# 인 사 말

안녕하십니까?

서울대학교 시스템면역의학연구소의 개소 4주년을 기념하여 개최하는 제5회 국제학술대회에 참석하여 주신 여러분께 깊은 감사의 말씀을 전합니다. 또한 강의를 수락하여 주신 연자분들께도 특별한 감사의 말씀을 드립니다.

서울대학교 시스템면역의학연구소는 종양 및 다양한 면역 질환의 기전 및 치료법에 대한 연구를 수행하고 있습니다. 또한 의생명과학 분야 여러 핵심 기술들을 지원하는 Lab on a Cloud를 구축하여 현재까지 100여 건의 공동협력 연구를 진행하였으며 이에 따른 우수한 성과가 나오고 있습니다. 이처럼 활발한 협력 연구를 통해 건강한 미래 사회를 구현하기 위해 최선의 노력을 다하겠습니다. 앞으로도 아낌없는 격려와 관심 부탁드립니다.

이번 국제학술대회에서는 아토피 피부염 연구를 선도하는 세계 각국의 연구자를 초청하여 분자 수준에서부터 임상에서의 진단과 치료에의 적용까지의 최신 연구를 소개하는 자리를 마련하였습니다. 본 학회를 통해서 참석자분들의 학문 증진 및 상호 교류를 유도하여 연구의 결실이 보다 풍성하게 무르익을 수 있기를 기원합니다.

제5회 국제학술대회가 성공적으로 마무리 될 수 있도록 여러분의 적극적인 성원을 부탁드리며 다시 한 번 감사의 말씀을 드립니다.

**서울대학교 시스템면역의학연구소장 성 승 용**

# 제5회 서울대학교 시스템면역의학연구소 국제학술대회

## The 5th International Symposium of Wide River Institute of Immunology
## Immunological and Clinical Aspects of Atopic Dermatitis

### Scientific Program

12:30~13:30 Registration

13:30~13:40 Opening Remark

Seung-Yong Seong (Seoul National University, Korea)

Chair: Kyu Han Kim (Seoul National University, Korea)

13:40~14:20 **IL-17A Producing ILC3s Elicits Type 2 Immune Responses to Promote Skin Inflammation**

Hye Young Kim (Seoul National University, Korea)

14:20~15:00 **Clinician Perspectives on Current Issues in Atopic Dermatitis in Korea**

Chong Hyun Won (Asan Medical Center, Korea)

15:00~15:40 **Live Imaging of Skin Immune Responses**

Kenji Kabashima (Kyoto University, Japan)

15:40~16:00 Break

Chair: Kenji Kabashima (Kyoto University, Japan)

16:00~16:40 **Roles of TRPV Channels in Keratinocytes in Itching Sensation**

Hye One Kim (Hallym University, Korea)

16:40~17:20 **Promises and Challenges of Improving Biomarkers for Atopic Dermatitis**

DirkJan Hijnen (Erasmus Medical Center, The Netherlands)

17:20~18:00 **Skin-resident Natural Killer T Cells Develop Cutaneous Allergic Inflammation in Atopic Dermatitis**

Chang Ook Park (Yonsei University, Korea)

18:00 Closing

# IL-17A Producing ILC3s Elicits Type 2 Immune Responses to Promote Skin Inflammation

**Hye Young Kim**
Seoul National University, Korea

Atopic dermatitis (AD) is a chronic inflammatory skin disorder known to be mediated by $T_H2$ cytokines. However, recent studies have shown that IL-17A may play a role in development of AD, although the mechanisms are unclear. It has become evident that innate lymphoid cells (ILCs) play a critical role in skin homeostasis and inflammation. This study was aimed to investigate the role of ILC3s which are relatively unexplored in the pathogenesis of AD. The numbers of ILC3s as well as ILC2s were increased in the blood from AD patients. The skin of AD-induced mice by house dust mite harbored increased numbers of IL-17A-producing ILC3s. Neutralizing IL-17A delayed AD development and adoptive transfer of ILC3s to the skin accelerated it. Finally, co-culture of human ILC3s with keratinocytes and fibroblasts induced IL-33 which is important for $T_H2$ cytokine production. Therefore, we propose that ILC3-IL-17A-IL-33 axis could be a potential target for AD.

Keywords : Innate lymphoid cell, ILC3, Atopic dermatitis, NC/Nga, House dust mite

# Curriculum Vitae

**Hye Young Kim, PhD**
Associate Professor, Department of Medical Science,
Seoul National University College of Medicine and Hospital
103 Daehak-ro, Jongno-gu, Seoul 03080, Korea
E-mail: hykim11@snu.ac.kr

**Education and Appointment**

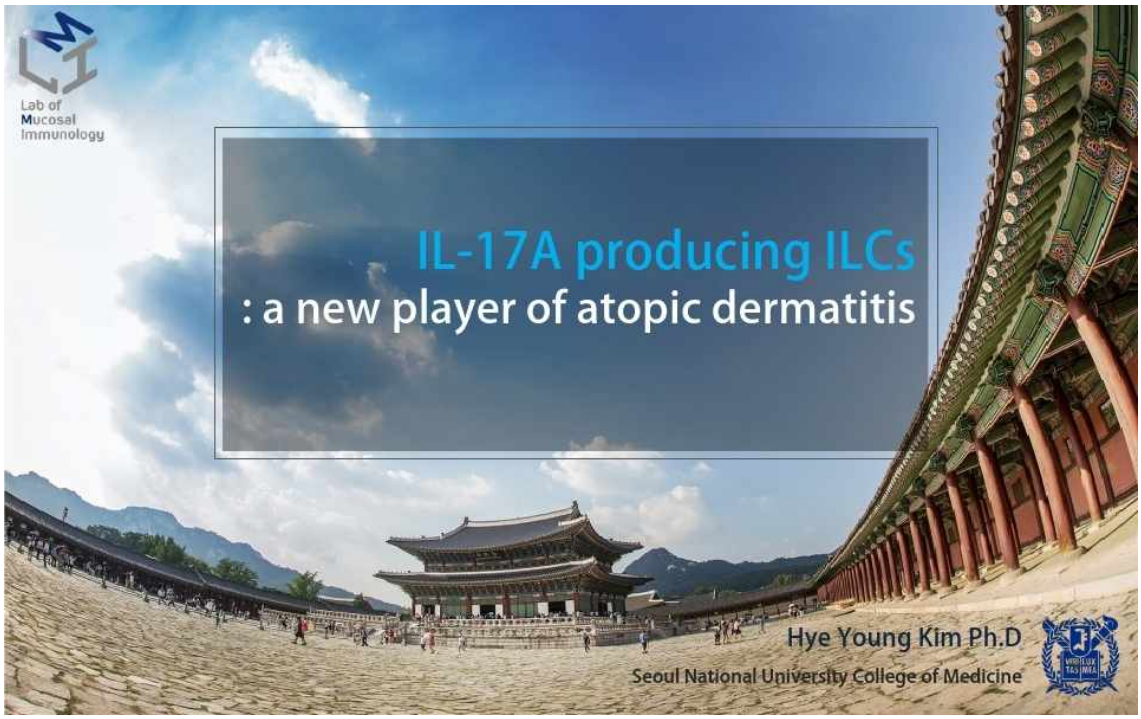
1997-2001 BS in Biology, Ewha Womans University
2001-2003 MS in Genetics, Seoul National University
2003-2006 PhD in Immunology, Seoul National University College of Medicine

**Professional Training and Employment**

2006-2011 Research fellow, Boston Children's Hospital/Harvard Medical School
2011-2013 Instructor (Research associate) Boston Children's Hospital/Harvard Medical School
2014-present Associate professor, Seoul National University College of Medicine, Department of Medical Science

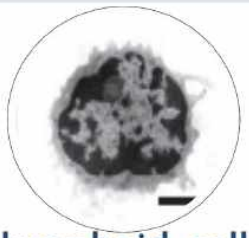
**Selected Publications**

1. IL-17 producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. *Nat Med, 20, 54–61, 2014* (first-author) **Faculty of 1000PRIME*
2. Invariant NKT cells recognized fungal glycophospholipid that can induce airway hyperreactivity. *Nat Med. 2013 Oct;19(10):1297-1304* (first-author)
3. T cell immunoglobuline and mucine domain 1 deficiency eliminate airway hyperreactivity triggered by the recognition of airway cell death. *J Allergy Clin Immunol. 2013 Aug;132(2):414-425.e6* (first-author)
4. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature. 2012 Feb 8;482(7385):395-9.* (co-author)
5. Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity. *J Allergy Clin Immunol. 2012 Jan;129(1):216-27.e1-6.* (first-author) ***** *Editor's Choice in the same issue of JACI*
6. Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity. *Nat Immunol. 2011 May 29;12(7):631-8.* (first-author) **Faculty of 1000, Editor's choice in Science (10, June 2011), News and views in Nature Immunol (12, 587) and J Allergy Clin Immunol(2011)*
7. A polymorphism in TIM1 is associated with susceptibility to severe hepatitis A virus infection in humans. *J Clin Invest. 2011 Mar;121(3):1111-8.*(first-author) **Commentary in the same issue of J.Clin Invest., Highlighted in Nature/SciBX (17 March 2011), Research Highlight in Nature Reviews Gastroenterology & Hepatology (4 May 2011)*




## Innate lymphoid cells





### Innate lymphoid cells (ILCs)

A group of Innate immune cells that belong to the lymphoid lineage, but do not respond in an antigen-specific manner, as they lack a B or T cell receptor

## Beyond T cells

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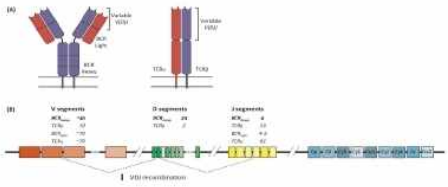
1. T cells are not the only source of cytokines

2. Inflammation/immune response can occur without adaptive immunity



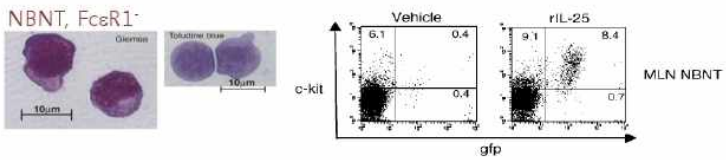
Published April 10, 2006
JEM
ARTICLE

Lab of Mucosal Immunology

# Identification of an interleukin (IL)-25–dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion

Padraic G. Fallon,[1] Sarah J. Ballantyne,[2] Niamh E. Mangan,[1] Jillian L. Barlow,[2] Ayan Dasvarma,[2] Duncan R. Hewett,[2] Ann McIlgorm,[2] Helen E. Jolin,[2] and Andrew N.J. McKenzie[2]

[1]Department of Biochemistry, Trinity College, Dublin 2, Ireland
[2]Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, England, UK



## Lin⁻c-Kit⁺Sca-1⁺ cells(Natural helper cells) in FALCs.

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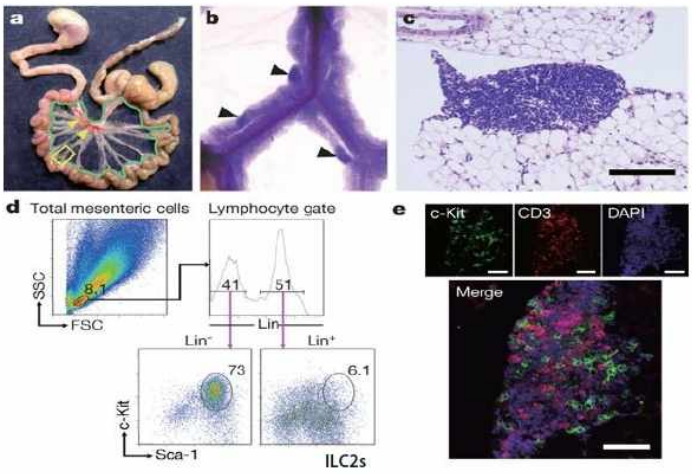


Moro et al, Nature 2010

nature



## The first identification of ILCs

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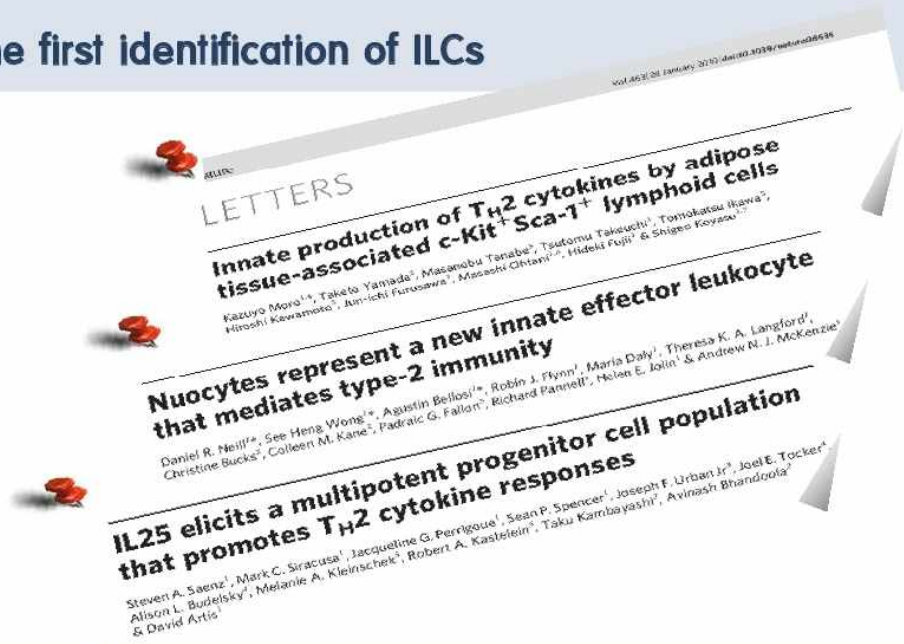


nature

LETTERS

**Innate production of $T_H2$ cytokines by adipose tissue-associated c-Kit⁺Sca-1⁺ lymphoid cells**

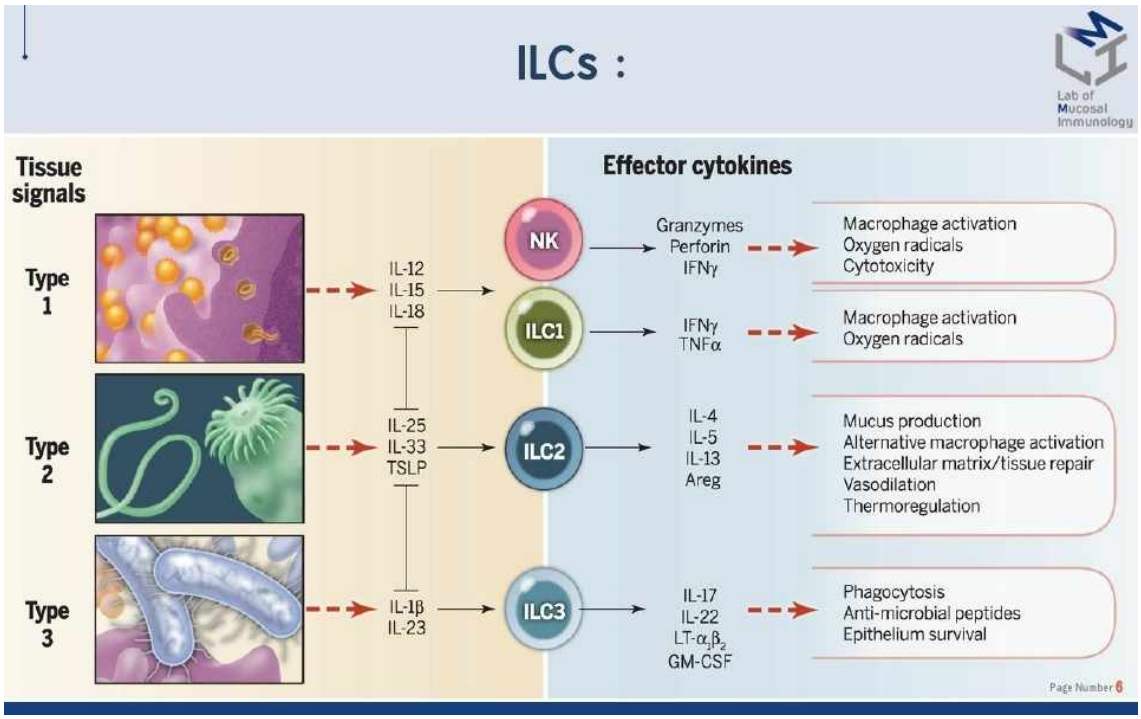
Kazuyo Moro[1,*], Taketo Yamada[2], Masamitsu Tanabe[2], Tsutomu Takeuchi[2], Tomokatsu Ikawa[3], Hiroshi Kawamoto[3], Jun-ichi Furusawa[4], Masaaki Ohtani[1,5], Hideki Fujii[2] & Shigeo Koyasu[1,*]

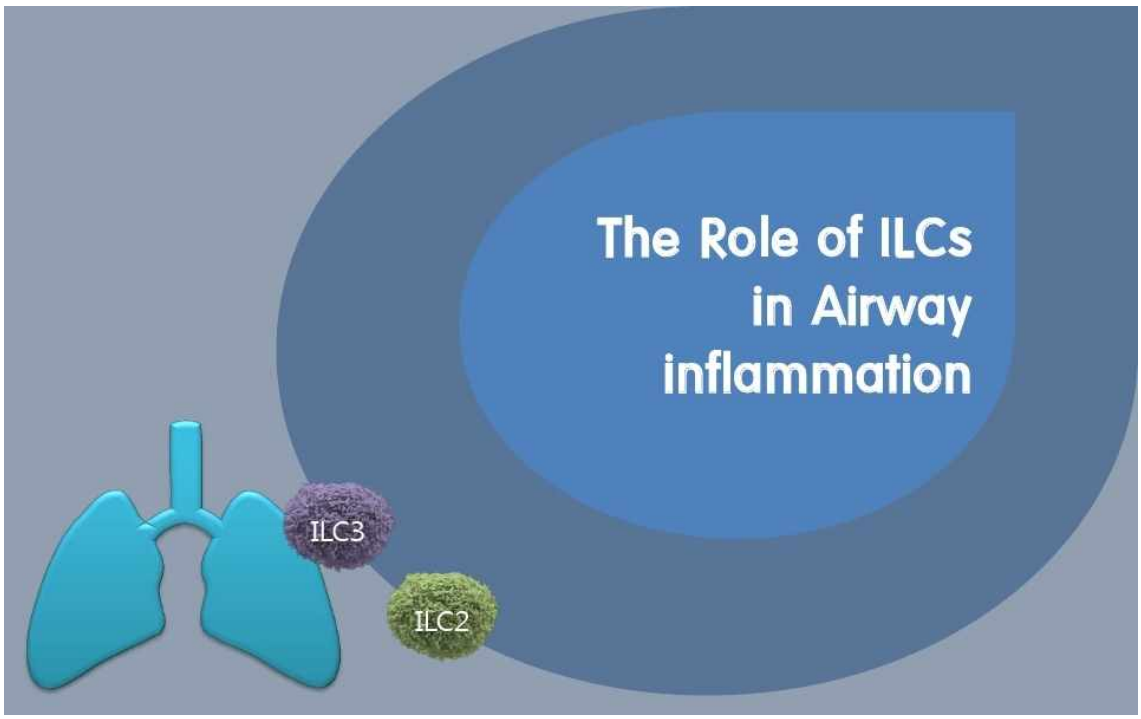
**Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity**

Daniel R. Neill[1,*], See Heng Wong[1,*], Agustin Bellosi[1,*], Robin J. Flynn[1], Maria Daly[1], Theresa K. A. Langford[1], Christine Bucks[2], Colleen M. Kane[2], Padraic G. Fallon[3], Richard Pannell[4], Helen E. Jolin[1] & Andrew N. J. McKenzie[1]

**IL25 elicits a multipotent progenitor cell population that promotes $T_H2$ cytokine responses**

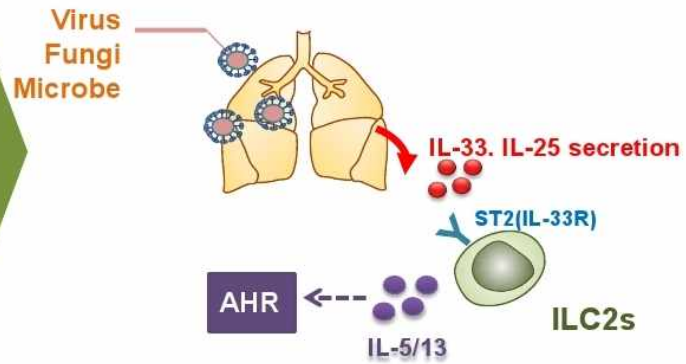
Steven A. Saenz[1], Mark C. Siracusa[1], Jacqueline G. Perrigoue[1], Sean P. Spencer[1], Joseph F. Urban Jr[2], Joel E. Tocker[1], Alison L. Budelsky[1], Melanie A. Kleinschek[3], Robert A. Kastelein[3], Taku Kambayashi[1], Avinash Bhandoola[1] & David Artis[1]


ILCs :
Lab of Mucosal Immunology
Tissue signals
Effector cytokines
Type 1
Type 2
Type 3
IL-12
IL-15
IL-18
IL-25
IL-33
TSLP
IL-1β
IL-23
NK
ILC1
ILC2
ILC3
Granzymes
Perforin
IFNγ
IFNγ
TNFα
IL-4
IL-5
IL-13
Areg
IL-17
IL-22
LT-α1β2
GM-CSF
Macrophage activation
Oxygen radicals
Cytotoxicity
Macrophage activation
Oxygen radicals
Mucus production
Alternative macrophage activation
Extracellular matrix/tissue repair
Vasodilation
Thermoregulation
Phagocytosis
Anti-microbial peptides
Epithelium survival
Page Number 6

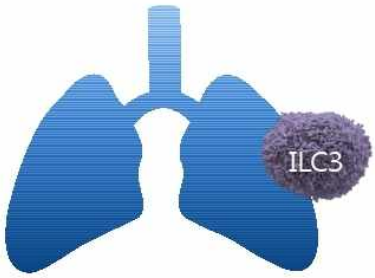

The Role of ILCs in Airway inflammation
ILC3
ILC2

## ILC2s in various forms of Asthma

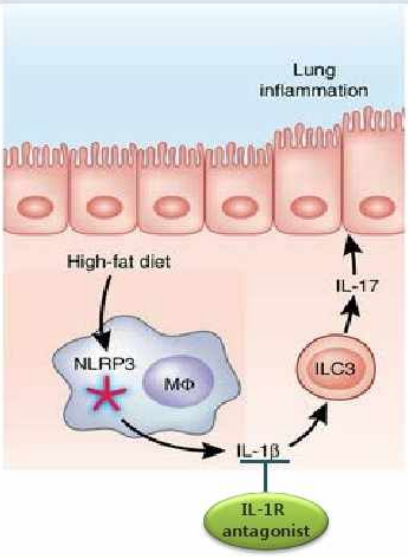
- ILC2 participate in asthma induced by
  - **Virus** (Chang and Kim. Nature Immunol 2011)
  - **Glycolipid** (Kim HY. JACI 2012)
  - **Protease** (Halim TYF. Immunity 2012)
  - **Fungi** (Lee and Kim HY, Nat med 2013, Bartemes, JI, 2012)
  - **LTD4** (Doherty, JACI, 2013)

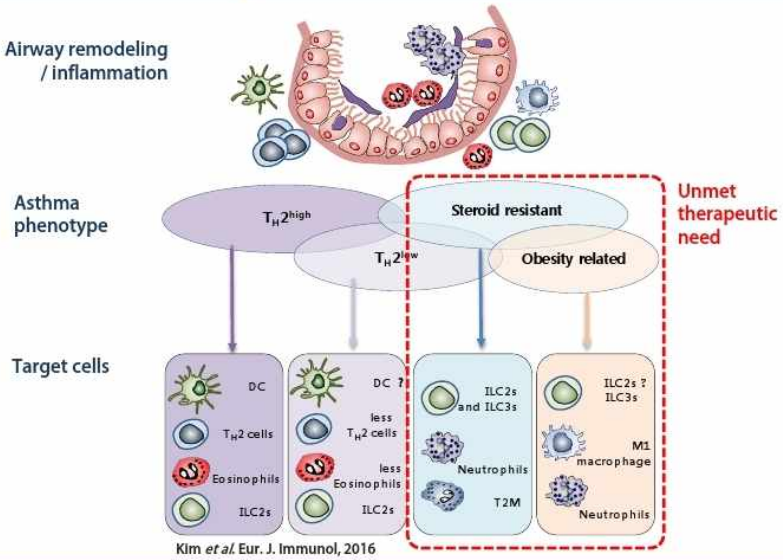

## ILC3s in Obesity induced Asthma




Kim *et al.* Nat. Med, 2014



## Asthma is an heterogeneous disease.



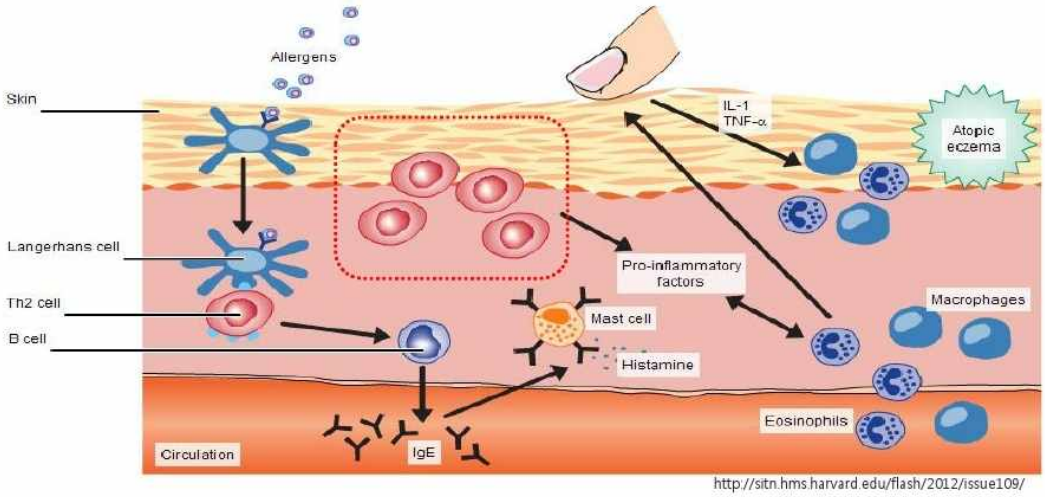

Kim *et al.* Eur. J. Immunol, 2016

## Atopic dermatitis

15~20% of children, 2~9% of adult
Chronic, relapsing inflammatory disease
Itchy, red, swollen, cracked skin(scaly), dry
Cause is not known.
Genetics, Environment, Immune system

