

IL-4

Effect of IL-4 Expressed by Recombinant Adenovirus on Collagen Induced Arthritis

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Abstract : Interleukin-4 (IL-4) is one of anti-inflammatory cytokines which has the potential to counteract pro-inflammatory cytokines often detected in rheumatoid arthritis. This study examined the effect of adenovirus-mediated gene transfer of IL-4 on collagen-induced arthritis (CIA), an animal model of rheumatoid arthritis. The IL-4 cDNA was cloned from a T lymphoma cell line after induction with PMA and was further cloned into an adenovirus shuttle vector. The recombinant adenovirus encoding rat IL-4, AdrIL-4, was constructed by homologous recombination between the cloned shuttle vector and a rescue vector, pJM17, and identified by polymerase chain reaction. CIA was induced in Dark agouti (DA) rats by immunizing type II collagen. On 12th day after immunization, DA rats were administrated with intra-articular injection of 4×10^8 plaque-forming units of AdrIL-4. The exogenous IL-4 expression was observed to inhibit CIA progression, compared to those of control groups, in which PBS or recombinant adenovirus without a transgene, Adnull, was administered. Whereas any significant difference was not observed on the production of anti-collagen antibodies among different groups of rats, IL-4 gene transfer but not with control treatments was shown to suppress CIA in a moderate manner.

Key Words : Collagen induced arthritis, Interleukin-4, Recombinant adenovirus

- Rheumatoid arthritis
가 .
[1].
rheumatoid arthritis
가
T- , B-
[2].
가
가
cytokine
tumor necrosis
factor α (TNF- α), interleukin-1(IL-1)
cytokine
IL-1 IL-1
IL-1 receptor antagonist(IL-1Ra)
[3]. TNF- α
TNF- α soluble receptor
[4].
TNF- α soluble receptor
rheumatoid arthritis
FDA [5]. IL-
1 TNF- α rheumatoid arthritis
cytokine
cytokine rheumatoid
arthritis 가 .
- IL-10, IL-4 TGF- β
cytokine ,
cytokine IL-10 TGF- β rheumatoid
arthritis ,
rheumatoid arthritis cytokine
[5].
rheumatoid arthritis
IL-1 β TNF- α T-
IL-4 IL-10
[6,7]. Rheumatoid arthritis
T- cytokine
Th1 Th2 . Rheumatoid
arthritis
Th1 cytokine IL-1 β TNF- α IL-6
Th2 cytokine IL-4, IL-10
[8]. rheumatoid arthritis
Th1 Th2 switch가
Th2 cytokine ,
cytokine
TNF- α IL-1 β IL-6
rheumatoid arthritis
Th2 cytokine IL-10 rheumatoid
arthritis 가
IL-10
IL-1 β TNF- α IL-6 cytokine
가 [9,10].
IL-4
T-
cytokine
[11]. IL-4 Th2
T-
[11]. IL-4
cytokine
soluble IL-IR type soluble
TNF receptor cytokine
[12-14].

[15]. minimum essential medium(EMEM) , HeLa Dulbecco's modified eagle medium(DMEM; JBI ,) 37 , 5% CO₂ .

2. Rat IL-4

[16,17].

type 5

36 kb

WKA-S1 1 × 10⁶ 12-

DNA well PMA 0.5 μg 5 μg

3

cytokine IL-4

TriPure™ isolation reagent RNA

(collagen-induced arthritis, CIA) dark agouti(DA) . Rat IL-4 cDNA upstream

downstream primers RT-PCR

(Table 1). cDNA 48

45 , PCR 94 30

, 60 1 , 68 2

35 . RT-PCR 1.0%

agarose gel rat IL-4

rat IL-4

1. cDNA RT-PCR ,

Pst I *Sac* I

. Rat IL-4 cDNA

pBluescript SK(+)

rat IL-4

pAd-Yc2

, rescue 293

DA rat Harlan Sprague-Dawley () . WKA-S1 Lewis-S1 10% fetal bovine serum(JBI ,), penicillin (100 units/mL) streptomycin(100 g/mL) , RPMI-1640 , 293 Earle's .

