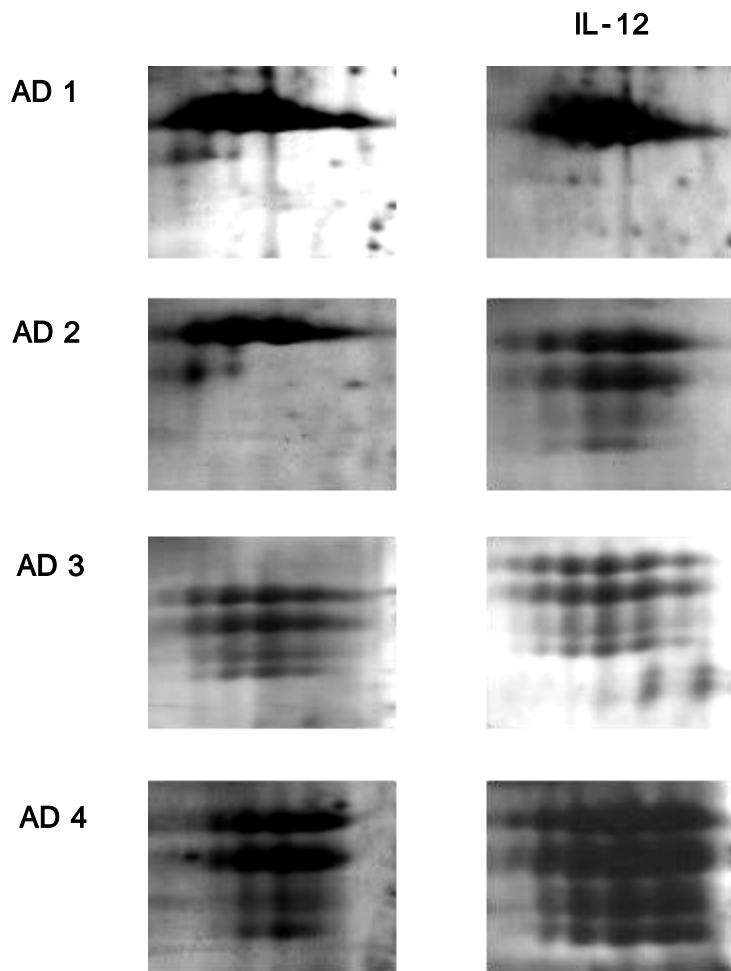
pl 3 ← non-linear IPG-strip → 10

3. IL-12

CD4+ T



4. CD4+ T . Silver  
 nitrate 1,500  