- Thomas Bieber (2008) NEJM

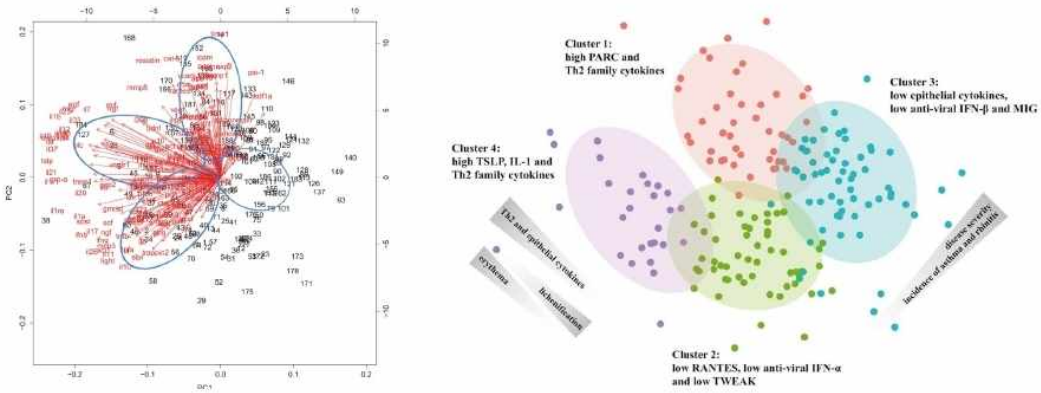
# Immune responses in the skin




http://sitn.hms.harvard.edu/flash/2012/issue109/

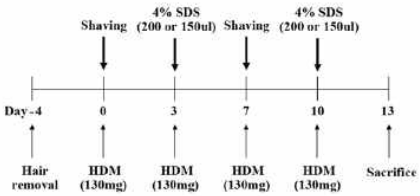


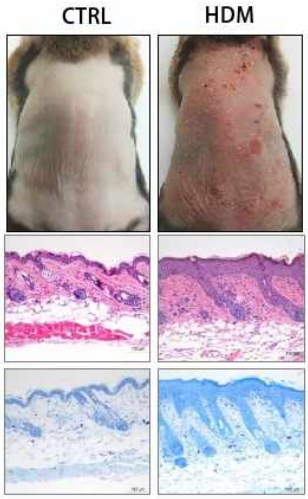
# Moving toward endotypes in atopic dermatitis:

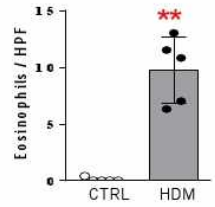


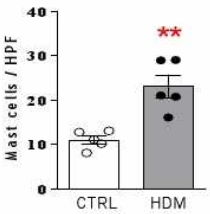

PCA and unsupervised cluster analysis reveals 4 clusters of patients with AD

# HDM-induced Atopic Dermatitis in NC/Nga mouse



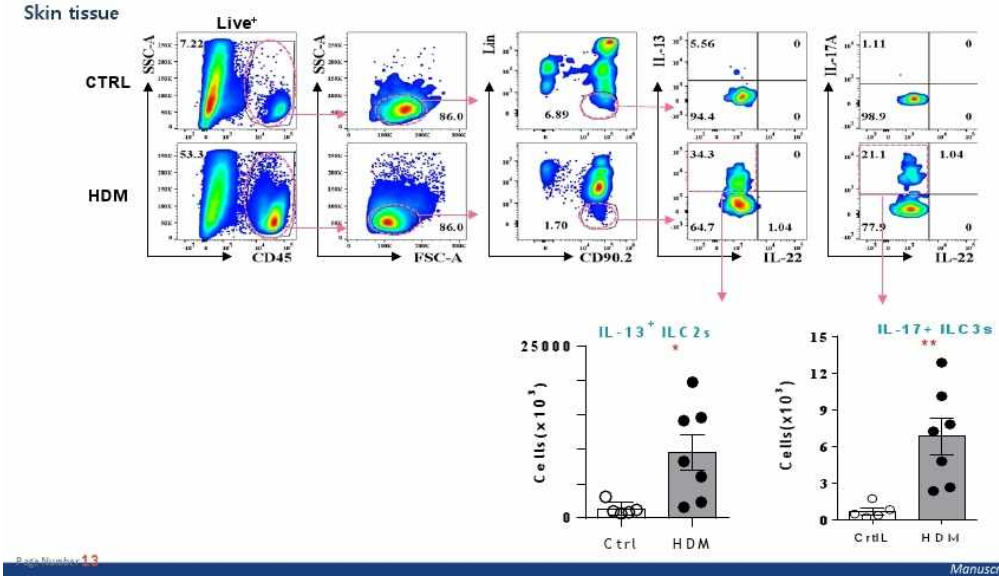



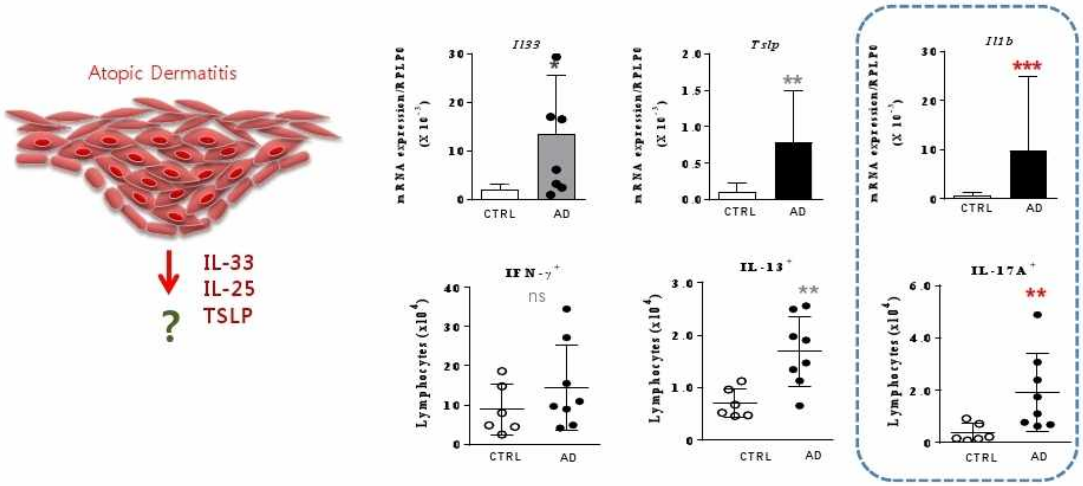

Manuscript in preparation

# IL-17 producing cells are increased in the skin of AD mice



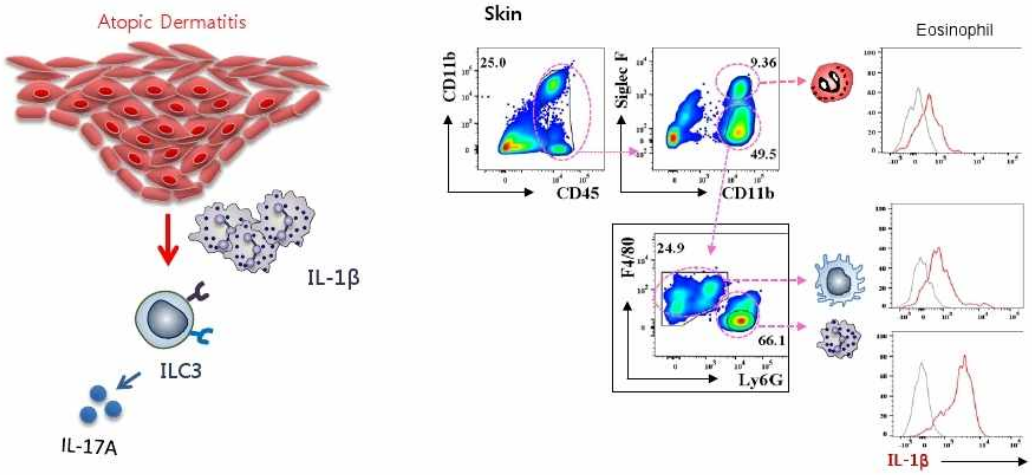

Manuscript in preparation

## Innate cytokines are increased in AD skin




Manuscript in preparation

## Neutrophils are the main producer of IL-1 β in the skin




Manuscript in preparation

# The role of IL-17A in Atopic Dermatitis

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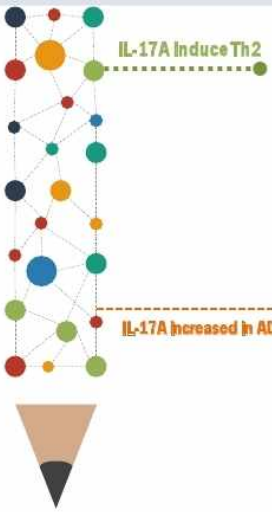
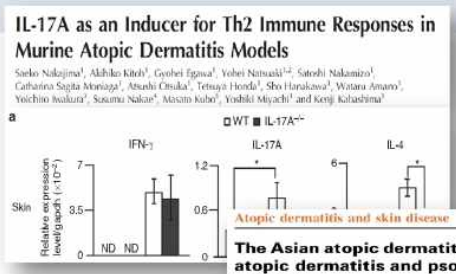


IL-17A as an Inducer for Th2 Immune Responses in Murine Atopic Dermatitis Models

Saeko Nakajima[1], Akihiko Kitoh[1], Gyohei Egawa[1], Yohei Natsuaki[1,2], Satoshi Nakamizo[1], Catharina Sagita Moniaga[1], Atsushi Otsuka[1], Tetsuya Honda[1], Sho Horakawa[1], Wataru Amano[1], Yoichiro Iwakura[3], Susumu Nakae[4], Masami Kubo[5], Yoshiki Miyachi[1] and Kenji Kabashima[1]

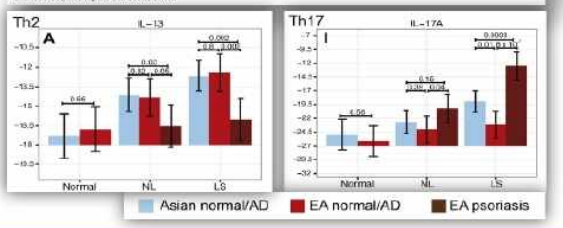



Atopic dermatitis and skin disease

The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased $T_H17$ polarization

Shinji Noda, MD, PhD,[a] Mayte Suárez-Fariñas, PhD,[a,b,c,d] Benjamin Ungar, BA,[a,b] Soo Jung Kim, MD, PhD,[b] Cristina de Guzman Strong, PhD,[a] Hui Xu, MSc,[b] Xiangyu Peng, MSc,[b] Yeriel D. Estrada, BSc,[a] Saeko Nakajima, MD, PhD,[f] Tetsuya Honda, MD, PhD,[f] Jung U. Shin, MD,[g] Hemin Lee, MD,[g] James G. Krueger, MD, PhD,[a] Kwang-Hoon Lee, MD, PhD,[g] Kenji Kabashima, MD, PhD,[f] and Emma Guttman-Yassky, MD, PhD[a,b,h]

New York, NY; St Louis, Mo; Kyoto, Japan; and Seoul, Korea

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# Working hypothesis

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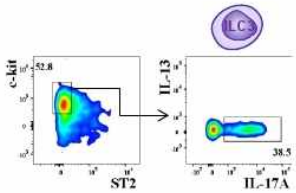
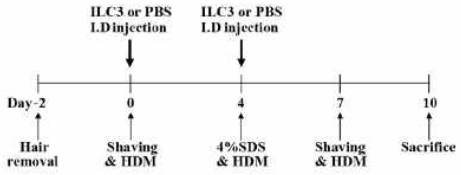
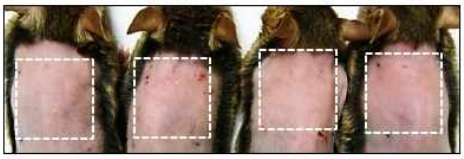
# Adoptive transfer of ILC3s induces the development of AD

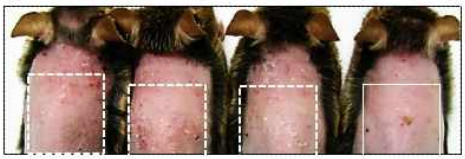



PBS +HDM

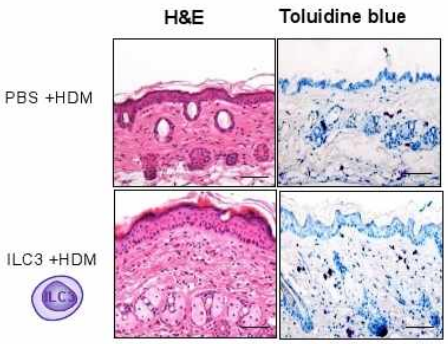


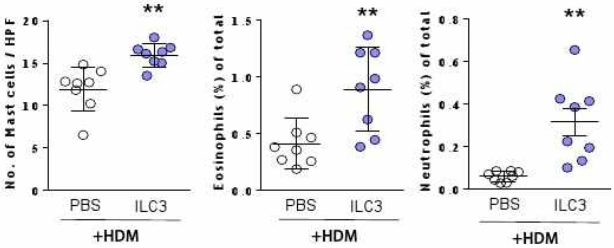
ILC3 +HDM



Manuscript in preparation


# Adoptive transfer of ILC3s induces the development of AD

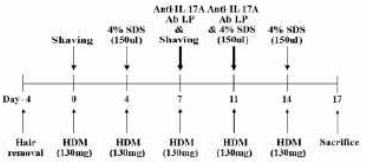



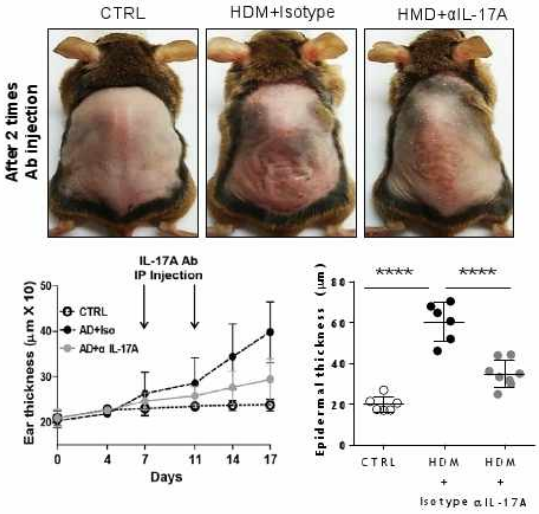





Manuscript in preparation

# Neutralizing of IL-17A delays the development of AD

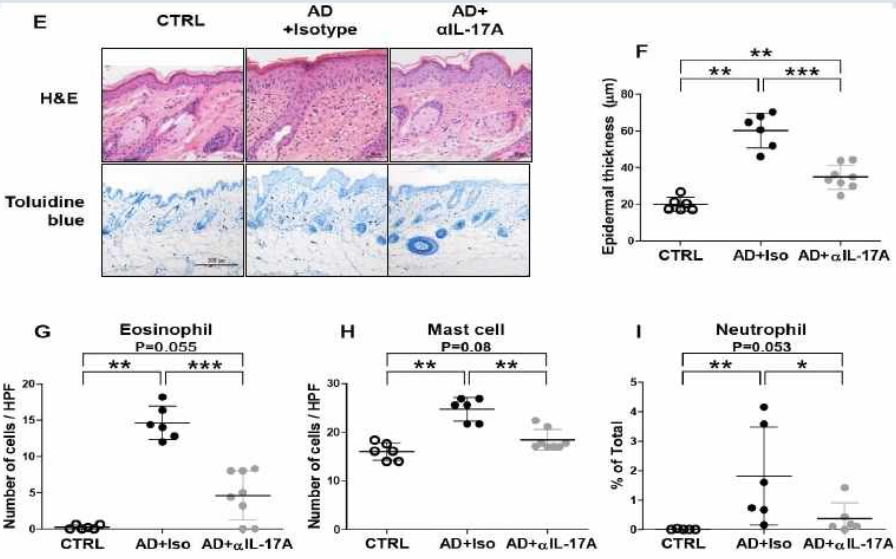



Manuscript in preparation

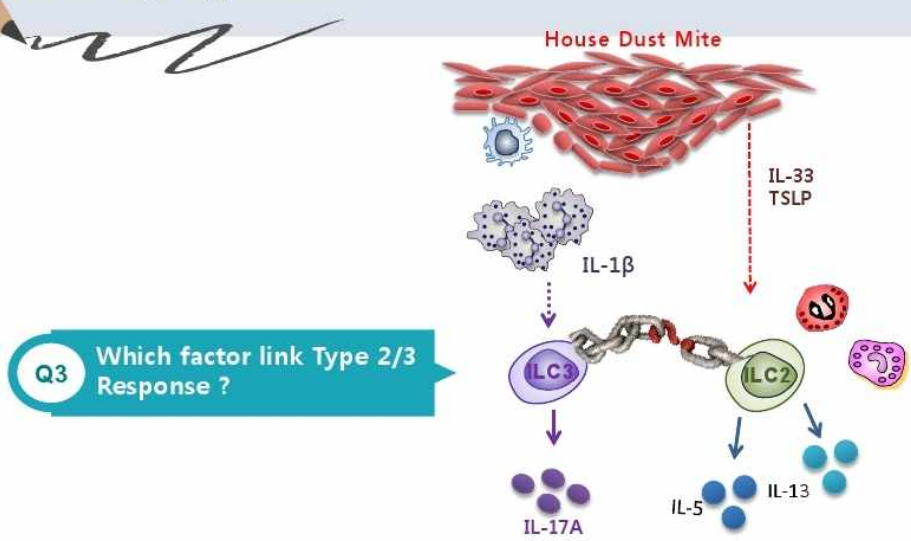
# Neutralizing of IL-17A delays the development of AD

Result 5.




Manuscript in preparation

## Working hypothesis




**Q3** Which factor link Type 2/3 Response ?

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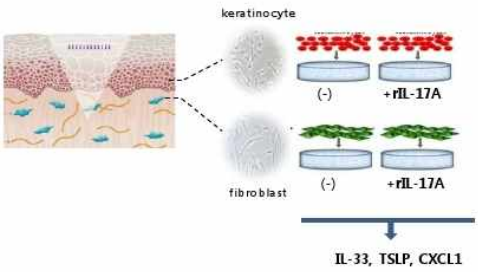
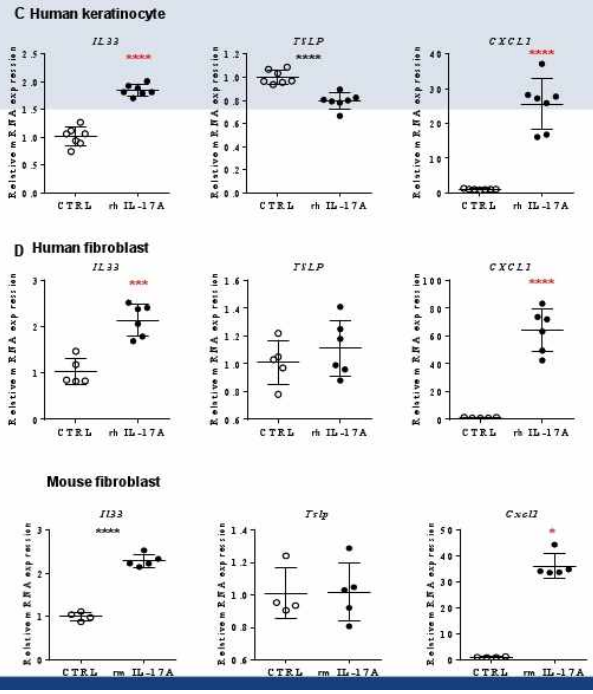
## IL-17A induces innate effector cytokines from epithelial cells




IL-33, TSLP, CXCL1

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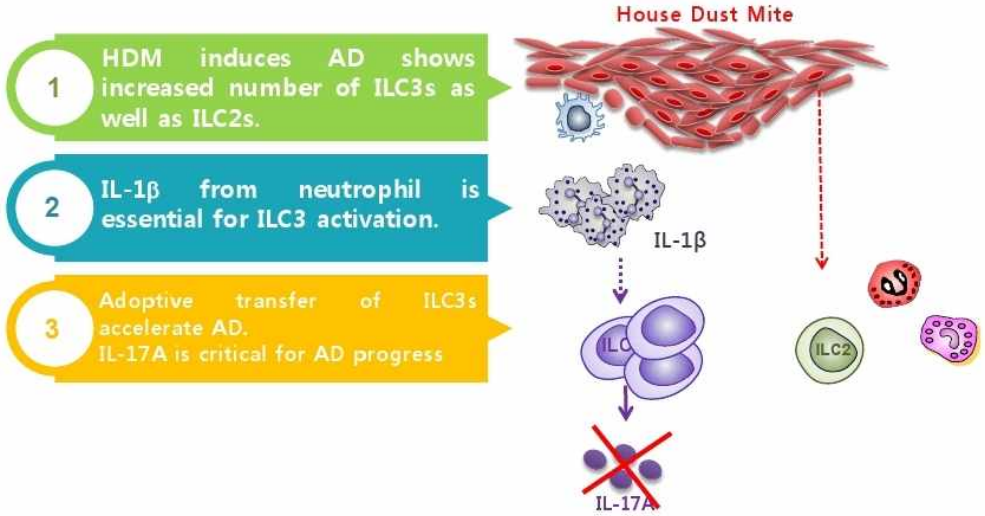
# Conclusions

IL-17A from ILC3s orchestrate skin inflammation

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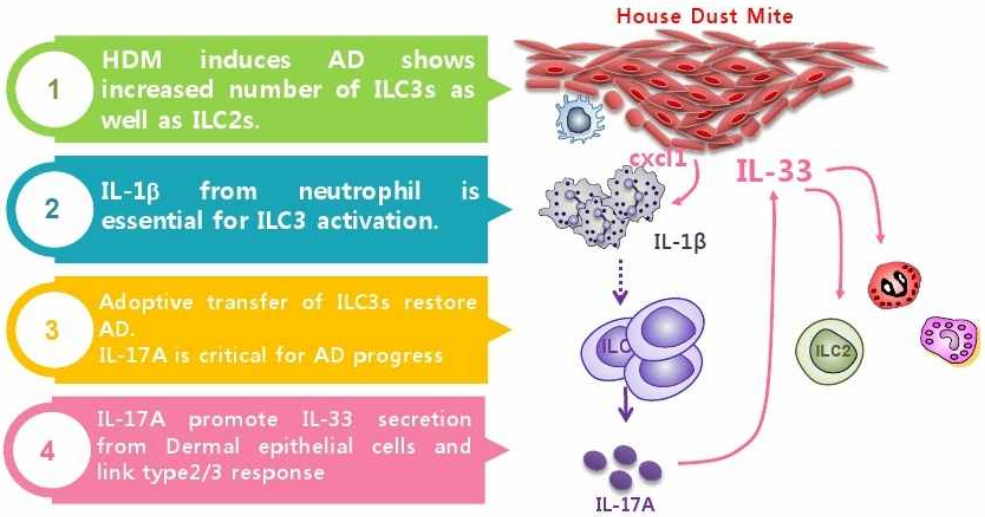
# Conclusions

IL-17A from ILC3s orchestrate skin inflammation




Page Number 22

# Acknowledgement

Minho Kim

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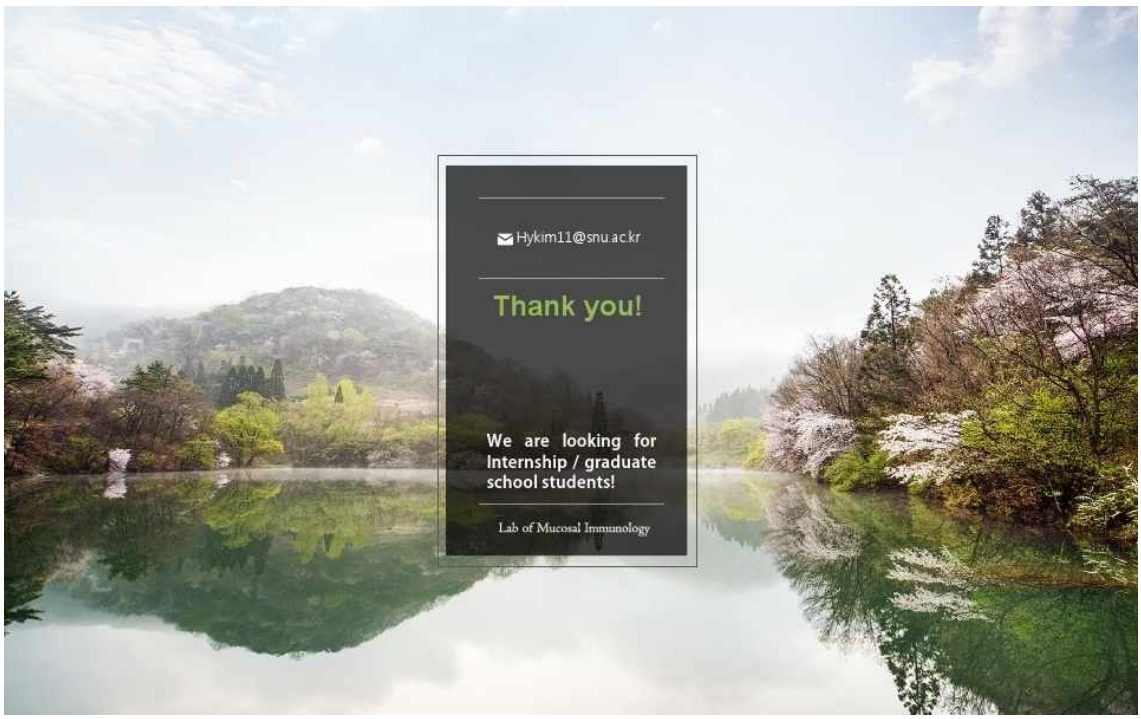
Lab of
Mucosal
Immunology

Pf. Kyuhan Kim
Pf. Donghoon Lee

Seoul National University
College of Medicine and Hospital

# Clinician Perspectives on Current Issues in Atopic Dermatitis in Korea

**Chong Hyun Won**
Asan Medical Center, Korea

Atopic dermatitis (AD) is a common chronic relapsing inflammatory skin disease and impacts substantially the quality of life of the patients. In this talk, I would like to review recently published Korean literature on AD. There are several issues raised in the current management of AD, which include hyperpigmentation in AD patients, epidemiological study, and adherence issues related with topical maintenance therapy. Several treatment modalities based on SNS communication can be used to improve an adherence and provide more adequate management of skin lesions.