Table 1. Primers used in PCR

	Sequence
Rat IL-4	
Upstream (5')	CTATTGATGGGTCTCAGCCCCCACC
Downstream (3')	ACTCATAAGTTAGGACATGGAAGTG
Recombinant adenovirus	
Upstream (in CMV promoter)	GCAGAGCTCTCTGGCTAAC
Downstream (in BGHpA)	GGGAGTGGCACCTTCCAGG
E1A	
Upstream (5')	ATTACCGAAGAAATGGCCGC
Downstream (3')	CCCATTTAACACGCCATGCA

3. CIA

[18]. Type II collagen
 0.1M acetic acid 2 mg/mL, 4
 type II collagen
 Freund's incomplete adjuvant
 . CIA DA rat
 200 μ L 가
 . CIA가 rat 6
 3 . Type II collagen
 12 , rat
 10 μ L PBS, Adnull, AdrIL-4
 4 .
 Adnull AdrIL-4 4×10^8 pfu
 . 1, 2, 4, 8,
 12, 16 rat ,
 enzyme-linked immunosorbent
 assay(ELISA) IL-4

4. CIA 가

Type II collagen 12
 30 2

arthritic score . 0
 , 1 slight swelling and/or
 erythema , 2 erythema and mild
 swelling , 3 erythema and severe
 swelling , 4 joint rigidity with
 severe swelling
 score 0 , 16

5. ELISA

AdrIL-4

rat IL-4
 ELISA kit
 Coating buffer 0.1 M carbonate(pH 9.5)
 anti-rat IL-4 250 ELISA
 plate well 100 μ L 4
 . 0.05% Tween-20
 PBS plate 3 block
 10% FBS가 PBS 200
 μ L 1
 3 1 : 2
 100 μ L 2
 rat IL-4
 5 detection Ab avidin-HRP
 100 μ L well
 1 0.05%
 Tween-20 PBS 5
 O-nitrophenyl benzene
 50 μ L 3 N HCl
 490 nm optical density(OD)

1. AdrIL-4

T- WKA-S1
 PMA 3 RNA
 RNA RT-PCR rat IL-4
 cDNA IL-4
 (AdrIL-4)
 COS-1 rat IL-4 RT-PCR
 . Adnull
 AdrIL-4
 rat IL-4 가

(Fig. 1).

HeLa 10 moi
AdrIL-4
ELISA
IL-4 가
AdrIL-4 24, 48,
72 가
가 (Fig.
2).

2. rat IL-4 가
AdrIL-4
rat IL-4
1, 2, 4, 8, 12, 16

1 : 2 ELISA
ELISA 490 nm

OD IL-4
pg/mL 가 IL-4 300
47 pg/mL, 22 pg/mL 2, 4
8 (Fig. 3).

3. CIA type II collagen

CIA type II
collagen
7, 10, 12, 16, 20, 30
ELISA
10 가
, 12 가
가

(Fig. 4).

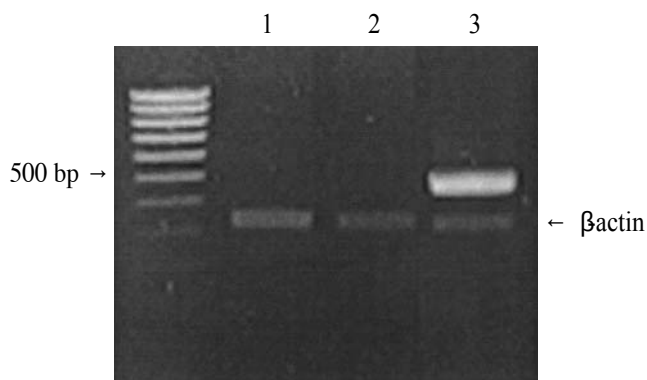


Fig. 1. Expression of rat IL-4 mRNA in COS-1 cells following recombinant adenovirus infection. Cells were infected with AdrIL-4 recombinant adenovirus at 100 moi for 4 hr in serum free medium. Cells were harvested at 24 hr after infection and RT-PCR was performed. 1: Sham-infected, 2: Adnull virus-infected, 3: AdrIL-4 virus-infected.