 A, B, C, D, E IL-12 .



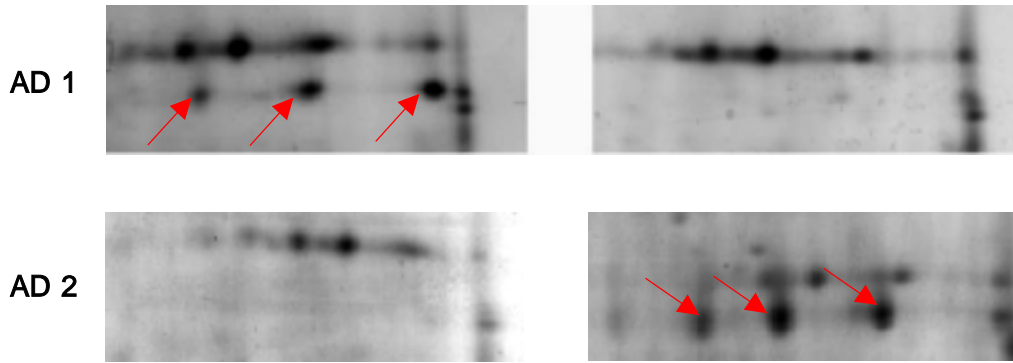
5. CD4+ T

4 (A) 가

, IL-12 가

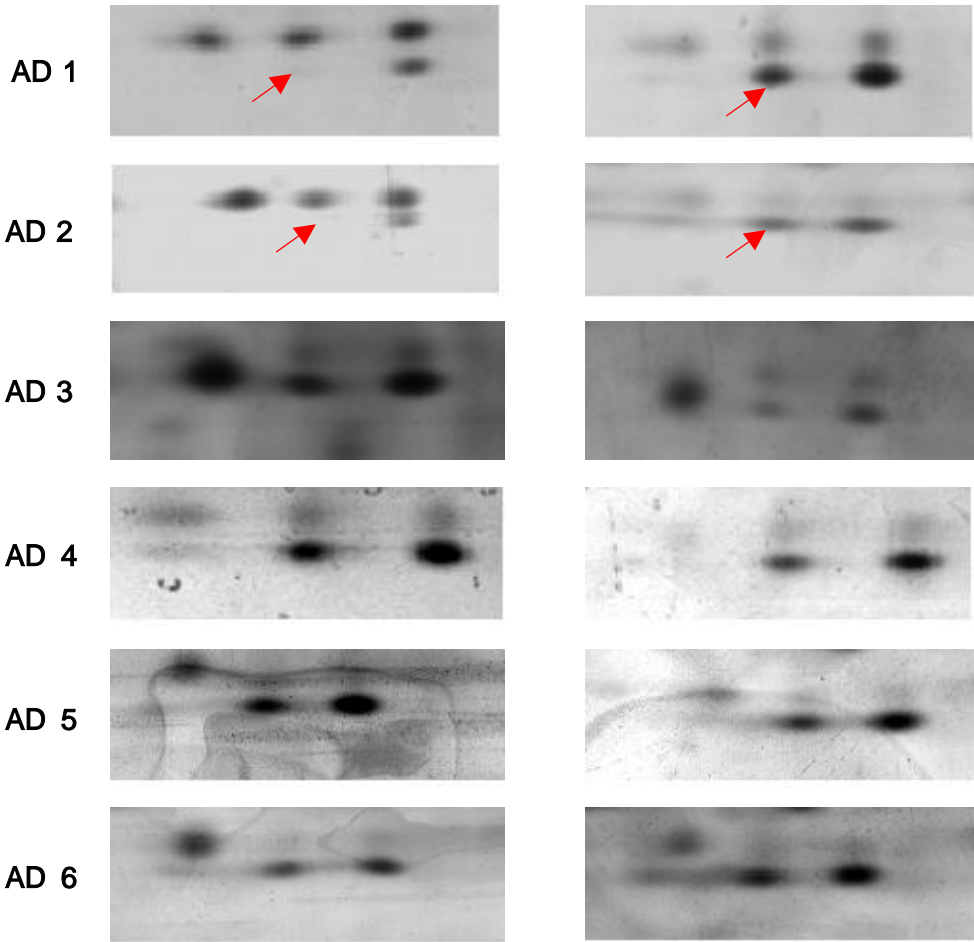
. (AD: )