In the era of biologics, still topical therapy will be the mainstay of AD. Even with a revolutionary emerging drug in atopic dermatitis, Dupilumab, study groups with topical agents showed better efficacy with Dupilumab. Atopic dermatitis is characterized by its intense itch, recurrent skin lesions and a fluctuating course. Given that chronic use of medication is needed for the control of this life long and uncomfortable disease of AD, other treatment options besides immune-suppression are required.

My research focuses on plasma medicine and recent clinical trials with TRPV antagonist. Medical device using low temperature and pressure plasma has proven its antibacterial effects, anti- inflammatory and enhanced wound healing ability. Plasma medicine lessens the signs and symptoms in murine AD model induce by house dust mite.

TRPV antagonist has been shown to significantly regulate the severity of inflammation of AD and the degree of pruritus in *in vitro* and *in vivo* studies. We performed clinical trials with TRPV antagonist through phase 2 and phase 3 studies. Clinical efficacy of TRPV antagonist was observed in these trials.

These studies suggest immunosuppressive agents including steroid can be used sparingly together with new treatment modalities on atopic dermatitis. Furthermore, these approaches will help to treat active disease, maintenance or prevention of flare with more optimal management of AD skin by topical therapies.

# Curriculum Vitae

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**Education and Appointment**

1991-1997 MD in Medicine, Seoul National University College of Medicine
2002-2005 MS in Medicine, Seoul National University College of Medicine
2005-2007 PhD in Medicine, Seoul National University College of Medicine

**Professional Training and Employment**

2001-2005 Residency in Dermatology, Seoul National University Hospital
2005-2006 Fellowship in Dermatology, Seoul National University Hospital
2006-2009 Assistant Professor, SMG-SNU Borame Medical Center
2009-2014 Assistant Professor in Dermatology, Ulsan University College of Medicine, Asan Medical Center
2014-present Associate Professor in Dermatology, Ulsan University College of Medicine, Asan Medical Center

**Selected Publications**

1. **Won CH**, Park GH, Wu X, Tran TN, Park KY, Park BS, Kim DY, Kwon O, Kim KH. The Basic Mechanism of Hair Growth Stimulation by Adipose-derived Stem Cells and Their Secretory Factors. *Curr Stem Cell Res Ther*. 2017;**12**(7):535-543.
2. Shin H, **Won CH**, Chung WK, Park BS. Up-to-date Clinical Trials of Hair Regeneration Using Conditioned Media of Adipose-Derived Stem Cells in Male and Female Pattern Hair Loss. *Curr Stem Cell Res Ther*. 2017;**12**(7):524-530.
3. Kim YJ, Moon IJ, Lee WJ, Chang SE, Lee MW, Choi JH, **Won CH**. Case of myoepithelial carcinoma on scalp. *J Dermatol*. 2018 Mar;**45**(3):375-376.
4. Lee WJ, Kang HJ, **Won CH**, Chang SE, Choi JH, Lee MW. Cutaneous Extranodal Natural Killer/T-Cell Lymphomas Histopathologically Mimicking Benign Inflammatory Disease. *Am J Dermatopathol*. 2017 Mar;**39**(3):171-176.
5. Lee WJ, Lee SH, Moon IJ, **Won CH**, Chang SE, Choi JH, Park CS, Suh C, Lee MW. Relative Frequency, Clinical Features, and Survival Outcomes of 395 Patients with Cutaneous Lymphoma in Korea: A Subgroup Analysis per 10-year Period. *Acta Derm Venereol*. 2016 Nov 2;**96**(7):888-893.
6. Lee WJ, Lee YJ, Lee MH, **Won CH**, Chang SE, Choi JH, Lee MW. Prognosis of 234 rosacea patients according to clinical subtype: The significance of central facial erythema in the prognosis of rosacea. *J Dermatol*. 2016 May;**43**(5):526-31.
7. Chung BY, Jung JM, Lee YJ, **Won CH**, Lee MW, Cho JH, Chang SE. Acquired bilateral telangiectatic macularis eruptiva perstans on the arms of Asian men: A common but unrecognized disorder. *J Dermatol*. 2015 Nov;**42**(11):1116-8.
8. Lee WJ, Lee YJ, **Won CH**, Chang SE, Choi JH, Lee MW. Clinical evaluation of 30 patients with localized nasal rosacea. *J Dermatol*. 2016 Feb;**43**(2):200-2.
9. Lee WJ, Rhee do Y, Bang SH, Kim SY, **Won CH**, Lee MW, Choi JH, Chang SE. The natural yeast extract isolated by ethanol precipitation inhibits melanin synthesis by modulating tyrosinase activity and downregulating melanosome transfer. *Biosci Biotechnol Biochem*. 2015;**79**(9):1504-11.
10. **Won CH**, Jeong YM, Kang S, Koo TS, Park SH, Park KY, Sung YK, Sung JH. Hair-growth-promoting

effect of conditioned medium of high integrin α6 and low CD 71 (α6bri/CD71dim) positive keratinocyte cells. *Int J Mol Sci*. 2015 Feb 19;**16**(3):4379-91.

11. Lee WJ, Jung JM, Won KH, **Won CH**, Chang SE, Choi JH, Moon KC, Lee MW. Clinical evaluation of 368 patients with nasal rosacea: subtype classification and grading of nasal rosacea. *Dermatology*. 2015;**230**(2):177-83.
12. Lee WJ, Won KH, **Won CH**, Chang SE, Choi JH, Moon KC, Park CS, Huh J, Suh C, Lee MW. Secondary cutaneous lymphoma: comparative clinical features and survival outcome analysis of 106 cases according to lymphoma cell lineage. *Br J Dermatol*. 2015 Jul;**173**(1):134-45.

# Live Imaging of Skin Immune Responses

**Kenji Kabashima**
Kyoto University, Japan

Various immune cells orchestrate cutaneous immune responses to external stimuli. To capture such dynamic phenomena, intravital imaging is an important technique and it may provide substantial information that is not available using conventional histological analysis. Multiphoton microscopy enables the direct, three-dimensional, and minimally invasive imaging of biological samples with high spatio-temporal resolution, and it has now become the leading method for in-vivo imaging studies. Using fluorescent dyes and transgenic reporter animals, both skin structures and cell- and humor-mediated cutaneous immune responses have been visualized.

In this symposium, I will introduce our recent findings in cutaneous immune responses and skin structural changes upon external stimuli in mice and humans.

# Curriculum Vitae

**Kenji Kabashima, MD, PhD**
Professor, Department of Dermatology, Kyoto University Graduate School of Medicine
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E-mail: kaba@kuhp.kyoto-u.ac.jp

**Education and Appointment**

1996 MD, Kyoto University, Faculty of Medicine
2003 PhD, Kyoto University, Faculty of Medicine

**Professional Training and Employment**

1996- Intern, United States Naval Hospital, Yokosuka
1997- Resident, University of Washington, Department of Internal Medicine
1998- Visiting Clinical Fellow, University of Washington, Department of Dermatology
1998- Clinical Fellow, Department of Dermatology, Kyoto University
2003- Assistant Professor, Department of Dermatology, Kyoto University
2003- Research Associate, Department of Microbiology and Immunology, University of California, San Francisco
2005- Associate Professor, Department of Dermatology, University of Occupational and Environmental Health
2008- Associate Professor, Department of Dermatology, Kyoto University Graduate School of Medicine
2010- Associate Professor, Department of Dermatology, Kyoto University Graduate School of Medicine
2015- Professor and Chairman, Department of Dermatology, Kyoto University Graduate School of Medicine
2015- Adjunct Principal Investigator, Singapore Immunology Network (SIgN)/Institute Medical Biology (IMB), A*Star, Singapore
2017- Visiting Senior Consultant, National Skin Centre, Singapore

**Selected Publications** (*) as a corresponding author

1. Ruzicka T *et al*. 2017. *N Engl J Med* 376:826-835.
2. Evald DA *et al*. (*). 2016. *J Allergy Clin Immunol* 139:562-571
3. Zhong FL *et al*. 2016. *Cell* 167: 187-202
4. Egawa G *et al*. (*). 2016. *J Allergy Clin Immunol* 138: 350-9
5. Sawada Y *et al*. (*). 2015. *J Exp Med* 212: 1921-30
6. Amano W *et al*. (*). 2015. *J Allergy Clin Immunol* 136: 667-677
7. Natsuaki Y *et al*. (*). 2014. *Nat Immunol* 15:1064-9
8. Otsuka A *et al*. (*) 2014. *J Allergy Clin Immunol* 133: 139-46 e1-10
9. Otsuka A *et al*. (*) 2013. *Nat Commun* 3:963
10. Otsuka A *et al*. 2013. (*) *J Allergy Clin Immunol* 132(6): 1448-51
11. Nakajima S, *et al*. (*) 2012. *J Allergy Clin Immunol* 129(4):1048-55
12. Tomura M, *et al*. (*) 2010. *J Clin Invest* 120(3):883-93
13. Kabashima K *et al*. 2007. *J Exp Med* 204(12):2865-74.
14. Kabashima K *et al*. 2006. *J Exp Med* 203:2683-2690.
15. Kabashima K *et al*. 2005. *Immunity* 22:439-450.
16. Kabashima K *et al*. 2003. *Nat Med* 9:744-749.
17. Kabashima K *et al*. 2003. *Nat Immunol* 4:694-701.
18. Kabashima K *et al*. 2002. *J Clin Invest* 109:883-893.
19. Matsuoka T *et al*. 2000. *Science* 287: 2013-2017

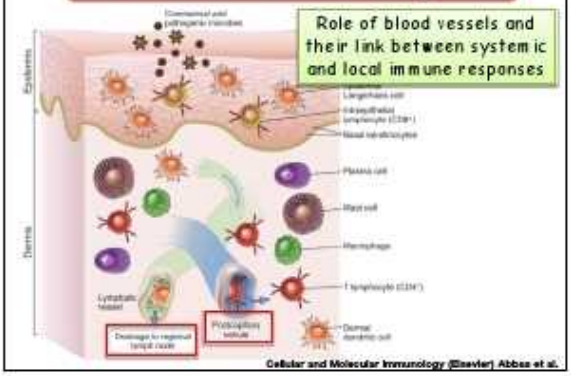
The 8th International Symposium of Wide River Institute of Immunology
Seoul National University, Korea
Oct 12th, 2018

# Live Imaging of Skin Immune Responses

Kenji Kabashima, MD, PhD
Kyoto University Graduate School of Medicine
Department of Dermatology, Japan
A*Star, Singapore

## Skin as an immune organ

Role of blood vessels and their link between systemic and local immune responses

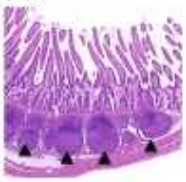



Cellular and Molecular Immunology (Elsevier) Abbas et al.

## Peripheral tissues as secondary/tertiary immune organs

GUT

- In 1974, Gut-associated lymphoid tissue (GALT)



BALT in lung, NALT in nasal area

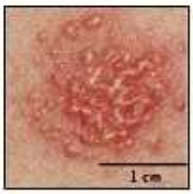
SKIN

- In 1978, Skin-associated lymphoid tissue (SALT) by Dr. Streilein.

## Question in contact dermatitis clinic

K Kabashima, et al
Contact dermatitis from lacquer in a "Go" player
Contact Dermatitis 49 (6), 306-307, 2003

## Question in contact dermatitis clinic



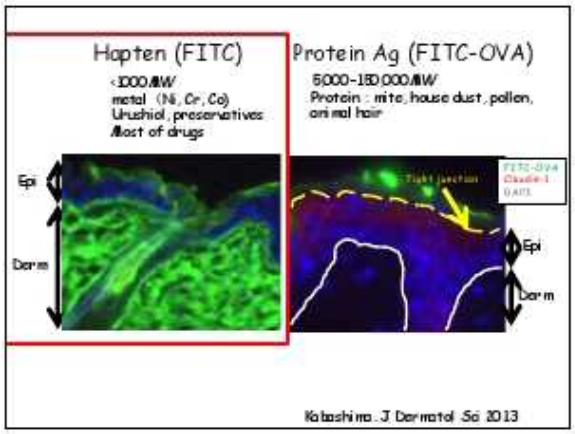

Where/how do memory T cells and DCs interact in the skin

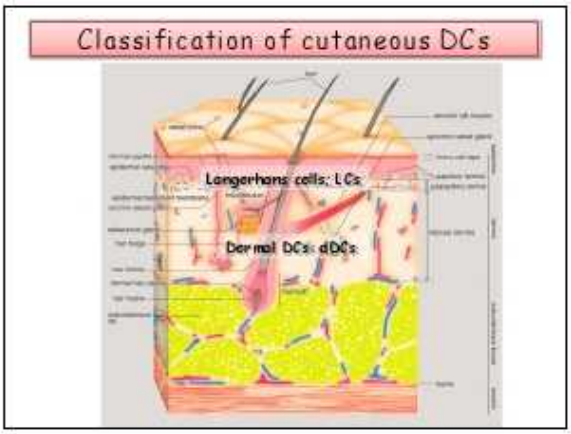
## Contact hypersensitivity (CHS)

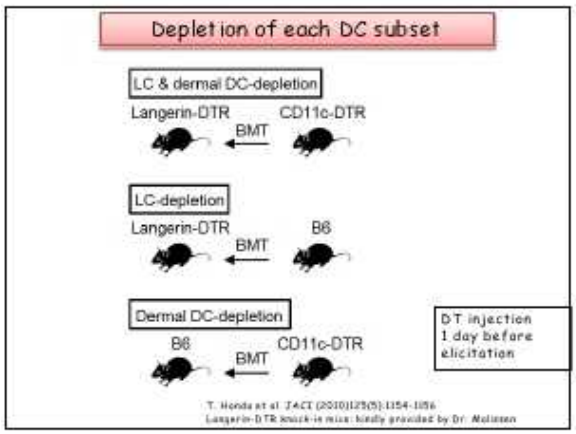
Mouse CHS = human contact dermatitis

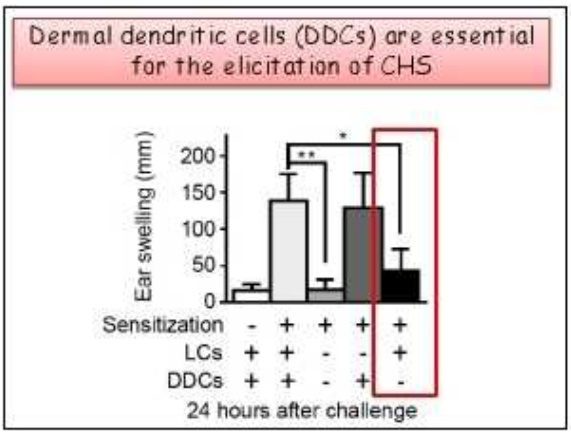
Day 5: Elicitation
DNFB on the ears
-> Measure ear thickness change

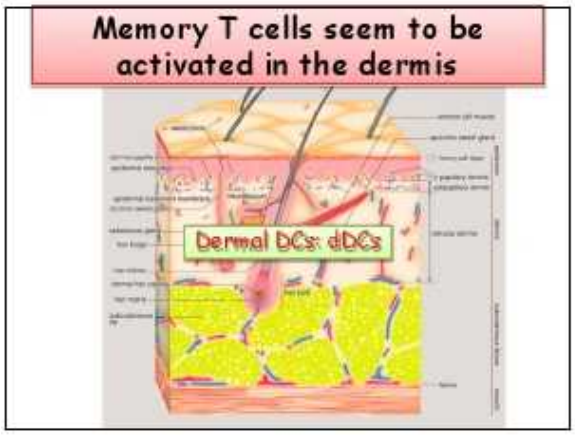
Day 0 : Sensitization
DNFB on the abdomen

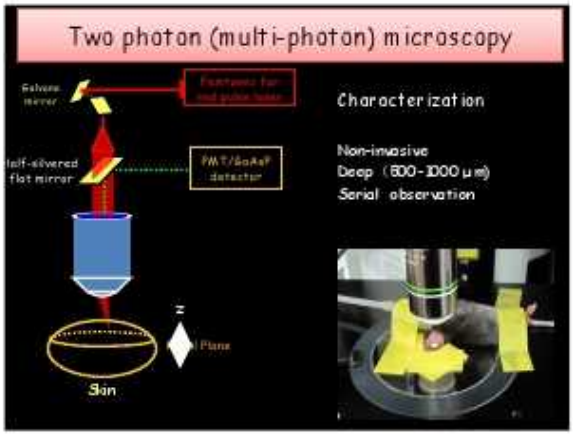

Hapten (FITC)
<1000MW
metal (Ni, Cr, Co)
Urushiol, preservatives
Most of drugs
Protein Ag (FITC-OVA)
5000-180000MW
Protein : mite, house dust, pollen, animal hair
Epi
Derm
Tight junction
Epi
Derm
Kabashima. J Dermatol Sci 2013

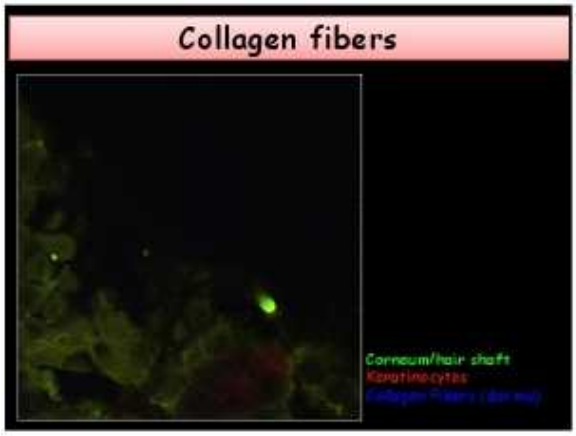

Classification of cutaneous DCs
Langerhans cells; LCs
Dermal DCs; dDCs

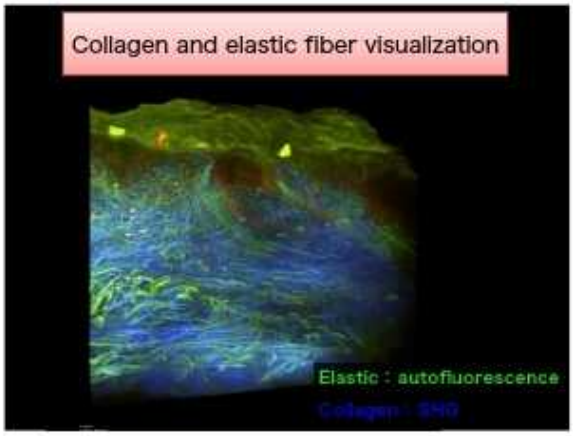

Depletion of each DC subset
LC & dermal DC-depletion
Langerin-DTR
BMT
CD11c-DTR
LC-depletion
Langerin-DTR
BMT
B6
Dermal DC-depletion
B6
BMT
CD11c-DTR
DT injection
1 day before
elicitation


Dermal dendritic cells (DDCs) are essential for the elicitation of CHS
Ear swelling (mm)
200
150
100
50
0
**
*
Sensitization - + + + +
LCs + + - - +
DDCs + + - + -
24 hours after challenge


Memory T cells seem to be activated in the dermis
Dermal DCs; dDCs


Two photon (multi-photon) microscopy
Galvano mirror
Half-silvered flat mirror
PMT/GaAsP detector
Skin
Characterization
Non-invasive
Deep (600-1000 μm)
Serial observation


Collagen fibers
Corneum/hair shaft
Keratinocytes


Collagen and elastic fiber visualization
Elastic : autofluorescence

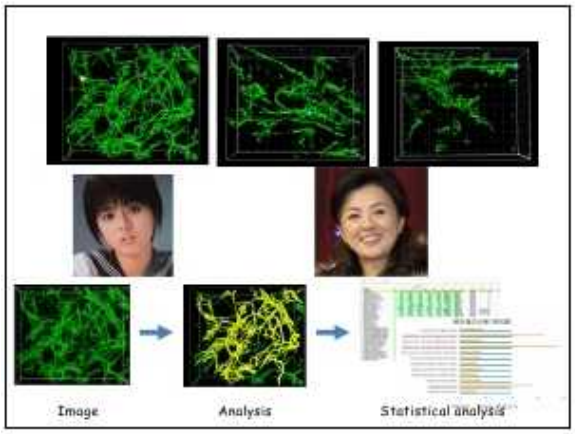
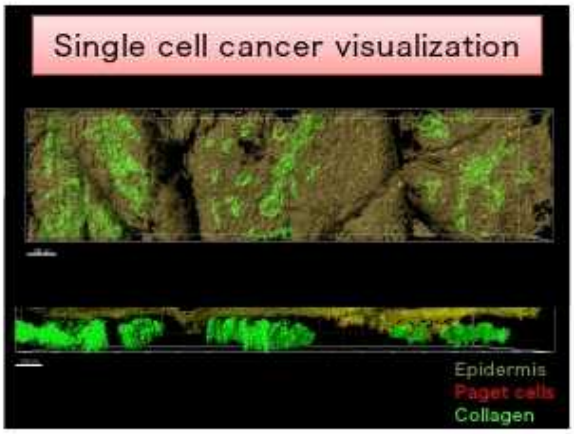
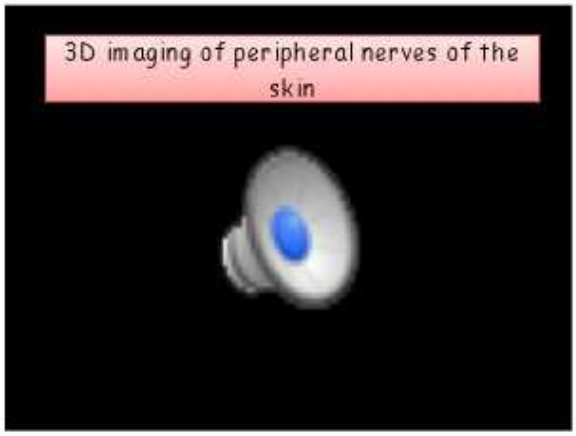
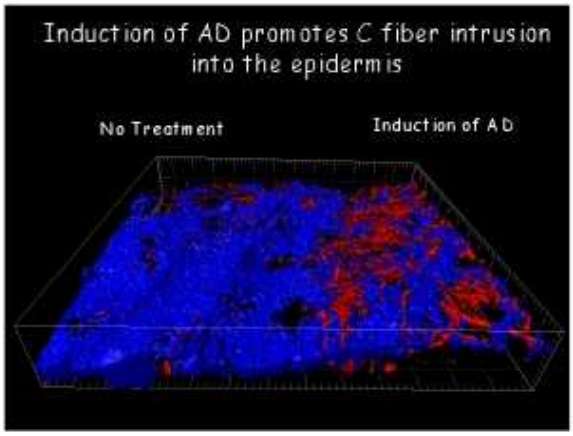
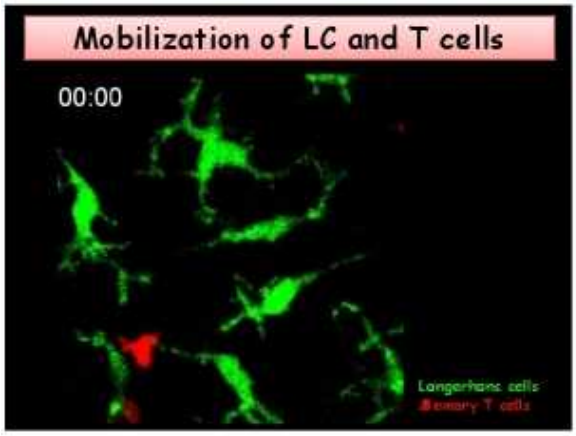