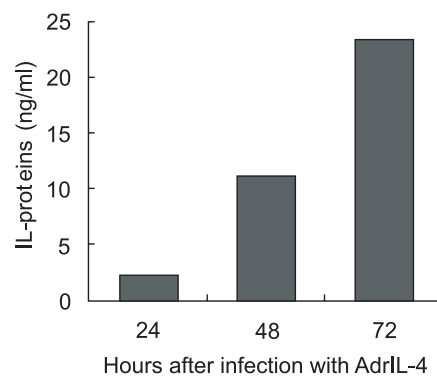


Fig. 2. Expression of rat IL-4 proteins in HeLa cells. Following adenovirus-mediated gene expression, HeLa cells were seeded on 12-well plates at 2×10^5 cells. Cells were infected with recombinant adenovirus at 10 moi and harvested at 24, 48 and 72 hr, respectively. IL-4 was measured by ELISA as described in Materials and Methods.

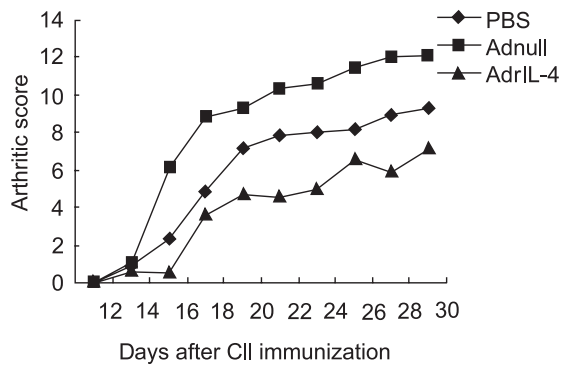


Fig. 5. Effects of rat IL-4 gene transfer on the severity of arthritis in DA rats. On day 12 after immunization with type II collagen, rats received the recombinant adenovirus encoding the rat IL-4 gene. Severity of arthritis in four paws was scored from 0 to 4 as described in Materials and Methods. Each data point represents the mean from six animals for each treatment group.

antagonist

[3,4]. IL-4, IL-10 cytokine
Th1 cytokine, TNF- α IL-1 β
soluble TNF- α receptor
soluble IL-1R type II cytokine
IL-10
, RA
[8,9,19]. IL-4
rheumatoid arthritis

IL-1 TNF- α
[20].
CIA PBS Adnull
AdrIL-4
AdrIL-4
가 가
가
AdrIL-4 IL-4 가
300 pg/mL
IL-4
IL-4
rheumatoid arthritis
CIA
rheumatoid arthritis
가
가
cytokine TNF- α IL-1
cytokine soluble TNF- α associated virus
receptor, TNF IL-1R 가

IL-4
cytokines
cytokines .
(CIA) IL-4
(AdrIL-4)
4)
IL-4 cDNA PMA T lymphoma
shuttle
vector . IL-4
, ADRIL-4 shuttle
vector rescue vector pJM17
(homologous recombination)
PCR . CIA
DA rats 2
12 PBS, Adnull,
ADRIL-4 (4×10^8 pfu) DA rats
30
IL-4
arthritic score .
IL-4
ADRIL-4 가
, 8
Adnull 가 , ADRIL-4
가
가 .

1. Arend WP, Dayer JM. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor alpha in rheumatoid arthritis. *Arthritis Rheum* 1995;**38**:151-60.