IL-12



6. CD4+ T 4 (B)  
CD4+ T IL-12  
가 40 kDa  
pI 가 6.0-10

IL-12



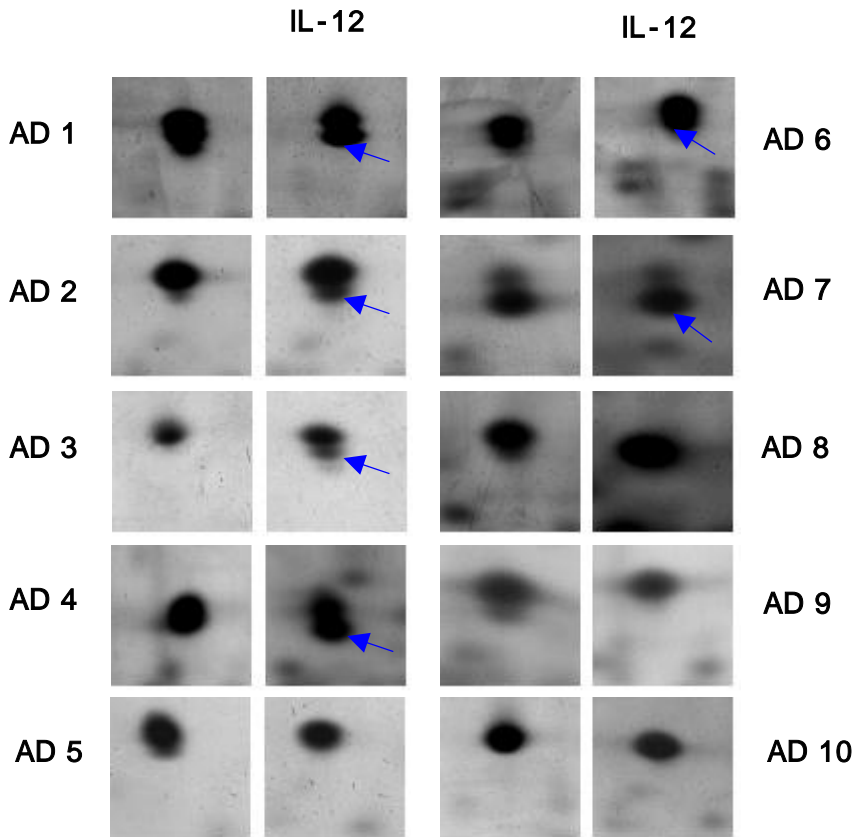
7. CD4+ T 4 (C)

CD4+ T IL-12

가

20

kDa pI 가 6.5-7.0



8. IL-12

CD4+ T

4

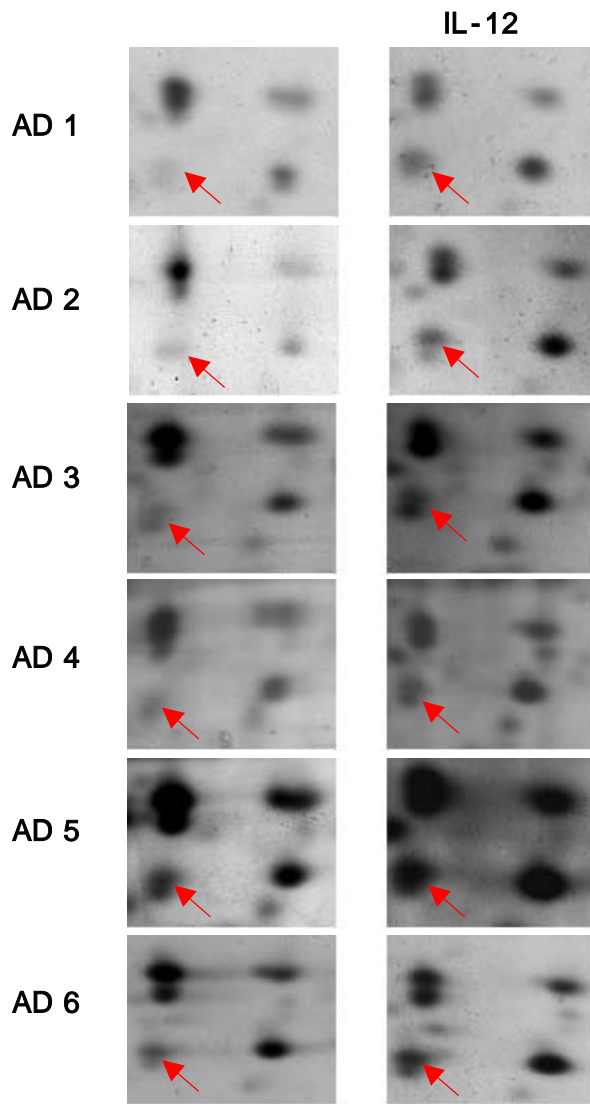
(D)

CD4+ T

IL-12      가

29 kDa

pI 가 5.7



9. IL-12

CD4+ T

4

(E)

IL-12

가

IL-12

40 kDa

pI 가 5.6

가가



IL-5, IL-13 Th2 IgE  
 IFN- IL-12 가 type 1 IL-4,<sup>1,2</sup>  
 Th2  
<sup>20,21</sup>  
 IL-12 IFN- Th1  
<sup>54</sup> T Th1  
 IL-12R<sub>2</sub> IL-12<sup>24,25</sup>  
 가 IL-12  
 IL-12R<sub>2</sub>  
 가 T IL-12R<sub>2</sub> 가  
 IL-12<sup>12,13</sup>  
 IL-12 CD4+ T  
 IL-12 CD4+ T  
 Th2 naive T  
 가  
 Th2 Th1  
 IL-12 가 IL-12R<sub>2</sub> 72 96