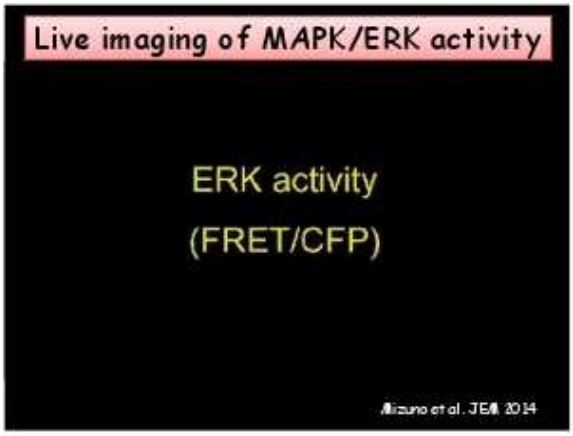
Image
Analysis
Statistical analysis

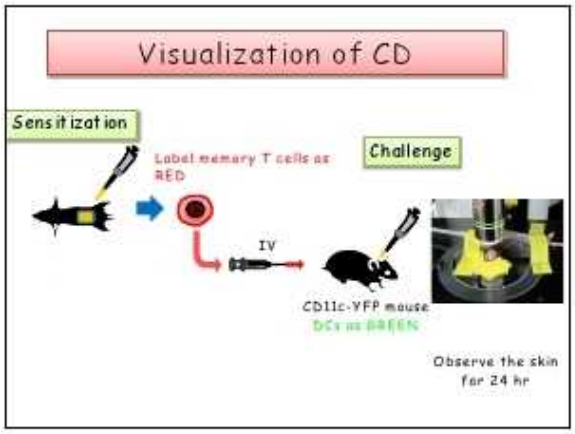

Single cell cancer visualization
Epidermis
Paget cells
Collagen

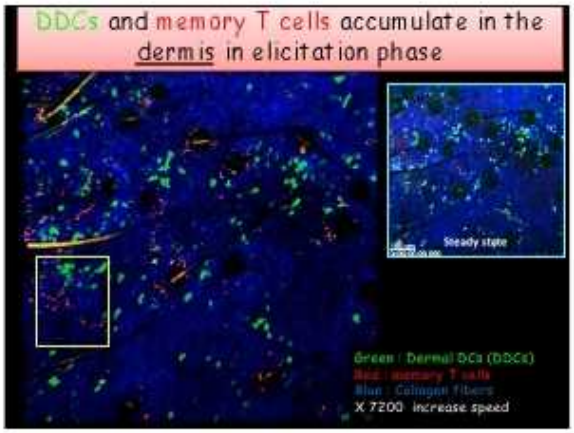

3D imaging of peripheral nerves of the skin

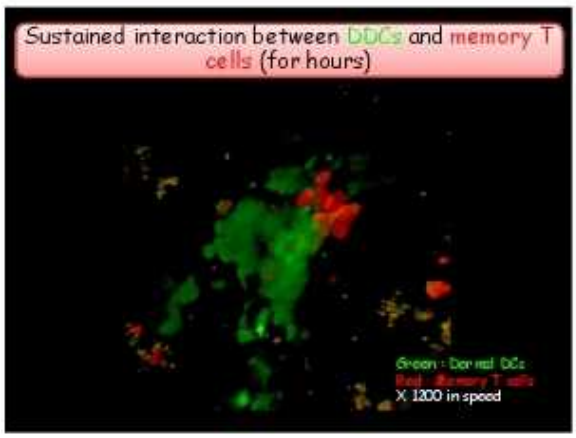

Induction of AD promotes C fiber intrusion into the epidermis
No Treatment
Induction of AD

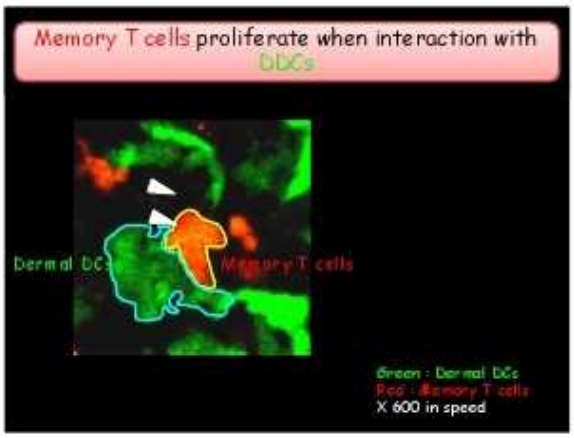

Mobilization of LC and T cells
00:00
Langerhans cells
Memory T cells


Live imaging of MAPK/ERK activity
ERK activity
(FRET/CFP)
Mizuno et al. JEM 2014

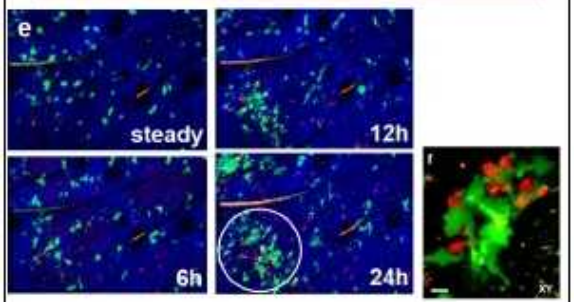

Visualization of CD
Sensitization
Label memory T cells as RED
Challenge
IV
CD11c-YFP mouse
DCs as GREEN
Observe the skin for 24 hr


DDCs and memory T cells accumulate in the dermis in elicitation phase
Steady state
Green : Dermal DCs (DDCs)
Red : memory T cells
Blue : Collagen fibers
X 7200 increase speed


Sustained interaction between DDCs and memory T cells (for hours)
Green : Dermal DCs
Red : Memory T cells
X 1200 in speed

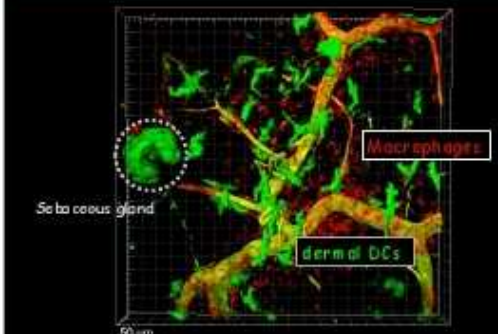

Memory T cells proliferate when interaction with DDCs
Dermal DCs
Memory T cells
Green : Dermal DCs
Red : Memory T cells
X 600 in speed

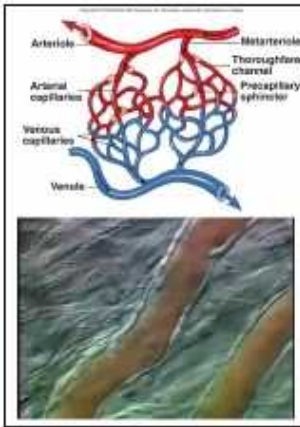
## Inducible skin-associated lymphoid tissue (iSALT)




Natsuaki Y et al. Nat Immunol

## DDCs accumulate at post capillary venules

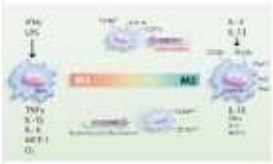


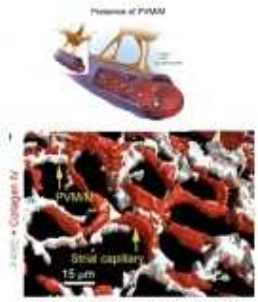

## Two types of VENULES:

1. Postcapillary Venules are the smallest venules. They form when several capillaries unite
   - Walls are very porous serve as the site for white blood cell emigration
2. Muscular Venules have 1-2 layers of smooth muscle
   - No more exchange with interstitial fluid

## (M2-like) Perivascular resident Mac

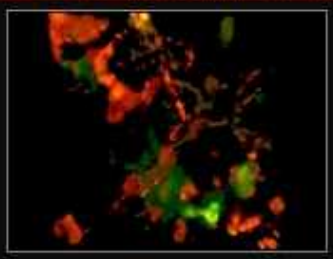


Claria et al. Frontiers Immunol 2012




Wenjing Zhang et al. PNAS 2012;109:10388-10393

## Perivascular macrophages attract dDCs in the dermis at PCV

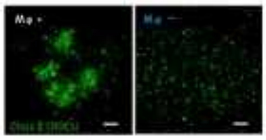



Green - Dermal DCs
Red - Macrophages (TRITC dextran-phagocytosing cells)
X 900 in speed

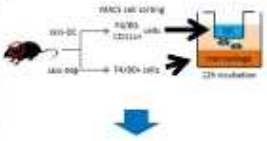
## Macrophages are essential for induction of iSALT

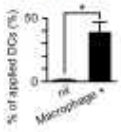
24h after challenge

■ LysM-DTR BM -> B6 chimeric mice



DC migration assay



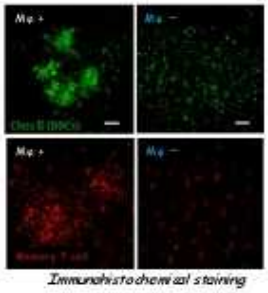



Immunohistochemical staining

## Macrophages are essential for induction of iSALT

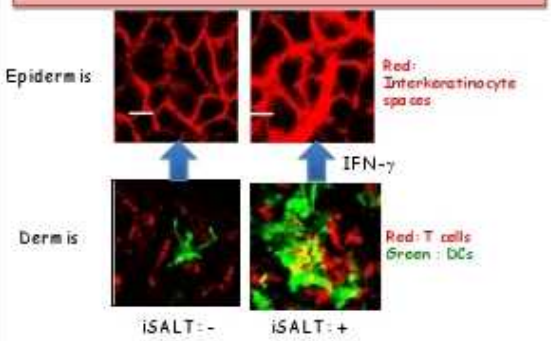
24h after challenge

■ LysM-DTR BM -> B6 chimeric mice

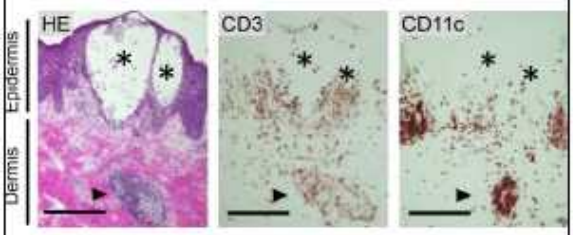



*Immunohistochemical staining*

## Epidermal edema is induced above DC-T cell clustering (iSALT) in mice

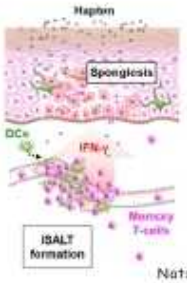



## DC-T cell cluster is observed at PCV in HUMAN contact dermatitis



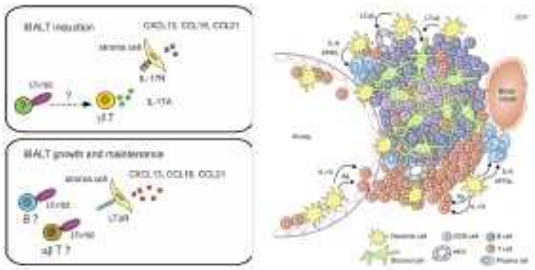

Collaboration with Dr. Emma Guttman-Yassky

## DC cluster (iSALT) formation is essential for the elicitation of CHS



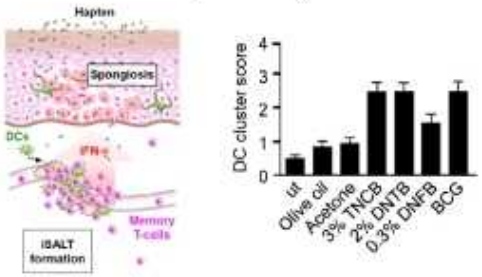

Natsuaki Y et al. Nat Immunol

## iBALT

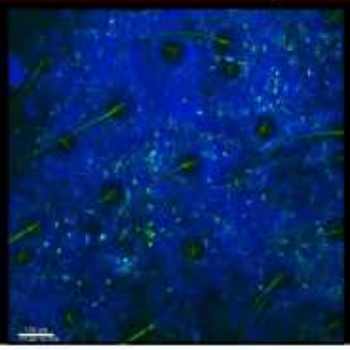



Randall TD et al. Forster R et al.

## DC cluster (iSALT) formation is induced by BCG injection

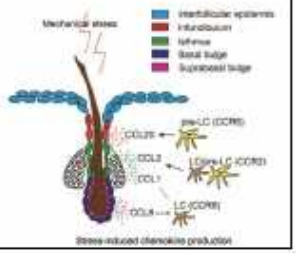


LysM+ neutrophil recruitment into hair follicles after IMQ

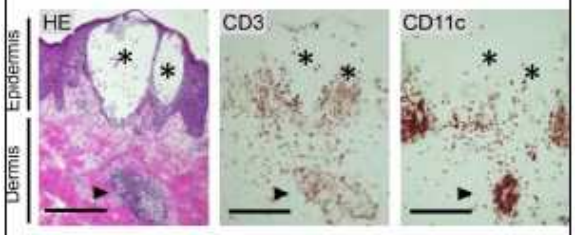


## Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin

Keisuke Nagao[1], Tetsuro Kobayashi[1], Kazuyo Moro[2], Mamoru Ohyama[1], Takeya Adachi[1], Daniela Y Kitashima[1], Satoshi Ueha[3], Keisuke Horiuchi[4,5], Hideoki Tanizaki[6], Kenji Kabashima[6], Akihiro Kubo[1,7], Yoong-Soo Cho[7], Björn E Clausen[8], Kenji Matsushima[3], Makoto Suematsu[9,10], Glaucia C Furtado[11], Sergio A Lira[11], Joshua M Farber[12], Mark C Udey[7] & Masayuki Amagai[1]



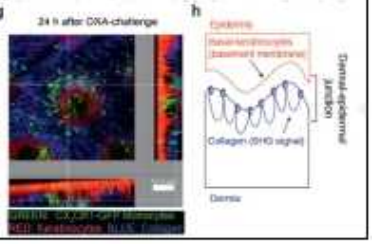

DC-T cell accumulation can be observed in epidermal-dermal junction

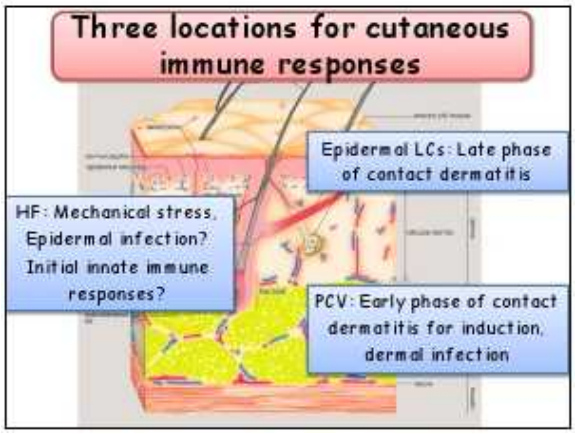



## Visualization of T Cell-Regulated Monocyte Clusters Mediating Keratinocyte Death in Acquired Cutaneous Immunity

Zheng Liu[1,2], Fei Yang[1,2], Hao Zheng[1,2], Zhan Fan[1,2], Sha Qiao[1,2], Lei Liu[1,2], Juan Tao[2], Qingming Luo[1,2] and Zhihong Zhang[1,2]

It remains unclear how monocytes are mobilized to amplify inflammatory reactions in T cell-mediated adaptive immunity. Here, we investigate dynamic cellular events in the cascade of inflammatory responses through intravital imaging of a multicolor-labeled murine contact hypersensitivity model. We found that monocytes formed clusters around hair follicl encountered dendritic cells unde CCR2-dependent monocyte–interc showed that hair follicles shaped t cytes, keratinocytes, and effector CXCR2 antagonization, monocyte-a provides a new perspective on effe ejection in acquired cutaneous im




## Is iSALT a really lymphoid tissue?

- Naïve T cell/B cell infiltration?
- Activation of naive T cells at iSALT?
- Lymphoid follicles/germinal center formation?

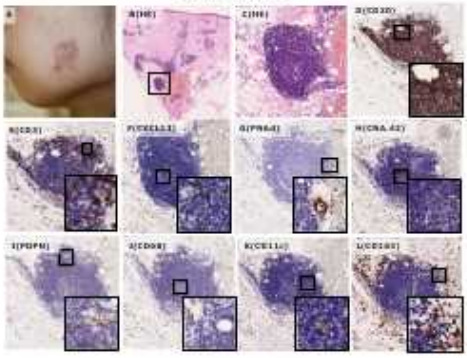
## Lymphoid clusters in certain skin diseases

SLE

Viral eruption

Melanoma

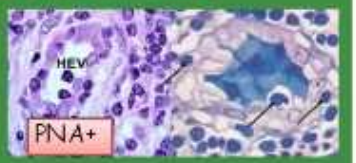
LE profundus



Kogame et al. in prep

## High endothelial venules

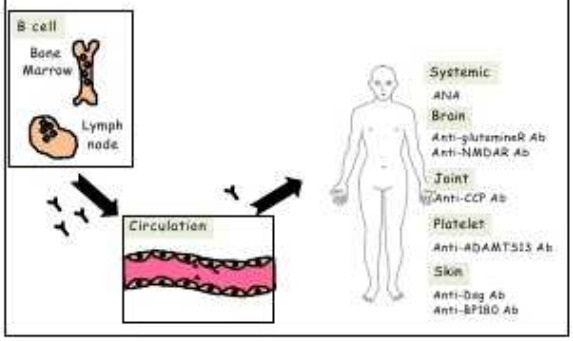
Post capillary venules in 2° lymphoid tissue HIGH ENDOTHELIAL VENULES Specialized to allow lymphocytes and nothing else into the lymph node




Post capillary venules in other tissues are lined by simple squamous epithelium


Can we induce HEV (-like) in the murine skin?

## Autoantibody-mediated disorders




## How is Ig distributed?

SLE

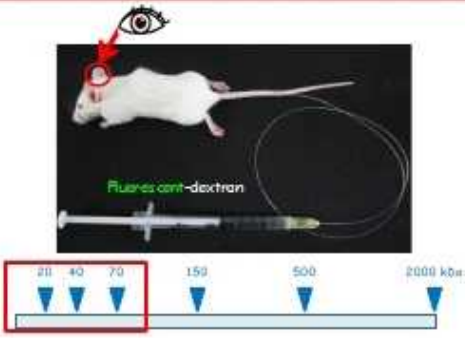


Bullous pemphigoid

**Question:**
**Auto-antibodies may tend to be distributed at the preceding inflammatory sites (ex. UV exposure)**

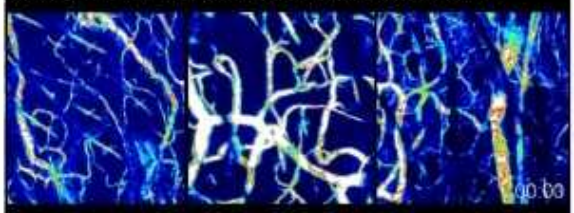
**Visualization of vascular permeability/barrier**

## Visualization of blood vessels



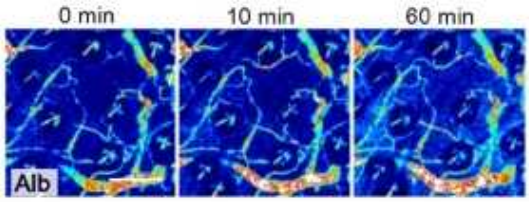

## In vivo analysis of vascular barrier

IV injection of fluorescent dextran and observe 60 min



Egawa et al. Scientific Reports 2013

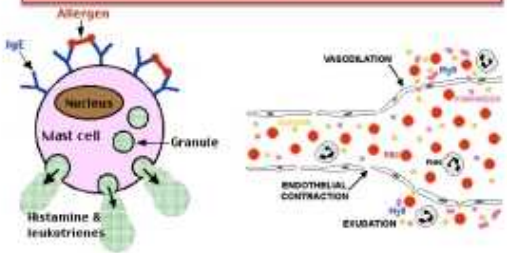
## Albumin (66kDa) does not traverse blood vessels in the steady states



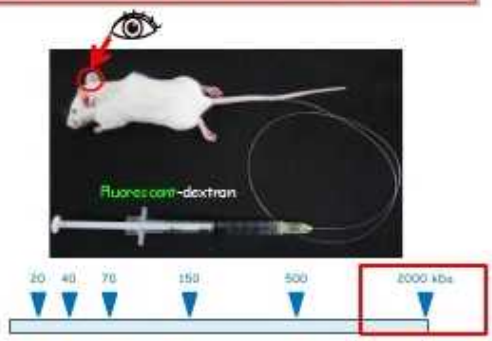

IV injection of fluorescent-labeled albumin

## Under inflammatory condition

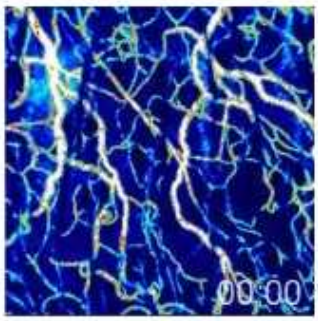
## Under inflammatory condition: Hives




## Visualization of blood vessels



## Histamine-induced inflammation @PCV

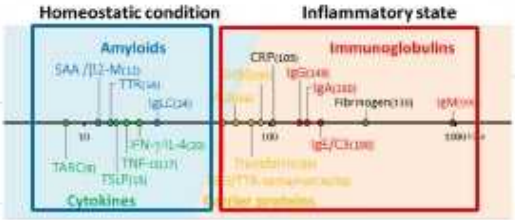



2000kD fluorescent Dextran iv

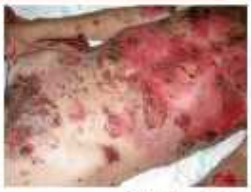
Histamine injection at 10 min

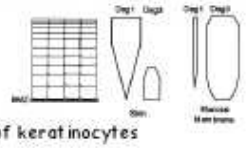
Leakage at the PCV

## Classification of plasma proteins by its molecular size

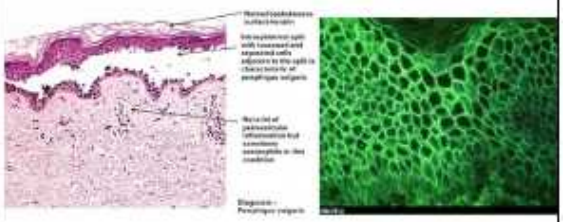



## Pemphigus vulgaris



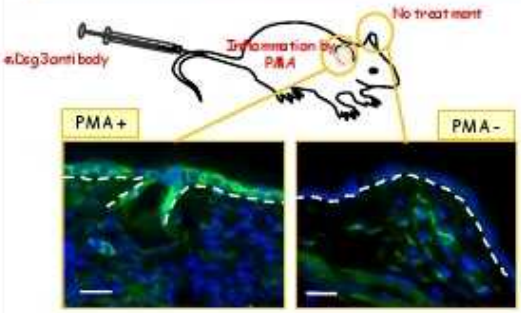


Autoimmune blistering disease
Induced by auto anti-Dsg3 Ab
Resulting in the loss of cohesion of keratinocytes

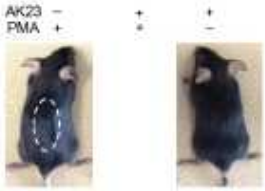


Skin biopsy should be performed at the skin lesions or peri-lesions (not at the normal appearing skin)

## Inflammation-dependent translocation of Igs to the skin in mice




## Inflammation-dependent translocation of Igs to the skin in mice




AK23: kindly provided by Dr. Amagai

Ono et al. JACI 2017

## Inflammation-dependent translocation of Igs to the skin in mice


AK23: kindly provided by Dr. Amagai

Ono et al. JACI 2017

## Factors to determine the distribution of Ig

Ag amount

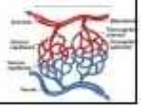
Amount of circulating Ab

Local inflammation

Endothelial paracellular way

## PCVs are key interface between central and peripheral immunity

1. Entry sites for memory (even naïve) T cells into the skin
2. Formation of iSALT by interaction between memory T cells and dermal DCs
3. PCV express PNAd+ ICAM$^{high}$ during chronic inflammation
4. Passive Ig transport under inflammation



## Department of Dermatology Kyoto University

# Roles of TRPV Channels in Keratinocytes in Itching Sensation

**Hye One Kim**
Hallym University, Korea

**Background:** Pruritus is one of the major symptoms of inflammatory skin diseases and strongly affects the quality of life in patients.