2. Koch AE, Kunkel SL, Strieter RM. Cytokines in rheumatoid arthritis. *J Invest Med* 1995;**43**:28-38.
3. Joosten LA, Helsen MM, van de Loo FA, van den Berg WB. Anticytokine treatment of established type II collagen-induced arthritis in DBA/1 mice. A comparative study using anti-TNF alpha, anti-IL-1 alpha/beta, and IL-1R α . *Arthritis Rheum* 1996;**39**:797-809.
4. Williams RO, Feldmann M, Maini RN. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc Natl Acad Sci USA* 1992;**89**:9784-8.
5. Calabrese LH. Molecular differences in anticytokine therapies. *Clin Exp Rheumatol* 2003;**21**:241-8.
6. Katsikis PD, Chu CQ, Brennan FM, Maini RN, Feldmann M. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. *J Exp Med* 1994;**179**:1517-27.
7. Chomarat P, Vannier E, Dechanet J, Rissoan MC, Banchereau J, Dinarello CA, *et al.* Balance of IL-1 receptor antagonist/IL-1 beta in rheumatoid synovium and its regulation by IL-4 and IL-10. *J Immunol* 1995;**154**:1432-29.
8. Doncarli A, Stasiuk LM, Fournier C, Abehsira-Amar O. Conversion in vivo from an early dominant Th0/Th1 response to a Th2 phenotype during the development of collagen-induced arthritis. *Eur J Immunol* 1997;**27**:1451-8.
9. Del Prete G, De Carli M, Almerigogna F, Giudiz MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* 1993;**150**:353-60.
10. Walmsley M, Katsikis PD, Abney E, Parry S, Williams RO, Maini RN, *et al.* Interleukin-10 inhibition of the progression of established collagen-induced arthritis. *Arthritis Rheum* 1996;**39**:495-503.

11. Miossec P, Naviliat M, Dupuy d'Angeac A, Sany J, Banchereau J. Low levels of interleukin-4 and high levels of transforming growth factor beta in rheumatoid synovitis. *Arthritis Rheum* 1990;**33**:1180-7.
12. Hart PA, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA. Potential anti-inflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor, interleukin 1, and prostaglandin E2. *Proc Nat Acad Sci USA* 1989;**86**:3803-7.
13. Cope AP, Gibbons DL, Aderka D. Differential regulation of tumor necrosis factor receptors (TNF-R) by IL-4: upregulation of p55 and p75 TNF-R on synovial joint mononuclear cells. *Cytokine* 1993;**3**:205-8.
14. Vannier E, Miller LC, Dinarello CA. Coordinated anti-inflammatory effects of interleukin-4: IL-4 suppresses IL-1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist. *Proc Natl Acad Sci USA* 1992;**89**:4076-80.
15. Missol EA, Sochanik, Szala S. Introduction of murine IL-4 gene into B16 (F10) melanoma tumors by direct gene transfer with DNA-liposome complexes. *Cancer Lett* 1995;**97**:189-93.
16. Kozarsky KF, Wilxon JM. Gene therapy: adenovirus vectors. *Curr Opin Genet Dev* 1993;**3**:499-503.
17. Sigalla J, David A, Anegon I, Fiche M, Huvelin JM, Boeffard F, *et al.* Adenovirus-mediated gene transfer into isolated mouse adult pancreatic islets: normal beta-cell function despite induction of an anti-adenovirus immune response. *Hum Gene Ther* 1997;**8**:1625-34.
18. Breedveld FC, Trentham DE. Progress in the understanding of inducible models of chronic arthritis. *Rheum Dis Clin N Am* 1987;**13**:531-44.
19. Taylor PC. Anti-cytokines and cytokines in the treatment of rheumatoid arthritis. *Curr Pharm Des* 2003;**9**:1095-106.
20. Wood JM, Tokushira M, Berry JC, Katschke KJ, Kurata H, Arai K, *et al.* Interleukin-4 adenoviral gene therapy reduces production of inflammatory cytokines and prostaglandin E2 by rheumatoid arthritis synovium ex vivo. *J Invest Med* 1999;**47**:285-92.