17,24

72, 96, 120

IL-12R<sub>2</sub>

72

96, 120

IL-12

Th1/Th2

Th2

가

IL-12

19,

IL-12

가

Th2

Th0/Th1

21

T

IL-12

IL-12R<sub>2</sub>

가

Th1/Th2

Th

CD4+ T

in vitro

IL-12

T

가 IL-12

Th2

가

IL-12

CD4+ T IL-12

CD4+ T

modified silver

가

MALDI-MS

CD4+ T

91

<sup>52</sup> IFN-

ubiquitin-conjugating

47

% , 가

가

CD4+ T

. PDQuest

CD4+ T

Th1 Th2 가

IL-12

가

F-actin polymerization, CD8+ T, CD4+ T

CD19+ rigidity

vimentin, tubulin cytokeratin CD19+ (B )

T 46

가

IL-12

29 kDa pI 가 5.7 10 6

IL-12

가 modification 2 kDa,

15-18 가 가 IL-12

Silver nitrate

modified silver

MALDI-MS

가

가

In-gel trypsin

digestion

MALDI-MS

가

가 T beta chain  
MALDI-MS

peptide coverage

MALDI-MS

가 pH IPG  
pH IPG (pH 3-10) loading  
2-3 가

IL-12

pSTAT 4

Western blot

FACS

55,56

IL-

12

CD4+ T

가

MALDI-MS ESI-MS

CD4+ T

가







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## Abstract

# Proteomic approach to CD4+ T cell differentiation protein by interleukin-12 in patients with atopic dermatitis

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Atopic dermatitis is based on an inflammatory mechanism involving type 2 cytokines such as interleukin (IL)-4 and IL-13. CD4+ T cells express predominantly the T helper (Th) 2 phenotype that down-regulates IL-12R<sub>2</sub> in atopic dermatitis. IL-12 is a major cytokine in the differentiation of naïve CD4+ T cells into Th1 cells. This mechanism is tightly regulated by expression of IL-12R<sub>2</sub>. However, it remains unclear that IL-12 signaling in polarized Th2 cells. The aim of this study was to identify IL-12 responsiveness in CD4+ T cells of patients with atopic dermatitis using a proteomic tools. CD4+ T cells isolated from peripheral blood of atopic dermatitis were treated with neutralizing anti-IL-4 antibody (200 ng/ml) and IL-12 (2 ng/ml) in RPMI-1640 (10% FBS, 50 μM 2-mercaptoethanol (2-ME), antibiotics) and parallel cultures of untreated cells were also prepared. On day 3, surface phenotype change was examined using IL-12R<sub>2</sub> antibody by FACS analysis and separate CD4+ T cells by two-dimensional (2-DE) electrophoresis. About 1500 protein spots were detected in the 2-DE gels

with modified silver staining. Several areas of the 2-DE map exhibited quantitative and qualitative change after IL-12 treatment. It was observed that several regions have different protein patterns. First, actin pattern, a group of spots of pI 6.0-10.0 with molecular weight about 40 kDa showed variation in each sample. In addition, decreased or increased spots were examined in the regions of pI 6.5-7.0 with molecular weight about 20kDa. Also, we detected that 2 spots were changed in CD4+ T cells with treatment of IL-12. Protein spots with pI 5.7 and 29 kDa molecular weight and pI 5.6 and 40 kDa molecular weight were increased in 60% of CD4+ T cells with treatment of IL-12. The identification of these spots is needed by mass spectrometry in further study in order to find targets regulated by IL-12 in CD4+ T cells of atopic patients. This study suggests that proteomic tools are suitable to understand a certain stage of pathological process of atopic dermatitis.

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Key Words: atopic dermatitis, proteomics, CD4+ T cells, interleukin-12