**Objective:** The aim of this study was to evaluate the manifestation of transient receptor potential (TRP) channels and other related receptors in itching sensation.

**Methods:** Sixty-five burn patients with (n=40) or without (n=25) pruritus were investigated, including skin biopsies. Keratinocytes and fibroblasts from those samples were separated. Immunohistochemical staining for TRPV3 and TRPA1; and immunofluorescence staining for TSLP, TSLPR, loricrin, involucrin, α-SMA, and TGF-β, were performed on samples of burn scars and normal skin. Real-time PCR and western blotting of TRPV3, TRPA1, PAR2 NK1R, TSLP, and TSLPR were done. We also measured intracellular $Ca^{2+}$ levels in keratinocytes from scars with or without pruritus, following TRPV3 activation and blocking, and measured the effects of PAR2 agonist on TRPV3 function. Expressions of TSLP after TRPV3 activation in keratinocytes were measured.

**Results:** In immunohistochemical and immunofluorescence staining, TRPV3, TSLP, and TSLPR stained more intensely the epidermis of the burn scars of post-burn-pruritus patients, than that of non-pruritic-burn patients. Real time-PCR showed that mRNA of TRPV3 and TSLP were significantly more abundant in keratinocytes from pruritic burn scars than in keratinocytes from non-pruritic burn scars. In addition, mRNA and protein levels of PAR2, NK1R, TSLP, and TSLPR were also significantly increased in pruritic burn scars. With TRPV3 activation, intracellular $Ca^{2+}$ concentrations were more significantly increased in keratinocytes from pruritic burn scars than in those from non-pruritic ones. In keratinocytes from pruritic burn scars, PAR2 activation markedly potentiated opening of TRPV3 channels. TRPV3 activation itself resulted in little increase of $Ca^{2+}$ influx with PAR2 inhibition in keratinocytes. In keratinocytes from all samples, PLC-β, PKA, PKCs, and PKD inhibitor markedly reduced intracellular $Ca^{2+}$ level by TRPV3 activation, as well as by PAR2 activation. TRPV3 activation also increased mRNA and protein expression of TSLP in keratinocytes.

**Conclusions:** In conclusion, we confirmed that TRPV3 of keratinocytes and PAR2, NK1R, TSLP, and TSLPR were highly expressed in pruritic burn scars. In addition, it seemed that PAR2 sensitized TRPV3 channels with PKA, PKC, PKD signaling pathways. It also seemed that TRPV3 activation induced TSLP expression.

# Curriculum Vitae

**Hye One Kim, MD, PhD**
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**Education and Appointment**

1996-2002 MD in Medicine, Hallym University College of Medicine, Chuncheon, Gangwondo, Korea
2008-2010 MS in Dermatology, College of Medicine, Hallym University, Seoul, Korea
2012-2016 PhD in Dermatology, College of Medicine, The Catholic University, Seoul, Korea

**Professional Training and Employment**

2008-2009 Fellowship, Department of Dermatology, Seoul National University Bundang Hospital, Seongnam, Gyeonggido, Korea
2009-2010 The Chief of Dermatologic Department, Seoul Medical Center, Seoul, Korea
2010-2011 Fellowship, Department of Dermatology, Hallym University Kangnam Sacred Heart Hospital, Seoul, Korea
2011-2012 Instructor, Department of Dermatology, Hallym University Kangnam Sacred Heart Hospital, Seoul, Korea
2013-2018 Assistant Professor, Department of Dermatology, Hallym University Kangnam Sacred Heart Hospital, Seoul, Korea
2018-present Associate Professor, Department of Dermatology, Hallym University Kangnam Sacred Heart Hospital, Seoul, Korea

**Selected Publications**

1. Yang YS, Cho SI, Choi MG, Choi YH, Kwak IS, Park CW, **Kim HO**. Increased expression of three types of transient receptor potential channels (TRPA1, TRPV4 and TRPV3) in burn scars with post-burn pruritus. *Acta dermato-venereologica*, 2015, 95.1: 20-24.
2. Chung BY, **Kim HO**, Kim, JH, Cho SI, Lee CH, Park CW. The proactive treatment of atopic dermatitis with tacrolimus ointment in Korean patients: a comparative study between once-weekly and thrice-weekly applications. *British Journal of Dermatology*, 2013, 168.4: 908-910.
3. Cho SI, Lee CH, Park GH, Park CW, **Kim HO**. Use of S-LANSS, a tool for screening neuropathic pain, for predicting postherpetic neuralgia in patients after acute herpes zoster events: a single-center, 12-month, prospective cohort study. *The Journal of Pain*, 2014, 15.2: 149-156.
4. **Kim HO**, Kim JH, Chung BY, Choi MG, Park CW. Increased expression of the aryl hydrocarbon receptor in patients with chronic inflammatory skin diseases. *Experimental dermatology*, 2014, 23: 278-281.
5. **Kim HO**, Cho YS, Park SY, Kwak IS, Choi MG, Chung BY, Park CW, Lee JY. Increased activity of TRPV3 in keratinocytes in hypertrophic burn scars with post burn pruritus. *Wound Repair and Regeneration*, 2016. 24: 841-850
6. Yang YS, Byun YS, Kim JH, **Kim HO**, Park CW. Food hypersensitivity in adult patients with atopic dermatitis in Korea. *Clinical and experimental dermatology*, 2015, 40.1: 6-10.
7. **Kim HO**, Cho SI, Chung BY, Ahn HK, Park CW, Lee CH. Expression of CCL1 and CCL18 in atopic dermatitis and psoriasis. *Clinical and experimental dermatology*, 2012, 37.5: 521-526.
8. Cho SI, Chung BI, Choi MG, Baek JH, Cho HJ, Park CW, Lee CH, **Kim HO**. Evaluation of the clinical efficacy of fractional radiofrequency microneedle treatment in acne scars and large facial pores. *Dermatologic Surgery*, 2012, 38.7: 1017-1024.

9. Cho HJ, Chung BY, Lee HB, **Kim HO**, Park CW, Lee CH. Quantitative study of stratum corneum ceramides contents in patients with sensitive skin. *The Journal of dermatology*, 2012, 39.3: 295-300.
10. Kwak IS, Park SY, Choi YH, Cho SI, Yang YS, Seo CH, Park CW, **Kim HO**. Clinical and Histopathological Features of Post Burn Pruritus. *J Burn Care Res*, 2016. 37: 343-349
11. Chun Wook Park, Hyun Ji Kim, Yong Won Choi, Bo Young Chung, So-Youn Woo, Dong-Keun Song, **Hye One Kim**. TRPV3 Channel in Keratinocytes in Scars with Post-Burn Pruritus. *Int. J. Mol. Sci*. 2017, 18, 2425

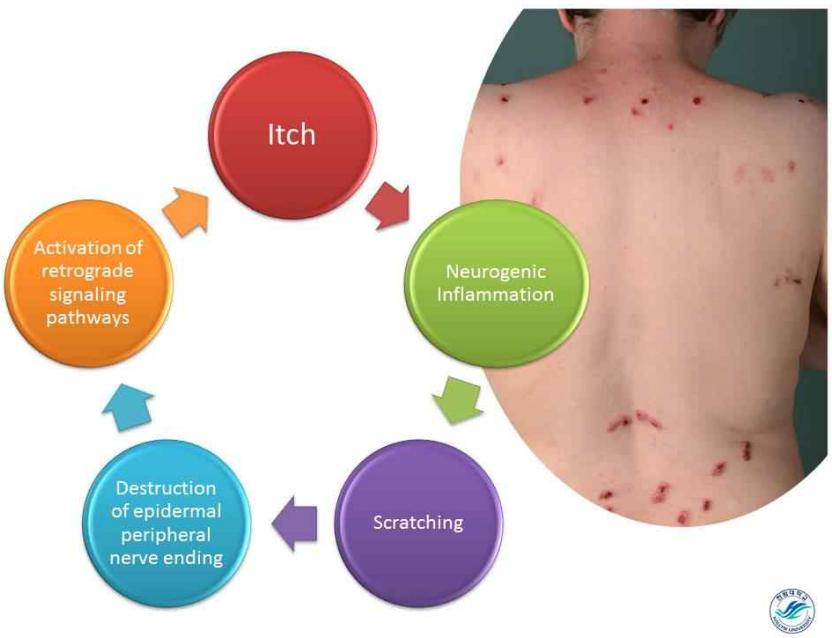

# Roles of TRPV Channels in Keratinocytes in Itching Sensation

**Hye One Kim**

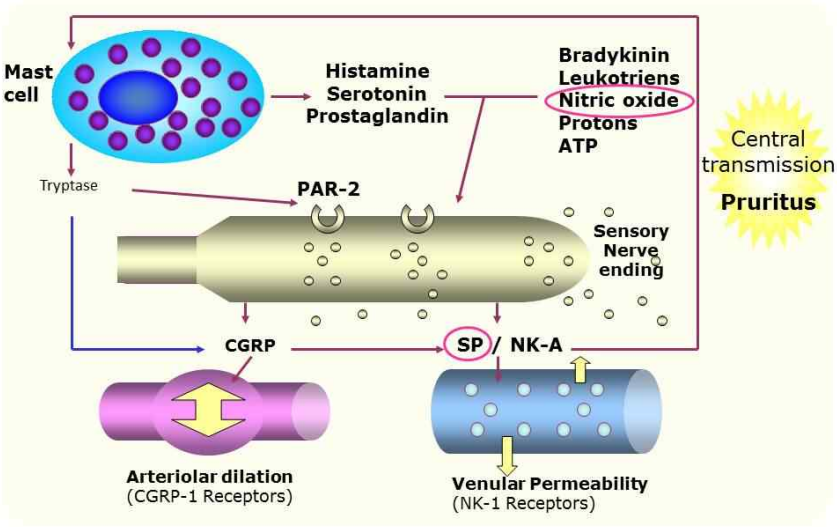
**Hallym Unv. Kangnam Scared Heart Hospital**

1

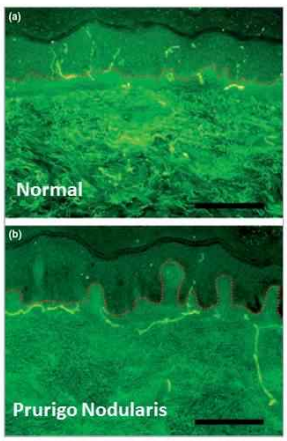
## MECHANISMS OF TRANSMISSION OF PRURITUS



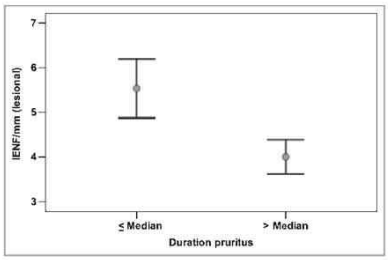
# Neurogenic inflammation




**Reduced** intraepidermal nerve fibre density in lesional and nonlesional prurigo nodularis skin

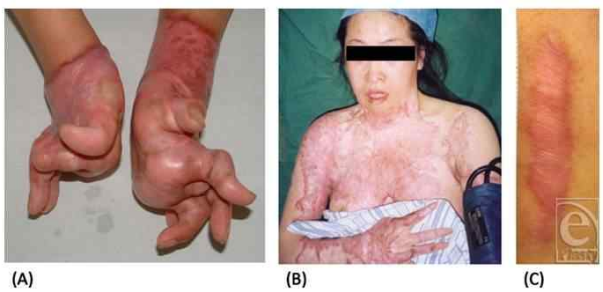



**Subclinical Cutaneous Neuropathy**




Br J Dermatol. 2011 Jul;165(1):85-91.

# Postburn Pruritus



(A) (B) (C)

# Postburn Pruritus - Incidence

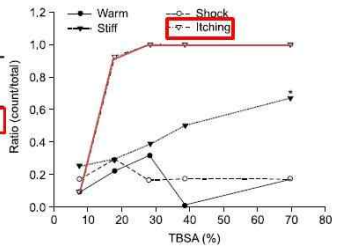
Korean J Pain Vol. 20, No. 2, 2007 대한통증학회지 2007; 20: 158-162
DOI : 10.3344/kjp.2007.20.2.158

## 한국인에서 화상 후 만성 감각이상의 특징에 대한 연구

한림대학교 의과대학 한강성심병원 마취통증의학과, 성균관대학교 의과대학 강북삼성병원 마취통증의학과*
김형석 · 장현묵 · 최도영 · 우철호 · 문성하* · 김현수* · 김광민

Table 2. Type and Prevalence of Paresthetic Sensations

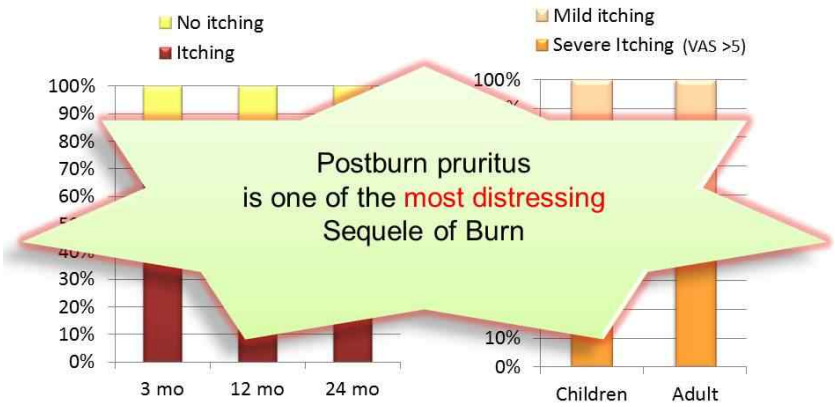
| | Number of cases (n = 51) | % |
|---|---|---|
| Itching | 39 | 76.5 |
| Tingling | 38 | 74.5 |
| Pin and Needles | 25 | 49.0 |
| Stiffness | 19 | 37.3 |
| Cold/freezing | 18 | 35.3 |
| Numbness | 15 | 29.4 |
| Tight sensation | 14 | 27.5 |
| Electrical shock sensation | 10 | 19.6 |
| Warm sensation | 9 | 17.7 |




Correlations between paresthetic sensations and total body surface area burned (TBSA)

Kim HS et al. Korean J Pain. 2007;20:158-62

# Postburn pruritus - Incidence




Van Loey NE et al. Br J Dermatol. 2008;158:95-100

# Classification of Pruritus

**Postburn pruritus**

| Clinical classification | Mediators and mechanisms | Diagnosis | Therapy |
|---|---|---|---|
| Itch caused by skin disorders | Histamine, interleukins, prostaglandin and proteases | Inflammatory dermatoses (atopic dermatitis, psoriasis, drug reactions, mites and urticaria) and dry skin | Antihistamines, anti-inflammatory, immuno-modulatory topical and systemic therapy (cyclosporine A, pimecrolimus, tacrolimus and corticosteroids) |
| Itch caused by systemic disorders | Opiates, interleukins? | Chronic liver disease and chronic renal failure | Naltrexone, κ-opioid receptor agonists and gabapentin |
| Neuropathic itch | Damage to nerve fibres, neuropeptides (such as substance P) and proteases | Postherpetic pruritus, notalgia paresthetica and brachoradial pruritus, itch post-CVA | Gabapentin, pregabalin and capsaicin |
| Psychogenic itch | Serotonin, noradrenaline | Delusions of parasitosis, stress and depression | Olanzapine, pimozide and SSRI antidepressants |
| Overlapping and mixed | | | Central-acting itch inhibitors and topical anti-inflammatory drugs |

CVA, cerebral vascular accident; SSRI, selective serotonin reuptake inhibitor.

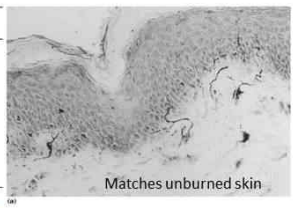
Ikoma et al. Nat Reviews Neurosci 2006:7:535–47

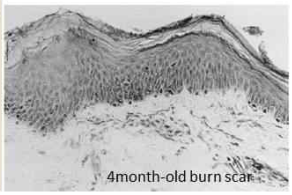
# Postburn Pruritus is neuropathic pruritus

Table 2
Number of nerve fibers in the dermis and epidermis of matched unburned skin and in the burn scars 1, 4 and 7 months post-burn

| Marker | Matched unburned skin (mean score ± SEM) | Time point post-burn (months) | Burned skin (mean score ± SEM) | P-value (Wilcoxons Signed Ranks test) |
|---|---|---|---|---|
| PGP 9.5 (epidermis) | 16.3 ± 3.5 | 1 | 2.0 ± 0.5 | 0.01 |
| | | 4 | 4.1 ± 1.1 | 0.01 |
| | | 7 | 9.0 ± 2.7 | 0.02 |
| PGP 9.5 (dermis) | 16.9 ± 3.6 | 1 | 3.7 ± 0.9 | 0.01 |
| | | 4 | 8.2 ± 2.2 | 0.02 |
| | | 7 | 7.0 ± 1.9 | 0.02 |
| SP (dermis) | 0.7 ± 0.2 | 1 | 1.6 ± 0.4 | * |
| | | 4 | 1.6 ± 0.2 | * |
| | | 7 | 0.8 ± 0.2 | * |
| NKA (dermis) | 0.7 ± 0.2 | 1 | 1.2 ± 0.3 | * |
| | | 4 | 1.8 ± 0.5 | * |
| | | 7 | 0.3 ± 0.1 | * |

* $P > 0.05$.




**Box 1** | Causes of neuropathic pruritus

CNS
- Tumours
- Abscesses
- Aneurysms
- Cerebrovascular incidents
- Creutzfeldt–Jakob disease
- Multiple sclerosis
- Syringomyelia
- Transverse myelitis
- Brown–Séquard syndrome

Proximal PNS
- Peripheral polyneuropathies
- Post-herpetic neuralgia and other ganglionopathies
- Brachioradial pruritus
- Notalgia paraesthetica and other entrapment neuropathies

Distal PNS
- Small-fibre neuropathies
- Sensitive skin
- Post-burn itch
- Itchy scars
- Prurigo nodularis (putative)

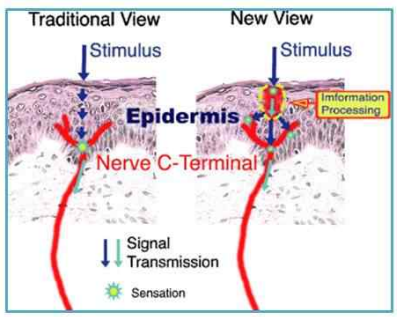
Mixed or undetermined
- Neurofibromatosis (putative)
- Ciguatera
- Drug-induced pruritus

## TRP channels and Postburn Pruritus

- Increased Expression of Three Types of Transient Receptor Potential Channels (TRPA1, TRPV4 and TRPV3) in Burn Scars with Post-burn Pruritus

## Epidermal keratinocytes as the Forefront of the Sensory System

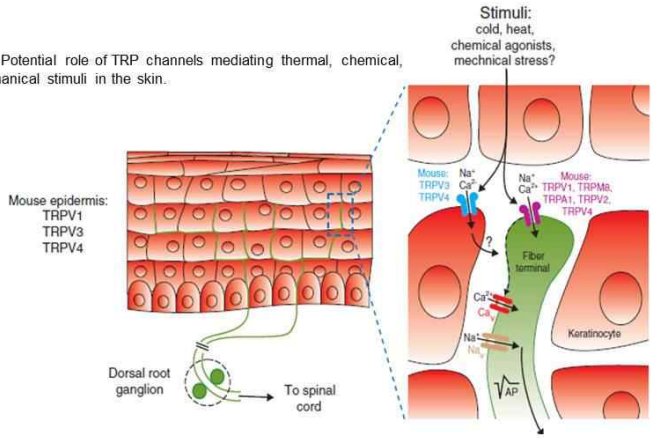
- Keratinocytes recognize various environmental factors,
- Then the information is processed and conveyed to the nervous system.




Denda M et al. Exp Dermatol. 2007 Mar;16:157-61

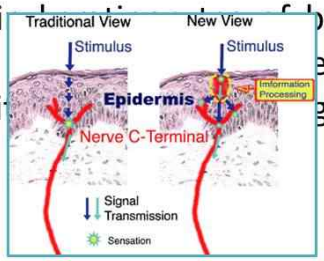
# TRP Channels in the Skin

Figure 2. Potential role of TRP channels mediating thermal, chemical, and mechanical stimuli in the skin.



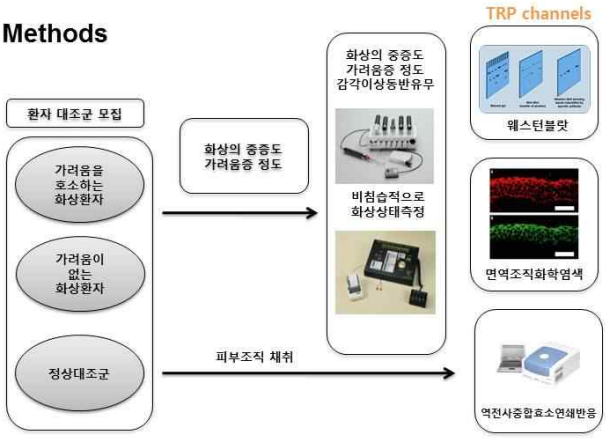

# Hypothesis

- The first signal that triggers post burn pruritus would not be the excitation of nerve endings but keratinocytes itself.
- $Ca^{2+}$ influx i urn scar and subsequent e information would exci gs delivering pruritus.




# TRP Channels and Postburn Pruritus

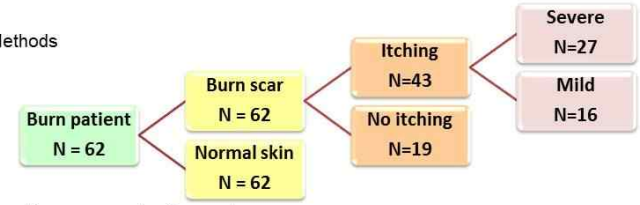
☐ **Methods**




Yang YS et al. Acta Derma Venereol 2015;95:20-4

## Clinical and Histopathological Features of Postburn Pruritus

- Methods




  - Burn scar evaluation scales
    - VAS, PSAS, OSAS etc.
  - Assessment of abnormal sensation
    - Tingling, Stiffness, Cold freezing, Numbness, Warmth, Pain needle, Electric shock
  - Histopathological analysis
    - H&E stain, Masson Trichrome stain, Giemsa stain
  - Other non-invasive methods
    - TEWL(trans epithelial water loss), Erythema/melanin index, Ultrasound

Kwak IS et al. J Burn Care Res. 2016;37:343-9

**Increased activity of TRPV3 in keratinocytes in hypertrophic burn scars with postburn pruritus**

Hye One Kim, MD[1]; Yong Se Cho, MD[1]; Sook Young Park, MSc[1]; In Suk Kwak, MD[2]; Min Gyu Choi, PhD[3]; Bo Young Chung, MD[1]; Chun Wook Park, MD[1]; Jun Young Lee, MD[4]

1. Department of Dermatology,
2. Department of Anesthesiology and Pain Medicine, College of Medicine, Hallym University, Seoul, Korea,
3. Department of Computer Science, Kwangwoon University, Seoul, Korea, and
4. Department of Dermatology, The Catholic University of Korea, Seoul, Korea

### Results

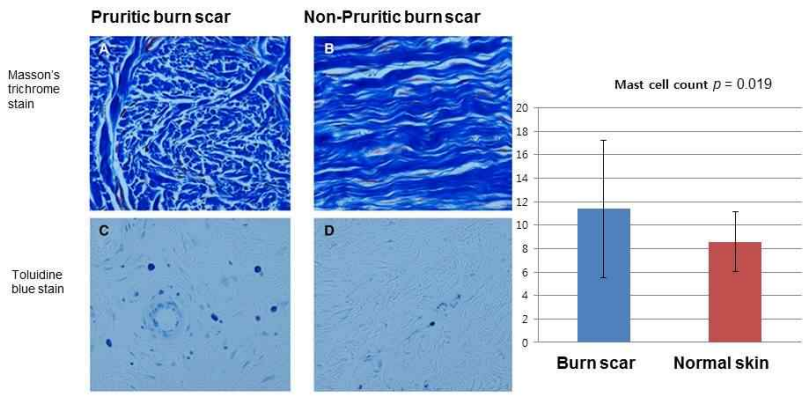
| | Non-pruritic burn scars (n=18) | Pruritic burn scars (n = 33) | p-value |
|---|---|---|---|
| Clinical characteristics | | | |
| Age | 29.39±16.84 | 38.03±12.78 | 0.068 |
| **TBSA** | **9.72±12.51** | **25.55±25.33** | **0.004*** |
| **Gender (male proportion)** | **50%** | **84.8%** | **0.008*** |
| Previous surgical procedures | 61.1% | 69.7% | 0.534 |
| Duration | 153.69±128.08 | 90.15±129.87 | 0.100 |
| **Total PSAS** | **21.64±6.07** | **35.74±9.73** | **<0.001*** |
| **Total OSAS** | **22.64±6.38** | **31.44±6.99** | **0.001*** |

Table 1. The clinical differences between burn patients with and without pruritus

• TBSA: total body surface area, PSAS: patient scar assessment scale, OSAS: observer scar assessment scale

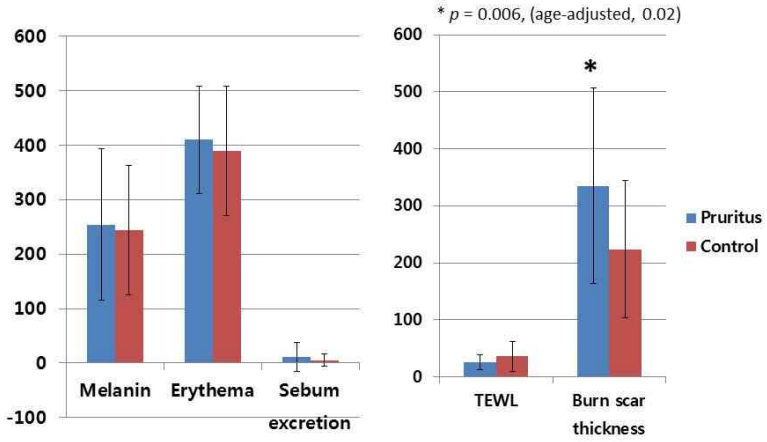
Kim HO et al. Wound Repair Regen 2016;24:841-50

## Histologic Difference
## Pruritic vs. Non-pruritic Scar




Kwak IS et al. J Burn Care Res. 2016;37:343-9

## Non Invasive Measurement – Pruritic vs. Non-pruritic Scar

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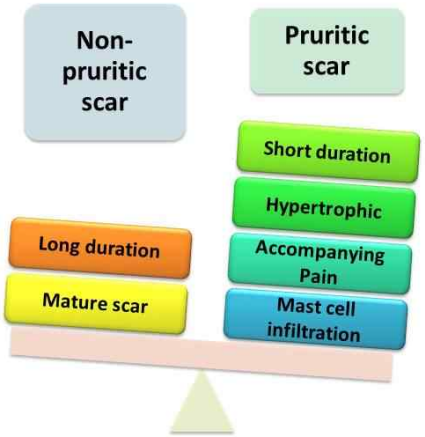
## Clinical and Histopathological Features of Postburn Pruritus



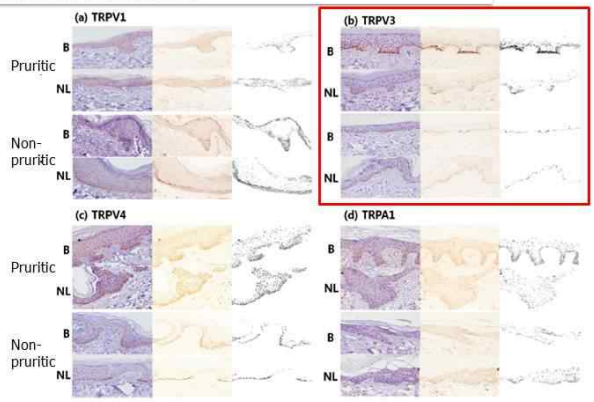

Kwak IS et al. J Burn Care Res. 2016;37:343-9

Acta Derm Venereol 2015; 95: 20–24

INVESTIGATIVE REPORT

### Increased Expression of Three Types of Transient Receptor Potential Channels (TRPA1, TRPV4 and TRPV3) in Burn Scars with Post-burn Pruritus

Yoon Seok YANG[1*], Soo Ick CHO[2*], Min Gyu CHOI[2], Young Hee CHOI[2], In Suk KWAK[3], Chun Wook PARK[1] and Hye One KIM[1]
*Departments of [1]Dermatology, [2]Pathology, and [3]Anesthesiology and Pain Medicine, College of Medicine, Hallym University, and [4]Department of Computer Science, Kwangwoon University, Seoul, Korea*
*These authors contributed equally and should be considered co-first authors.*

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## Increased activity of TRPV3 in keratinocytes in hypertrophic burn scars with postburn pruritus

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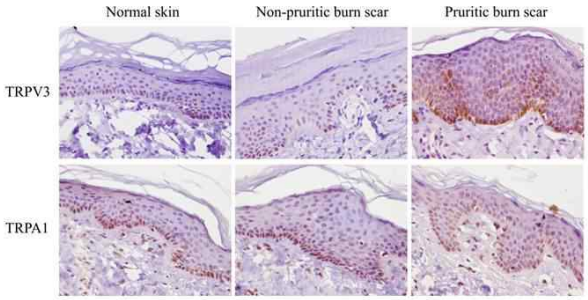


Fig. 1. Immuno-histochemical study of normal skin and burn scars with transient receptor potential vanilloid 3 (TRPV3) and TRP ankyrin 1 (TRPA1) (400×): All could be demonstrated on basal and supra-basal keratinocytes of burn scars and normal skin. Especially in the pruritic burn scars, there was prominent staining of TRPV3 in basal keratinocytes. TRPA1 expression was also increased in the pruritic burn scars, but that was not statistically significant.

Kim HO et al. Wound Repair Regen 2016;24:841-50

Acta Derm Venereol 2015; 95: 20–24

INVESTIGATIVE REPORT

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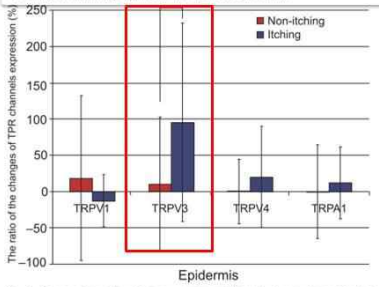



*Fig. 2.* Comparison of protein expression of Transient receptor potential (TRP) channels of non-itching and itching burn scars. The relative differences in the immunohistochemical staining intensity of various TRP channels are presented as the ratio of the changes of TPR channels expression between itching ($n=33$) or non-itching ($n=18$) burn scars and the normal skin of the same patients using software. Only TRPV3 expression in the epidermis was more significantly increased in itching burn scars than in non-itching burn scars. Other TRP channels did not show a significant difference in the epidermis and dermis.

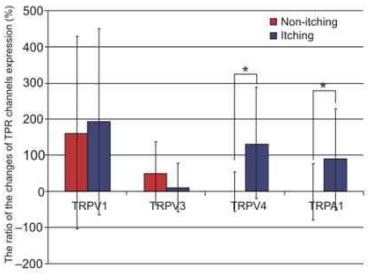



*Fig. 3.* Comparison of quantity of mRNA of various Transient receptor potential (TRP) channels in non-itching ($n=18$) and itching ($n=33$) burn scars. Data are presented as the ratio of the changes of mRNA expression between burn scars and the normal skin of the same patients with real time PCR. TRPV4 and TRPA1 were more significantly elevated in the itching group than in the non-itching group.

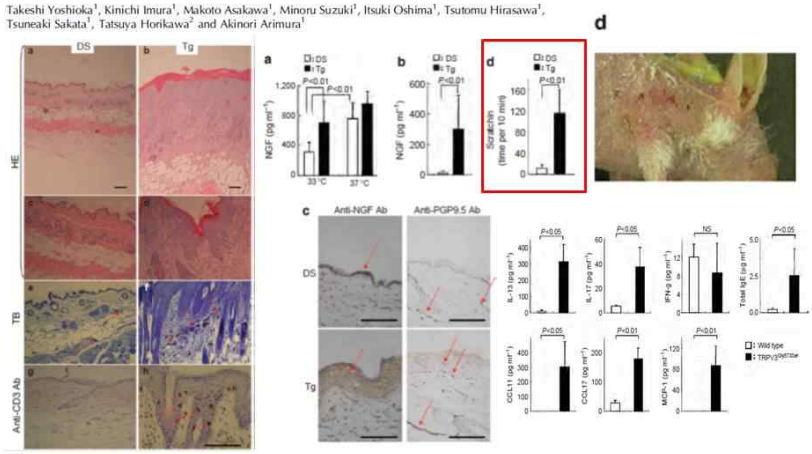
Yang YS et al. Acta Derma Venereol 2015;95:20-4

# TRPV3 and Post-burn Pruritus

- Increased activity of TRPV3 in keratinocytes in burn scars with post-burn pruritus

## Impact of the Gly573Ser Substitution in TRPV3 on the Development of Allergic and Pruritic Dermatitis in Mice

Takeshi Yoshioka[1], Kinichi Imura[1], Makoto Asakawa[1], Minoru Suzuki[1], Itsuki Oshima[1], Tsutomu Hirasawa[1], Tsuneaki Sakata[1], Tatsuya Horikawa[2] and Akinori Arimura[1]

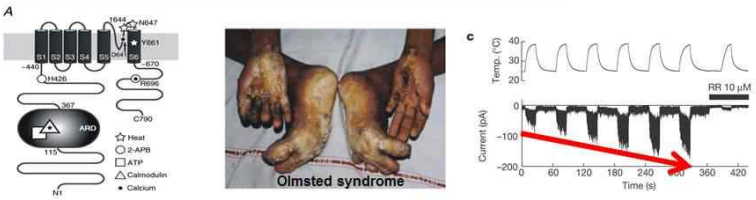



Yoshika T et al. J Invest Dermatol. 2009 Mar;129:714-22

## TRPV3: time to decipher a poorly understood family member!

Bernd Nilius[1], Tamás Bíró[2] and Grzegorz Owsianik[1]

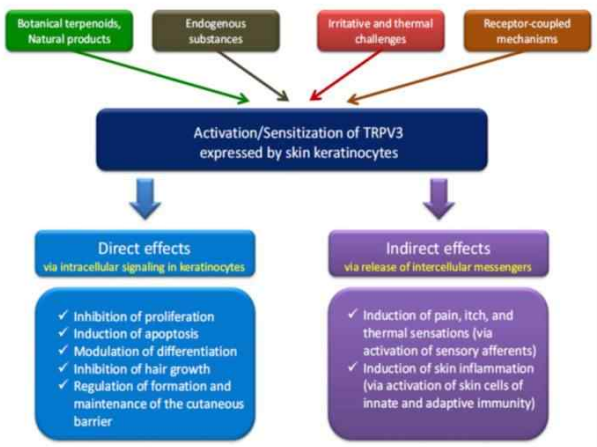
[1]KU Leuven, Department of Cellular and Molecular Medicine, Laboratory Ion Channel Research, Campus Gasthuisberg, Herestraat 49, bus 802, Leuven, Belgium
[2]DE-MTA 'Lendület' Cellular Physiology Research Group, Department of Physiology, University of Debrecen, Medical and Health Science Center, Research Center for Molecular Medicine, Debrecen, Hungary




- **TRPV3 is most abundantly expressed by skin cells**
  - Regulates proliferation, differentiation and apoptosis of human skin
    - Keratinocyte migration and wound healing via NO release
    - Hair follicle cycling
  - Olmsted syndrome; Multiple 'gain-of-funtion' mutations of TRPV3
    - hyper-orthokeratosis and keratomas, diffuse alopecia and **extreme pruritus**
  - Activation of TRPV3 on keratinocytes led to **pruritus and pro-inflammatory actions**
- **Shows unique activation properties**
  - **TRPV3 channel activity successively increases upon repeated stimulation**
  - **Intracellular ATP lowers sensitivity of TRPV3** to chemical agonist and **stabilizes** the sensitization induced by repeated stimulation by TRPV3 agonist

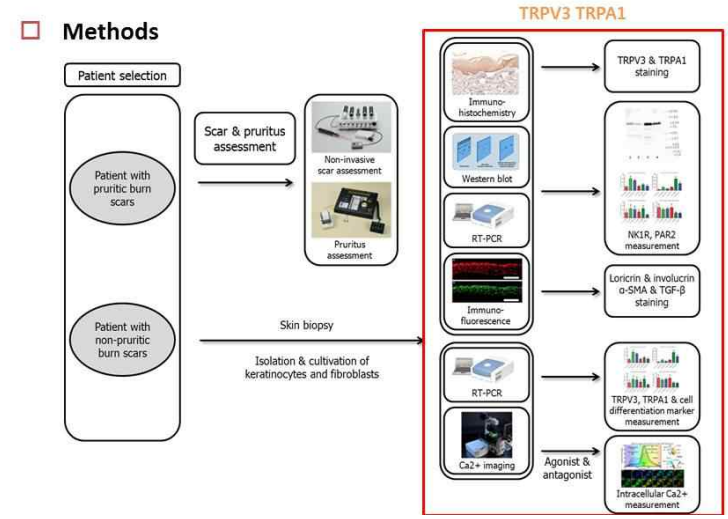
Xu et al. Nature. 2002;418:181-6
Bernd N et al. J Physiol. 2014;592:295-304

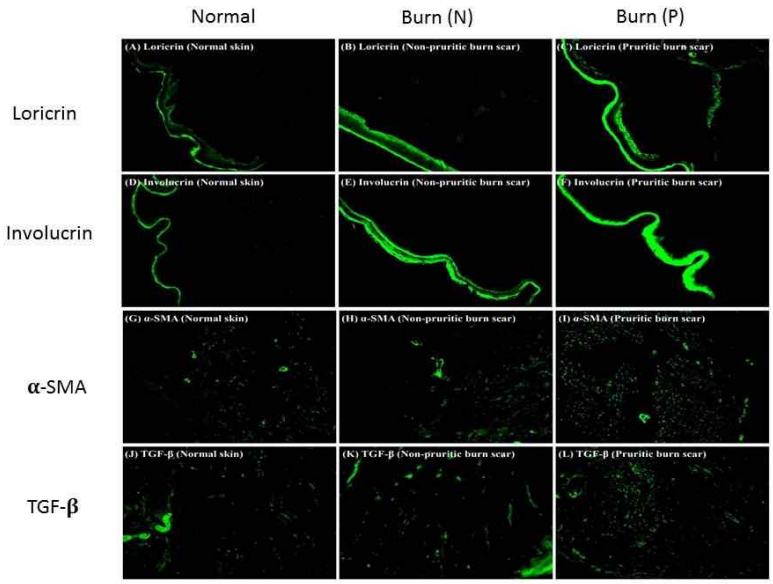
## Role of TRPV3 Channel in KC




NILIUS B et al. Exp Dermatol. 2013;22:447-52

# Increased Activity of TRPV3 in Keratinocytes in Burn Scars with Post-burn Pruritus




Kim HO et al. Wound Repair Regen 2016;24:841-50

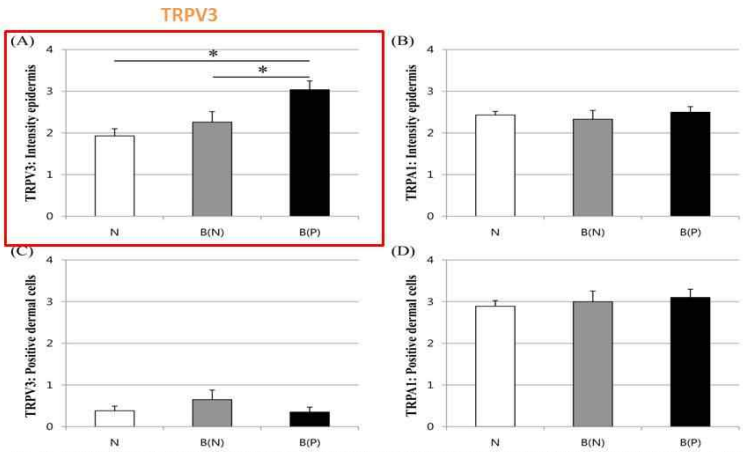



Fig. 2. Comparison of epidermal and dermal staining intensity of transient receptor potential vanilloid 3 (TRPV3) and TRP ankyrin 1 (TRPA1) in pruritic and non-pruritic burn scars, and normal skin. (A, B) Only TRPV3 expression in the epidermis was significantly increased in pruritic burn scars compared to non-pruritic burn scars and normal skin. (C, D) In the dermis, there was no significant difference of TRPV3 and TRPA1 expression among the three groups.
N: normal skin, B(N): non-pruritic burn scars, B(P): pruritic burn scars

Kim HO et al. Wound Repair Regen 2016;24:841-50

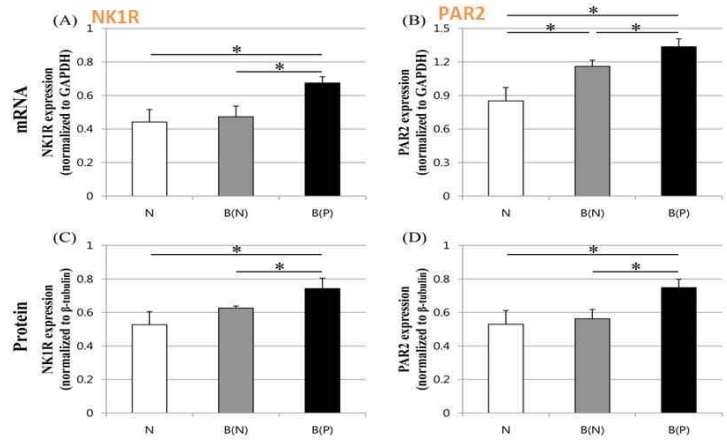



Fig. 3. Quantities of mRNA and protein of neurokinin-1 receptor (NK1R) and proteinase-activated receptor 2 (PAR2) among pruritic, non-pruritic burn scars and normal skin. (A, B) The mRNA of NK1R and PAR2 were significantly elevated in pruritic burn scars compared to non-pruritic burn scars and normal skin. Particularly, the quantity of mRNA in PAR2 was more elevated in non-pruritic burn scars than in normal skin. (C, D) The proteins NK1R and PAR2 were significantly elevated in pruritic burn scars compared to non-pruritic burn scars and normal skin. There was no difference in protein between non-pruritic burn scars and normal skin.
N: normal skin, B(N): non-pruritic burn scars, B(P): pruritic burn scars

Kim HO et al. Wound Repair Regen 2016;24:841-50

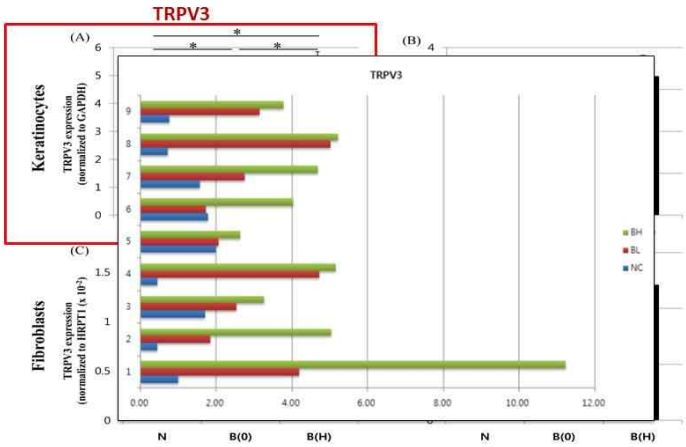
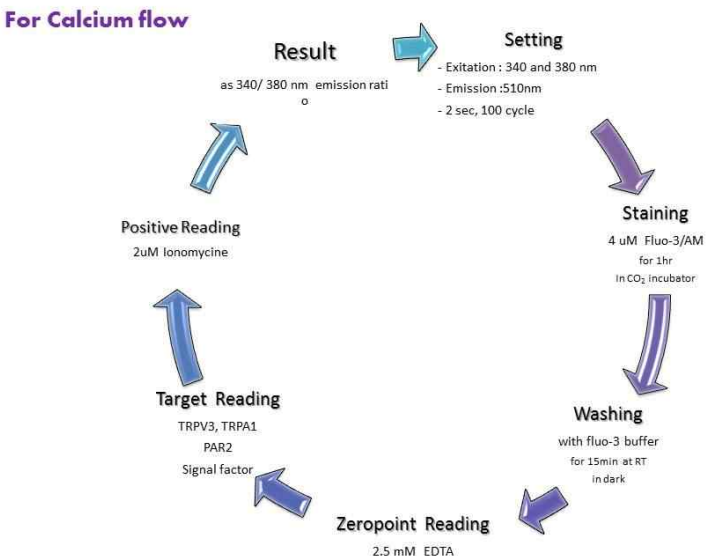


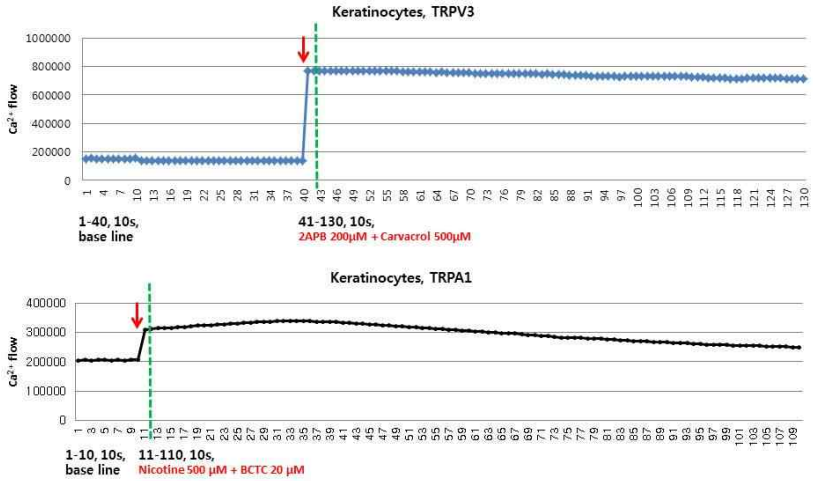

Fig. 4. Comparison of the quantity of mRNA of transient receptor potential vanilloid 3 (TRPV3) and TRP ankyrin 1 (TRPA1) in keratinocytes and fibroblasts. (A, B) In the keratinocytes, Only TRPV3 mRNA expression of pruritic burn scars was significantly higher than in that of non-pruritic burn scars and normal skin. (C, D) In the fibroblasts, quantities of mRNA of TRPV3 and TRPA1 exhibited no significant difference among the three groups.
N: normal skin, B(N): non-pruritic burn scars, B(P): pruritic burn scars

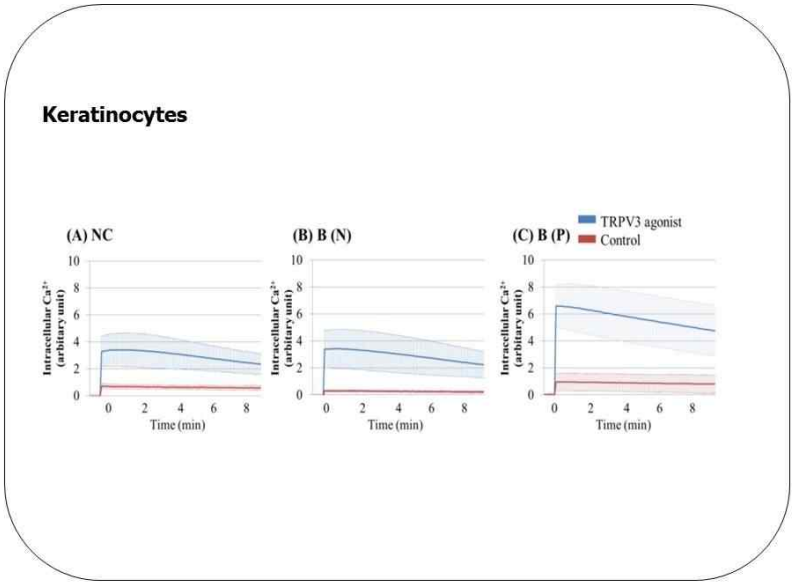
Kim HO et al. Wound Repair Regen 2016;24:841-50



## Finding Specific Activator/Deactivator

- TRPV3 agonist: **2ABP+carvacrol**, camphor
- TRPV3 antagonist: **DPTHF**
- TRPA1 agonist : **Nicotine+BCTC**, icillin
- TRPA1 antagonist: **HC-030031**




## Increased Activity of TRPV3 in Keratinocytes in Burn Scars with Post-burn Pruritus

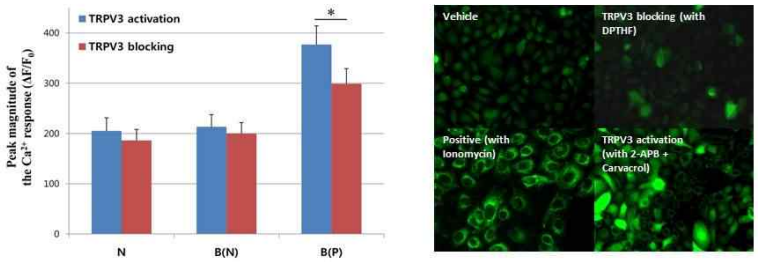
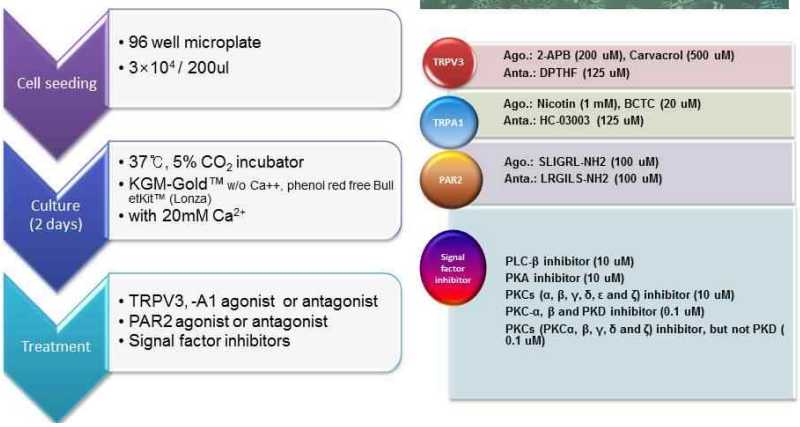



Fig. 7. Peak magnitude of the Ca2+ response on transient receptor potential vanilloid 3 (TRPV3) activation in the keratinocytes of normal skin, and in non-pruritic and pruritic burn scars: Compared to TRPV3 blocking, intracellular calcium levels in the keratinocyte were only increased in pruritic burn scars after TRPV3 activation.
N: normal skin, B(N): non-pruritic burn scars, B(P): pruritic burn scars

## Methods

- Experiments on cultured keratinocytes from
  - Normal skins, non-pruritic burn scars, pruritic burn scars
  - Looking for PAR2 effecting TRPV3 activation

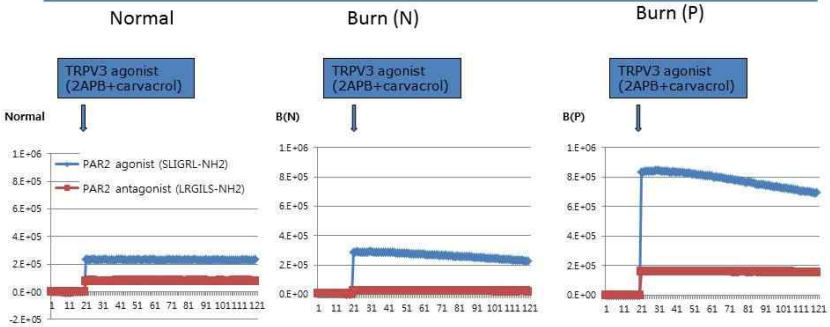



# Why PAR2?

- In our present study, PAR2 is highly expressed in the epidermis of pruritic burn scar tissue.
- Our previous study showed higher mast cell counts in pruritic burn scar tissue than non-pruritic burn scar.
- PAR2, expressed by sensory neurons and keratinocytes, is considered as a key molecule in itch sensation.
- It is considered that such as trypsin degraded from mast cell may have a role in postburn pruritus by affecting keratinocytes or nerve ending.

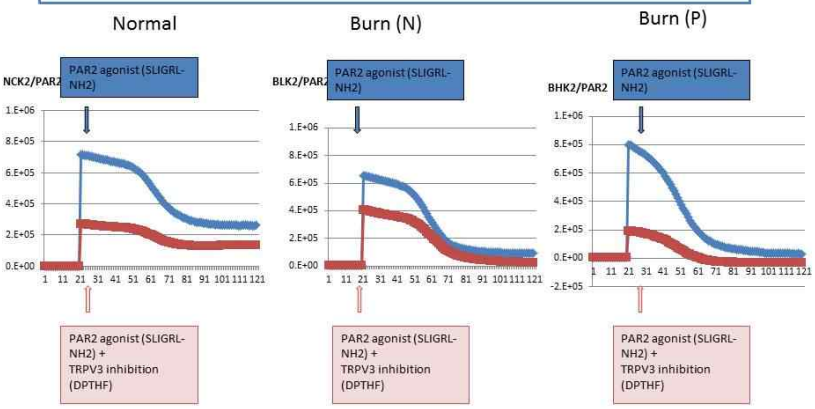
## TRPV3 Activation w or w/o PAR2 Activation

- In KCs from pruritic burn scar, PAR2 activation markedly potentiate opening TRPV3 channel.
- TRPV3 activation itself increase little $Ca^{++}$ influx with PAR2 inhibition in KCs.



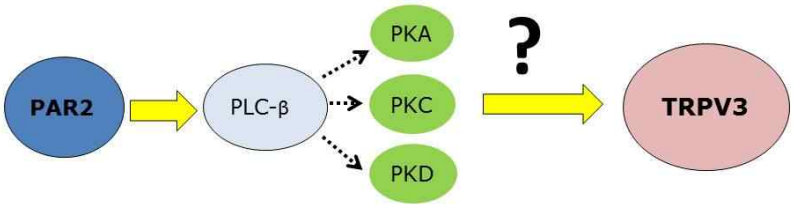
# TRPV3 Inhibition after PAR2 Activation

In KCs from all samples, activation of PAR2 effectively increase intracellular $Ca^{++}$ concentration.
Otherwise TRPV3 inhibition markedly abrogate PAR2-mediated calcium influx.

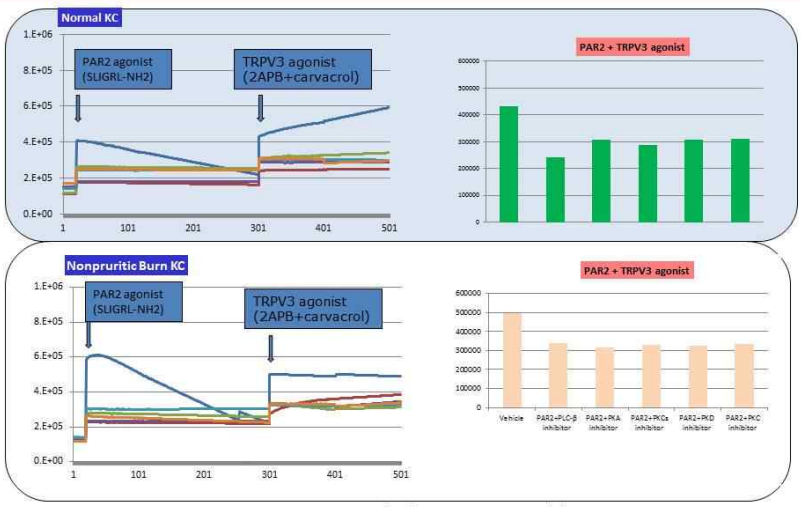



## Does PAR2 sensitize TRPV3 in KC?

- PAR2 (Protease-Activated Receptor 2)
  - **PAR2** has been reported to couple with PLC-β(Phospholipase-β) and to activate Protein Kinase A, C, D and sensitize **TRPV4**
  - Currently working on effects and mechanism of PAR2 on **TRPV3**



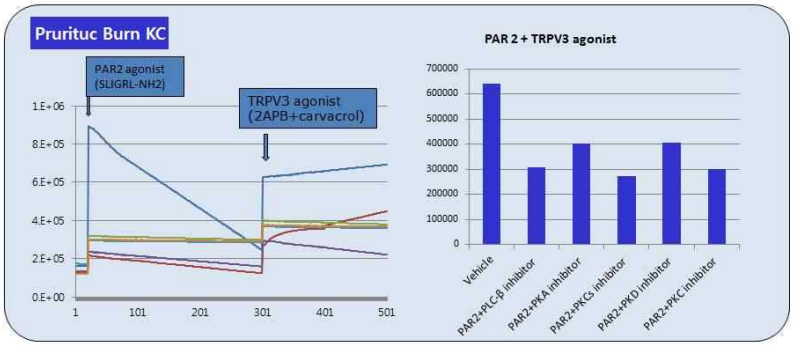

## TRPV3 Activation After PAR2 Pathway Inhibition




PLC-β inhibitor: U73122, 10um, PKA inhibitor: H-89, 10um
PKCs (PKCα, β, γ, δ, ε and ζ) inhibitor: GF109203X (bisindolylmaleimide 1), 10um
PKC-α, β and PKD inhibitor: Go6976, 0.1um
PKCs (PKCα, β, γ, δ and ζ) inhibitor, but not PKD: Go6983, 0.1um

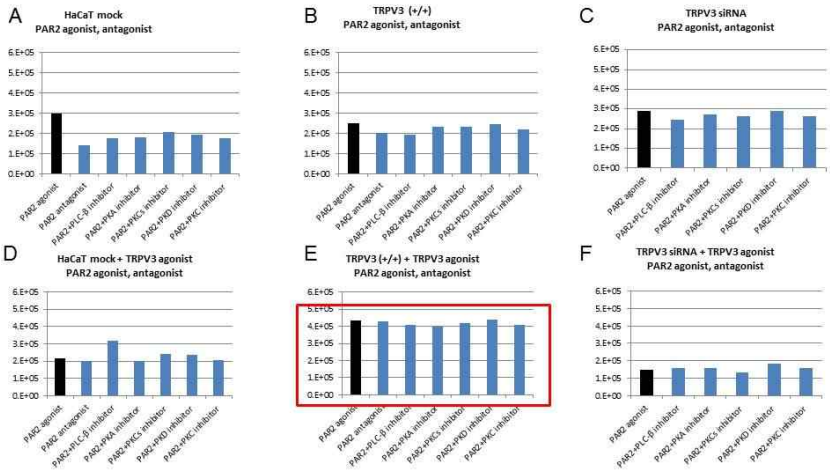
## TRPV3 Activation After PAR2 Pathway Inhibition

❖ In KCs from all samples, PLC-β, PKA, PKCs, PKD inhibitor markedly reduce intracellular $Ca^{++}$ level by TRPV3 activation as well as by PAR2 activation.



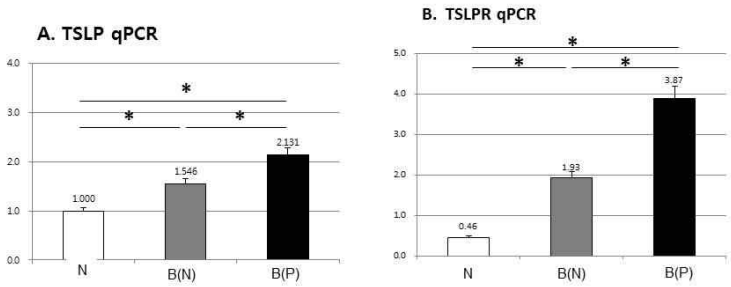

PLC-β inhibitor: U73122, 10um, PKA inhibitor: H-89, 10um
PKCs (PKCα, β, γ, δ, ε and ζ) inhibitor: GF109203X (bisindolylmaleimide 1), 10um
PKC-α, β and PKD inhibitor: Go6976, 0.1um
PKCs (PKCα, β, γ, δ and ζ) inhibitor, but not PKD: Go6983, 0.1um

## TRPV3 Activation w or w/o PAR2 Activation




**Comparision of calcium peak influx in HaCat mock, TRPV3 Plasmid inserted, and TRPV3 siRNA inserted keratonicytes.**
PAR2 agonist alone didn't make high calcium peak influx even in overexpressed TRPV3 keratinocytes (B).But TRPV3 overexpressed keratinocyte showed relatively high level of calcium peak influx with TRPV agonist even after PAR2 pathway inhibitors (E).
PLC-β inhibitor: U73122, 10um, PKA inhibitor: H-89, 10um
PKCs (PKCα, β, γ, δ, ε and ζ) inhibitor: GF109203X (bisindolylmaleimide 1), 10um
PKC-α, β and PKD inhibitor: Go6976, 0.1um
PKCs (PKCα, β, γ, δ and ζ) inhibitor, but not PKD: Go6983, 0.1um

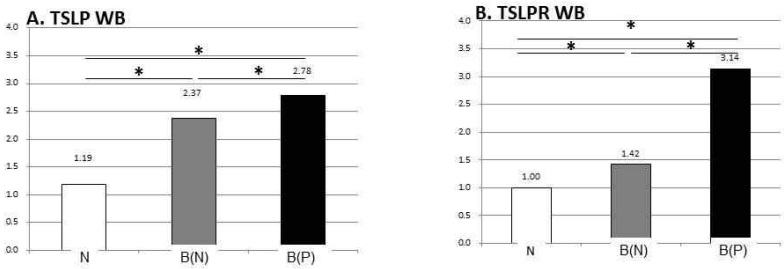
## Expression of TSLP and TSLPR in burn scar




**Comparision of quantity of mRNA of TSLP (A) and TSLPR (B) in keratonicytes in normal, nonpruritic, and pruritic burn keratinocyte.**
mRNA of TSLP was measured by qPCR. Pruritic burn scar showed the highest expression of TSLP & TSLPR mRNA compared to normal and non-pruritic burn scars.

TSLP, thymic stromal lymphopoietin; TSLPR thymic stromal lymphopoietin receptor
N: normal skin, B(N): non-pruritic burn scars, B(P): pruritic burn scars

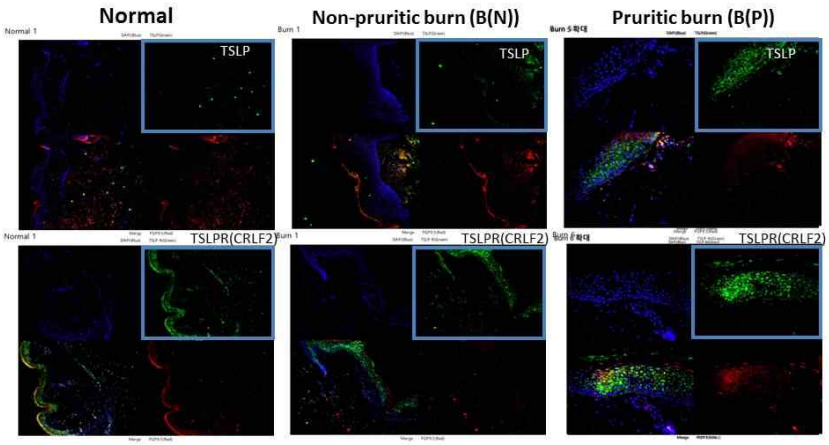
## Expression of TSLP and TSLPR in burn scar




**Comparision of quantity of protein of TSLP (A) and TSLPR (B) in keratonicytes in Normal, nonpruritic, and pruritic burn keratinocyte.**
TSLP and TSLPR protein were measured by western blotting. Pruritic burn scar showed the highest protein level of TSLP and TSLPR compared to normal and non-pruritic burn scars.

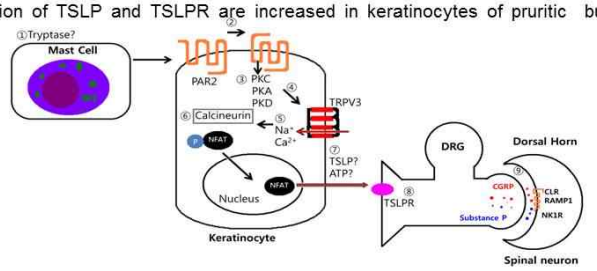
TSLP, thymic stromal lymphopoietin; TSLPR thymic stromal lymphopoietin receptor
N: normal skin, B(N): non-pruritic burn scars, B(P): pruritic burn scars

## Expression of TSLP and TSLPR in burn scar



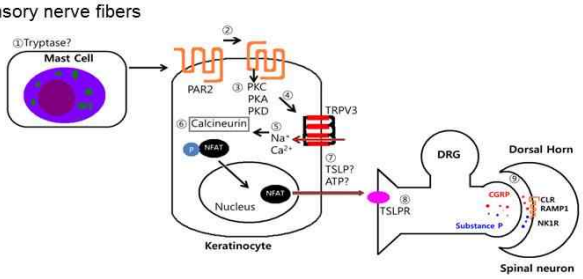

# Conclusion

- Expression of TRPV3 is increased and activated in keratinocytes of burn scar with post-burn pruritus.
- Activation of PAR2 on keratinocytes induce rapid TRPV3 activation and induce inward current of $Ca^{++}$.
- PAR2 sensitize TRPV3 channels with PKA, PKC, PKD signaling pathway.
- PAR2 itself can increase intracellular $Ca^{++}$ but it is markedly decreased if TRPV3 is blocked.
- Expression of TSLP and TSLPR are increased in keratinocytes of pruritic burn scar.

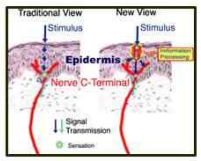


# Further study

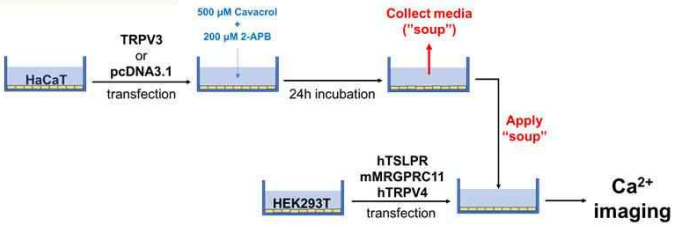
- How can TRPV3 calcium flow in keratinocytes, being non-excitable cell, be transmitted to nerves? Hypothesis
  - $Ca^{2+}$ waves in keratinocytes are transmitted to sensory neurons mediated by extracellular ATP
  - TSLP excretion from KC increased by TRPV3 activation
- Currently studying for
  - The role and mechanism of TSLP and ATP, released by keratinocytes, on sensory nerve fibers




**In vitro Model for itching**

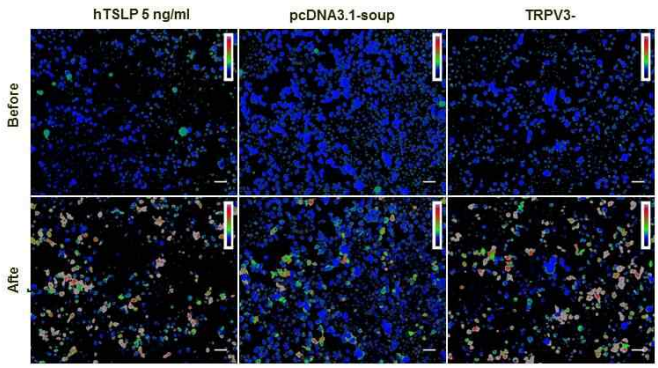


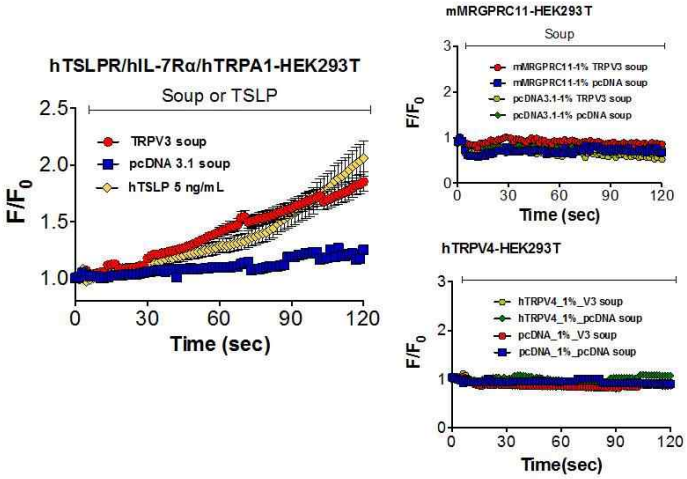

**Experimental scheme**





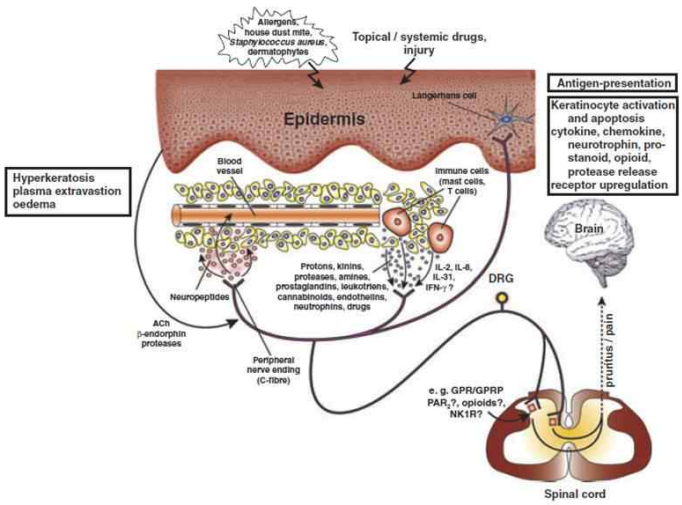

**hTSLPR/hIL-7Rα/hTRPA1-HEK293T**








## Pathophysiology of Itching in AD






Buddenkotte J et al. Allergy 2010: 65; 805-821

감사합니다.

# Promises and Challenges of Improving Biomarkers for Atopic Dermatitis

**DirkJan Hijnen**
Erasmus Medical Center, The Netherlands

Atopic dermatitis (AD) is a challenging disease in several ways. A major challenge is the heterogeneity of the disease. There have been many attempts to stratify the disease by using aspects such as allergic comorbidity (asthma and allergic rhinitis), and age of onset. These patient characteristics do however not predict response to treatment or the course of the disease. Recent technical advances have allowed determination of large numbers of potential biomarkers in different tissues. With many new targeted treatments for treatment of AD in development, these biomarkers may be helpful in predicting response to treatment on our route to precision medicine.

Determination of disease severity has also been a challenging topic for AD research. Many different tools for measuring disease severity have been used in the past (eg. EASI and SCORAD), each of which had advantages as well as disadvantages. We now have biomarkers that can be determined in skin or blood may provide better tools for objective determination of disease severity.

Keywords : Atopic dermatitis, Biomarkers, Disease severity, Precision medicine

# Curriculum Vitae

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| | |
|---|---|
| 1986-1992 | Secondary School (Atheneum B); Macropedius College |
| 1992-1993 | Medical School; University of Antwerp (RUCA) – Belgium |
| 1993-1999 | Medical Biology, University of Amsterdam (graduation November 1999, MSc) |
| 1997-2002 | Medical School, University of Utrecht (graduation March 2002, MD) |
| 2002-2007 | PhD Student, Department of Dermatology, University Medical Center Utrecht. |

**Professional Training and Employment**

| | |
|---|---|
| 2007-2008 | Post-Doctoral Research Fellow, Brigham and Women's Hospital, Department of Dermatology & Harvard Skin Disease Research Center, Harvard Medical School, Boston, MA. |
| 2006-2011 | Residency in Dermatology, Department of Dermatology, University Medical Center Utrecht |
| 2011-2015 | Dermatologist, Diakonessenhuis Utrecht & Zeist |
| 2011-2017 | Dermatologist & Physician Scientist, University Medical Center Utrecht |
| 2017-present | Dermatologist & Physician Scientist, Erasmus MC Rotterdam, DirectorExpertise Center for Atopic Dermatitis Rotterdam |

**Selected Publications**

1. Thijs JL, StricklandI, Bruijnzeel-Koomen CAFM, Nierkens S, Giovannone B, Knol EF, Csomor E, Sellman BR, Mustelin T, SleemanMA, de Bruin-Weller MS, Herath A, Drylewicz J,May RD, **Hijnen D**. Serum biomarker profiles suggest that atopic dermatitis is a systemic disease. *J Allergy Clin Immunol*. 2018 Apr;141(4):1523-1526
2. Petrelli A,Mijnheer G, van Konijnenburg DPH, van der Wal MM, Giovannone B, Mocholi E, VazirpanahN, Broen JC, **Hijnen D**, Oldenburg B, Coffer PJ, Vastert SJ, Prakken BJ, Spierings E, Pandit A, MokryM, vanWijk F. PD-1+CD8+ T cells are clonally expanding effectors in human chronic inflammation, *J Clin Invest*. 2018 Sep 10
3. Thijs JL, Drylewicz J, Fiechter R, Strickland I, SleemanMA, Herath A, May RD, Bruijnzeel-Koomen CAFM, Knol EF, Giovannone B, deBruin-WellerMS, Nierkens S, **Hijnen DJ**. EASI p-EASI: Utilizing a combination of serum biomarkers offers an objective measurement toolfor disease severity in atopic dermatitis patients. *J Allergy Clin Immunol*. 2017 Sep;140(3):730-737
4. Thijs JL, StricklandI, Bruijnzeel-Koomen CAFM, Nierkens S, Giovannone B, Csomor E, Sellman BR, Mustelin T, Sleeman, de Bruin-Weller MS, Herath A, Drylewicz J, May RD, **Hijnen DJ**. Moving towards endotypes in atopic dermatitis: identification of patient clusters based on serum biomarker analysis, *J Allergy Clin Immunol*. 2017 Sep;140(3):730-737
5. Thijs JL, Knipping K, Bruijnzeel-Koomen CA, Garssen J, de Bruin-Weller MS, **Hijnen DJ**. Immuno-globulin free light chains in adult atopic dermatitis patients do not correlate with disease severity. *Clin Transl Allergy*. 2016 Dec 6;6:44
6. Thijs JL, Herath A, de Bruin-Weller MS, **Hijnen DJ**. Multiplexplatform technology and bioinformatics are essential for development of biomarkers in atopic dermatitis. *J Allergy Clin Immunol*. 139(3), 1065
7. Knol EF, **Hijnen D**. Atopic dermatitis: A tale of two distinct pathomechanisms that make you itch. *Eur J Immunol*. 2016 Nov; 46(11): 2512-2515

8. Thijs J, Krastev T, Weidinger S, Buckens CF, de Bruin-Weller M, Bruijnzeel-Koomen C, Flohr C, **Hijnen D**. Biomarkers for atopic dermatitis: a systematic review and meta-analysis. *Curr Opin Allergy Clin Immunol*. 2015 Oct;15(5):453-460.
9. Thijs JL, van Seggelen W, Bruijnzeel-Koomen C, de Bruin-Weller M, **Hijnen D**. New Developments in Biomarkers for Atopic Dermatitis. *J ClinMed*. 2015 Mar 16;4(3):479-87
10. Thijs JL, Nierkens S, Herath A, Bruijnzeel-Koomen CA, Knol EF, Giovannone B, deBruin-Weller MS, **Hijnen DJ**. A panel of biomarkers for disease severity in atopic dermatitis. *Clin Exp Allergy*. 2015 Mar;45(3):698-701.
11. **Hijnen D**, Knol EF, Gent YY, Giovannone B, Beijn SJ, Kupper TS, Bruijnzeel-Koomen CA, Clark RA. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22. *J Invest Dermatol*. 2013;133(4):973-9.
12. Landheer J, Giovannone B, Mattson JD, Tjabringa S, Bruijnzeel-Koomen CA, McClanahan T, de Waal Malefyt R, Knol E, **Hijnen D**. Epicutaneous application of house dust mite induces thymic stromal lymphopoietin in nonlesional skin of patients with atopic dermatitis. *J Allergy Clin Immunol*. 2013;132(5):1252-4.

# Skin-resident Natural Killer T Cells Develop Cutaneous Allergic Inflammation in Atopic Dermatitis

**Chang Ook Park**
Yonsei University, Korea

Natural killer T (NKT) cells are unconventional T cells that bridge innate and adaptive immunity. NKT cells have been implicated in the development of atopic dermatitis (AD). We demonstrated that CXCR4 and its cognate ligand CXCL12 were significantly up-regulated in human AD skin in global transcriptomic analysis; proteomic analysis revealed that $CXCR4^+$ NKT cells were enriched in AD skin and were consistently elevated in our AD mouse model. Adoptive transfer of allergen-specific NKT cells conferred antigen-specific cutaneous inflammation, and predominant skin NKT cells were $CXCR4^+$ and $CD69^+$, similar to tissue-resident memory T (TRM) cells. Skin-resident NKT cells uniquely expressed CXCR4, which was confirmed using a parabiosis system. Intravital imaging showed that $CXCR4^+$ NKT cells preferentially trafficked to a CXCL12-rich area, forming an enriched $CXCR4^+$ NKTRM/$CXCL12^+$ cell cluster, which developed acute and chronic allergic inflammation. $CXCR4^+$ NKTRM cells may form a niche to develop AD, where CXCL12 is highly expressed.

Keywords : Atopic dermatitis, Natural killer T cells, Tissue-resident memory T (TRM) cells, CXCR4, CXCL12, Thymic stromal lymphopoietin

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**Education and Appointment**

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1998-2002 MD in Medicine, Yonsei University College of Medicine, Seoul, Korea
2004-2006 MS in Medicine, Yonsei University College of Medicine, Seoul, Korea
2010-2015 PhD in Medicine, Yonsei University College of Medicine, Seoul, Korea

**Professional Training and Employment**

2002-2003 Internship, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea
2003-2007 Dermatology Residency, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea
2007-2009 Chief Dermatologist, The Armed Forces Gangneung Hospital, Gangneung, Korea
2009-2010 Chief Dermatologist, The Armed Forces Byukjae Hospital, Goyang, Korea
2010-2011 Instructor, Deparment of Dermatology, Yonsei University College of Medicine, Seoul, Korea
2011-2012 Clinical Research Assistant Professor, Deparment of Dermatology, Yonsei University College of Medicine, Seoul, Korea
2012-2016 Postdoc in Skin T cells Skin Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston
2014-2016 Clinical Research Assistant Professor, Deparment of Dermatology, Yonsei University College of Medicine, Seoul, Korea
2016-present Assistant Professor, Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea

**Selected Publications**

1. Park CO, Fu X, Jiang X, Pan Y, Teague JE, Collins N, Tian T, O'Malley JT, Emerson RO, Kim JH, Jung Y, Watanabe R, Fuhlbrigge RC, Carbone FR, Gebhardt T, Clark RA, Lin CP, Kupper TS. Staged development of long lived TCRαβ Th17 resident memory T cell population to Candida albicans after skin infection. J Allergy Clin Immunol. 2018 Aug;142(2):647-662.
2. Lee JW, Kim DS, **Park CO**. Circumscribed palmoplantar hypokeratosis: successful treatment with the 10 600-nm carbon dioxide fractional laser. *J Eur Acad Dermatol Venereol*. 2017 Oct;31(10):e473-e474.
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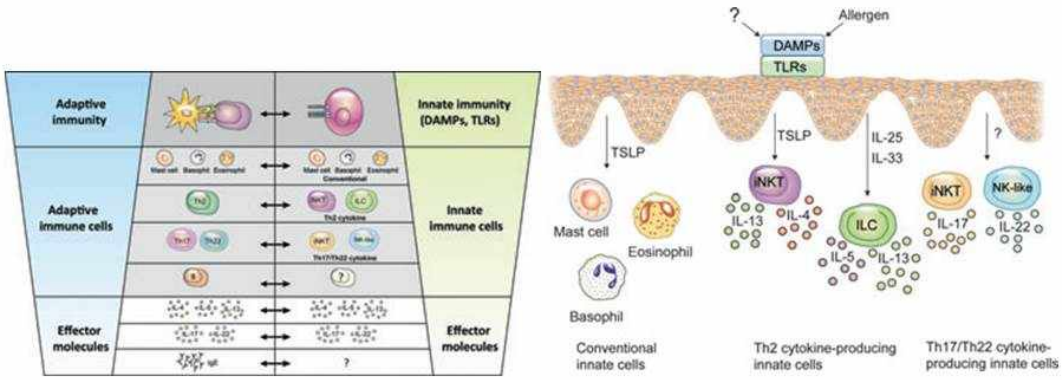
# New immunologic approaches: innate immunity in Atopic dermatitis

| Extrinsic | Intrinsic |
|---|---|
| ● Most common form of AD (70-80%).<br>● Also allergic AD.<br>● Elevated total and allergen-specific IgE in serum and skin. | ● Remaining (20-30%) patients have non-allergic AD.<br>● Normal total IgE levels and negative serum allergen-specific IgE. |

# New immunologic approaches: innate immunity in Atopic dermatitis

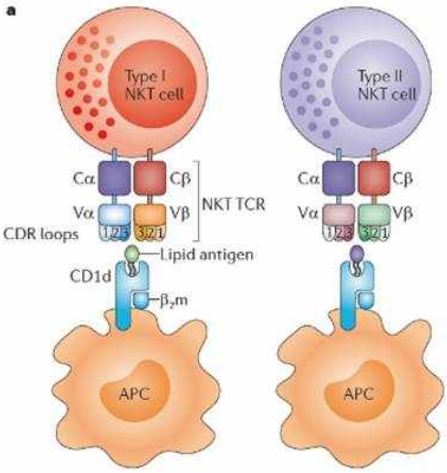
- The treatments of non-allergic AD patients are often challenging, thus new immunologic approach such as thymic stromal lymphopoietin (TSLP) activated invariant natural killer T (iNKT) cells is required.




CO Park, et al. **Exp Dermatol.** 2013 Jan;22

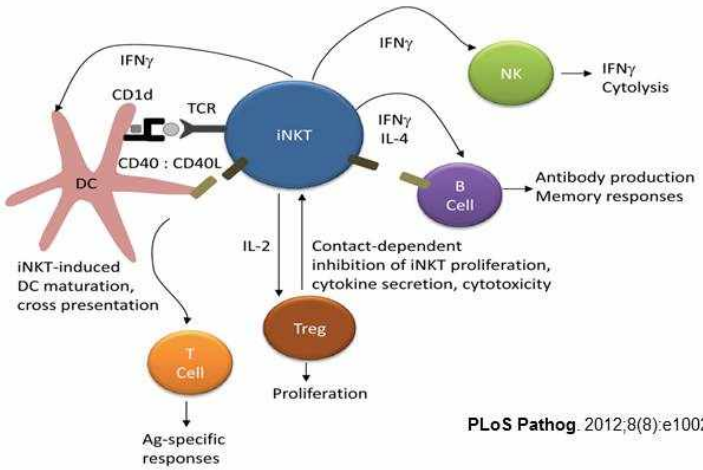
# Natural killer T (NKT) cell

- Natural killer T (NKT) cells co-express a T cell receptor (TCR) along with typical surface receptors for natural killer cells.
- More than 80% of NKT cells express invariant TCRα chain Vα24;Vβ11 in humans and are referred to as invariant NKT (iNKT) cells.




**Nat Rev Immunol**. 2012 Dec;12(12):845-57

# Natural killer T (NKT) cell



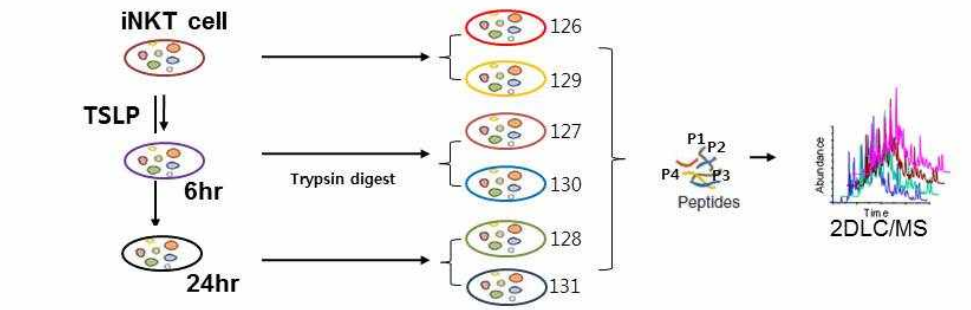

**PLoS Pathog**. 2012;8(8):e1002838

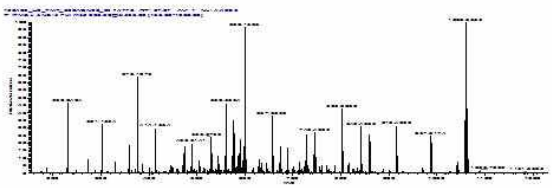
- iNKT cells recognize CD1d-associated glycolipid and rapidly produce cytokines including IL-4 and IFN-γ.
- This cytokines regulates adaptive immune responses by activation of dendritic cells (DCs), natural killer cells, B cells, and conventional T cells, thus linking innate and adaptive immunity.

# Objective

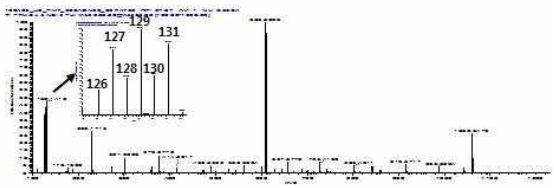
**To identify the AD-related innate allergic immune modulatory proteins and validate its role in iNKT cells**

## Protein quantification using in-vitro labeling, Tandem Mass Tag (TMT)



CID-MS/MS for peptide identification



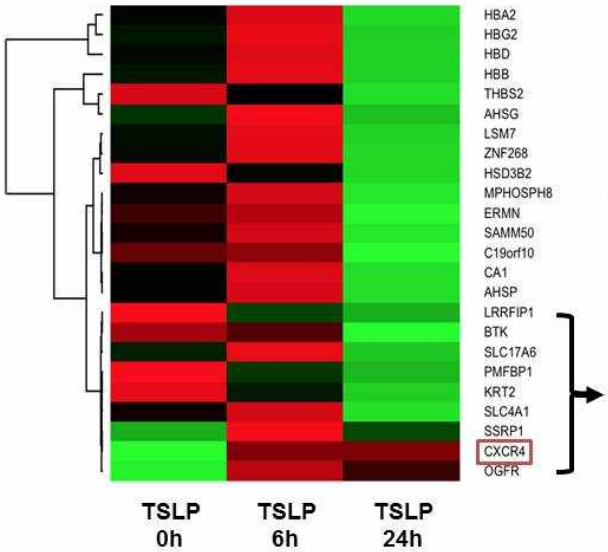

HCD-MS/MS for quantification

# iNKT Cell Protein list of ratio

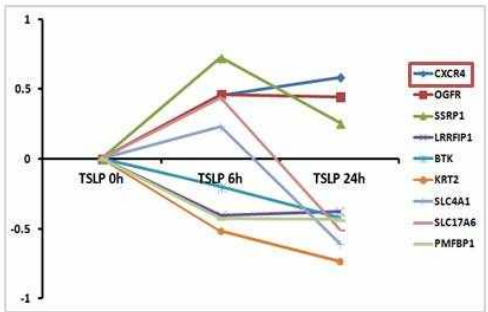
| Index | DESCRIPTION | avg(-) | avg(6hr) | avg(24hr) | Log2 avg(-) | Log2 avg(6hr) | Log2 avg(24hr) | Fit ratio 6hr/(-) | Fit ratio 24hr/(-) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | GS=ERMN Isoform 2 of Ermin | 20518.75 | 23889.25 | 11376.5 | 14.32 | 14.54 | 13.47 | 0.096 | -0.839 |
| 2 | GS=KRT2 Keratin, type II cytoskeletal 2 epidermal | 4813.25 | 3664.125 | 2867.875 | 12.23 | 11.84 | 11.49 | -0.517 | -0.735 |
| 3 | GS=HBA2;HBA1 Hemoglobin alpha-2 | 317319.46 | 450704.73 | 193931.34 | 18.28 | 18.78 | 17.57 | 0.383 | -0.698 |
| 4 | GS=HBA2;HBA1 Hemoglobin subunit alpha | 329927.01 | 467712.4 | 202272.28 | 18.33 | 18.84 | 17.63 | 0.380 | -0.694 |
| 5 | GS=HBD Hbbm fused globin protein (Fragment) | 370413.3 | 539026.95 | 232897.44 | 18.50 | 19.04 | 17.83 | 0.418 | -0.657 |
| 6 | GS=THBS2 Thrombospondin-2 | 92871.96 | 76466.595 | 58523.265 | 16.50 | 16.22 | 15.84 | -0.403 | -0.654 |
| 7 | GS=HBB Hemoglobin subunit beta | 296839.05 | 425536.77 | 190167.88 | 18.18 | 18.70 | 17.54 | 0.397 | -0.630 |
| 8 | GS=HBG2 Hemoglobin subunit gamma-2 | 267729.92 | 397001.77 | 172117.56 | 18.03 | 18.60 | 17.39 | 0.445 | -0.625 |
| 9 | GS=HBB Truncated beta-globin (Fragment) | 164620.29 | 242638.93 | 106622.43 | 17.33 | 17.89 | 16.70 | 0.437 | -0.615 |
| 10 | GS=LSM7 R30783_1 | 42283.165 | 61396.33 | 27457.665 | 15.37 | 15.91 | 14.74 | 0.415 | -0.611 |
| 11 | GS=HBD Hemoglobin subunit delta | 175224.65 | 252391.8 | 114006.54 | 17.42 | 17.95 | 16.80 | 0.403 | -0.608 |
| 12 | GS=SLC4A1 Solute carrier family 4, anion exchanger, member 1 | 5428.5 | 6950.335 | 3541.33 | 12.41 | 12.76 | 11.79 | 0.233 | -0.604 |
| 13 | GS=HBD Hemoglobin Lepore-Baltimore (Fragment) | 150925.98 | 215449.41 | 101266.51 | 17.20 | 17.72 | 16.63 | 0.390 | -0.564 |
| 14 | GS=CA1 Carbonic anhydrase 1 | 15367.86 | 20284.035 | 10536.595 | 13.91 | 14.31 | 13.36 | 0.277 | -0.532 |
| 15 | GS=AHSG Alpha-2-HS-glycoprotein | 68062.52 | 111812.1 | 47272.8 | 16.05 | 16.77 | 15.53 | 0.593 | -0.514 |
| 16 | GS=SLC17A6 Vesicular glutamate transporter 2 | 9310.335 | 13721 | 6502.835 | 13.18 | 13.74 | 12.67 | 0.436 | -0.506 |
| 17 | GS=ZNF268 zinc finger protein 268 isoform c | 35780 | 47307 | 25798.83 | 15.13 | 15.53 | 14.66 | 0.280 | -0.460 |
| 18 | GS=HSD3B2 Isoform 1 of 3 beta-hydroxysteroid dehydrogenase/ Delta 5-- 4-isomerase type 2 | 36153.75 | 30982.5 | 26365.25 | 15.14 | 14.92 | 14.69 | -0.346 | -0.443 |
| 19 | GS=SAMM50 Sorting and assembly machinery component 50 homolog | 18207 | 21886.165 | 13280.83 | 14.15 | 14.42 | 13.70 | 0.142 | -0.443 |
| 20 | GS=PMFBP1 Isoform 3 of Polyamine-modulated factor 1-binding protein 1 | 5754.665 | 4665.5 | 4227.165 | 12.49 | 12.19 | 12.05 | -0.426 | -0.433 |
| 21 | GS=C19orf10 UPF0556 protein C19orf10 | 19095.75 | 19680 | 14042 | 14.22 | 14.26 | 13.78 | -0.080 | -0.431 |
| 22 | GS=MPHOSPH8 Isoform 2 of M-phase phosphoprotein 8 | 19986.25 | 24059.25 | 14709 | 14.29 | 14.55 | 13.84 | 0.145 | -0.430 |
| 23 | GS=BTK Tyrosine-protein kinase BTK | 7233.93 | 6859.785 | 5362.145 | 12.82 | 12.74 | 12.39 | -0.200 | -0.420 |
| 24 | GS=AHSP Alpha-hemoglobin-stabilizing protein | 14324.5 | 17662.25 | 10814.25 | 13.81 | 14.11 | 13.40 | 0.179 | -0.394 |
| 25 | GS=LRRFIP1 Isoform 1 of Leucine-rich repeat flightless-interacting protein 1 | 7914.07 | 6521.785 | 6041.5 | 12.95 | 12.67 | 12.56 | -0.402 | -0.377 |
| 26 | GS=SSRP1 FACT complex subunit SSRP1 | 2843.5 | 5130 | 3380.5 | 11.47 | 12.32 | 11.72 | 0.728 | 0.262 |
| 27 | GS=OGFR Isoform 2 of Opioid growth factor receptor | 455.75 | 683.5 | 615.25 | 8.83 | 9.42 | 9.27 | 0.462 | 0.445 |
| 28 | GS=CXCR4 Isoform 2 of C-X-C chemokine receptor type 4 | 2417.5 | 3600.5 | 3595 | 11.24 | 11.81 | 11.81 | 0.452 | 0.585 |

# Differentially expression profiles of TSLP 0h, 6h, and 24h-treated iNKT cell groups

- Heat map analysis

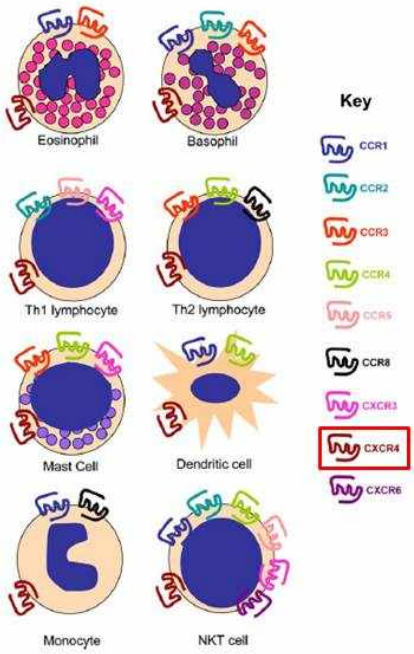



- Gaussian fit ratios of 9 proteins clustering in the same gene group



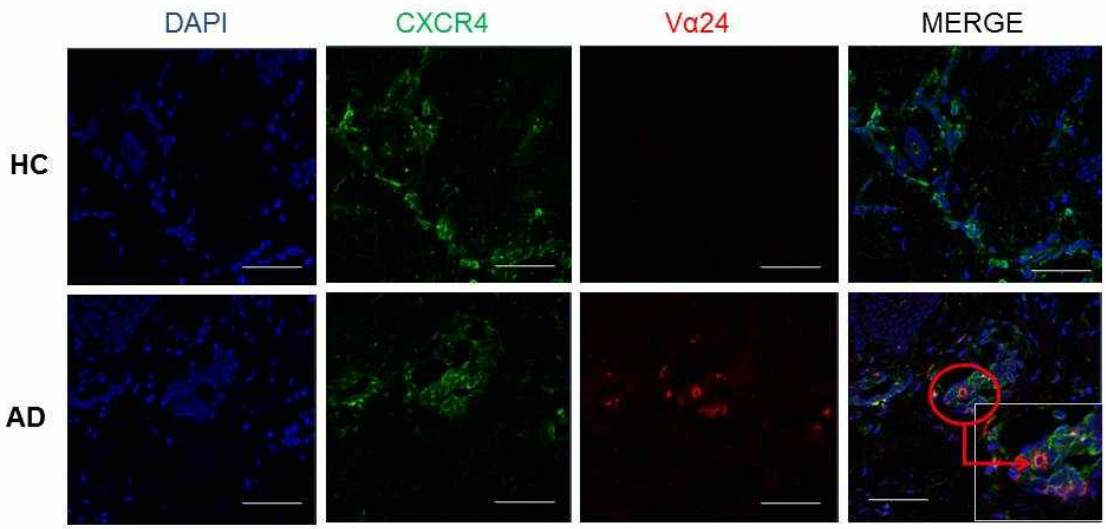
# CXCR4

- An alpha-chemokine receptor having a 7 transmembrane domain, G-protein-coupled cell surface receptor which was initially cloned in 1994.
- specific for stromal-derived-factor 1 (SDF1α, also called CXCL12).
- A molecule endowed with potent chemotactic activity for lymphocytes (B cell, pDC, NK cell).




**J Allergy Clin Immunol**. 2006 Aug;118(2):305-18

## Expression of CXCR4 in Vα24+ iNKT cells in the human skin of healthy controls and AD patients

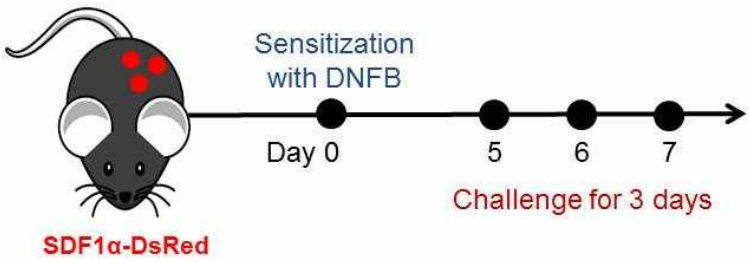


# SDF1α: cognate ligand for CXCR4

- Now designated as CXCL12.
- Produced in bone marrow, spleen, liver, lymph nodes, and fibroblasts.
- Binding of SDF1α to CXCR4 activates signaling pathways that control cell proliferation, survival, and migration.
- The CXCR4/SDF1α chemokine axis is essential for leukocyte trafficking, for immune homeostasis in secondary lymphoid organs.
- In AD, the role of CXCR4/SDF1α is rarely discussed.

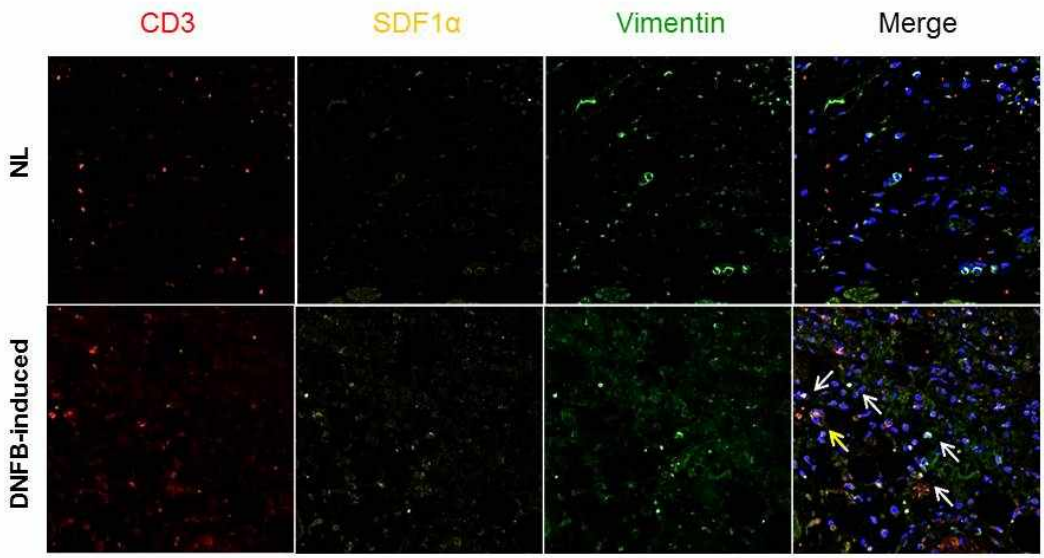
## DNFB-induced in SDF1α-DsRed transgenic mouse

- SDF1α-DsRed transgenic mouse: express DsRedE2 from the endogenous *Cxcl12* (chemokine (C-X-C motif) ligand 12), mouse promoter.
- DsRed (stained with anti-RFP) is primarily expressed by perivascular stromal cells and endothelial cells throughout the bone marrow.
- DNFB were treated to SDF1α-DsRed mouse for 3 days, 5 day after the sensitization.



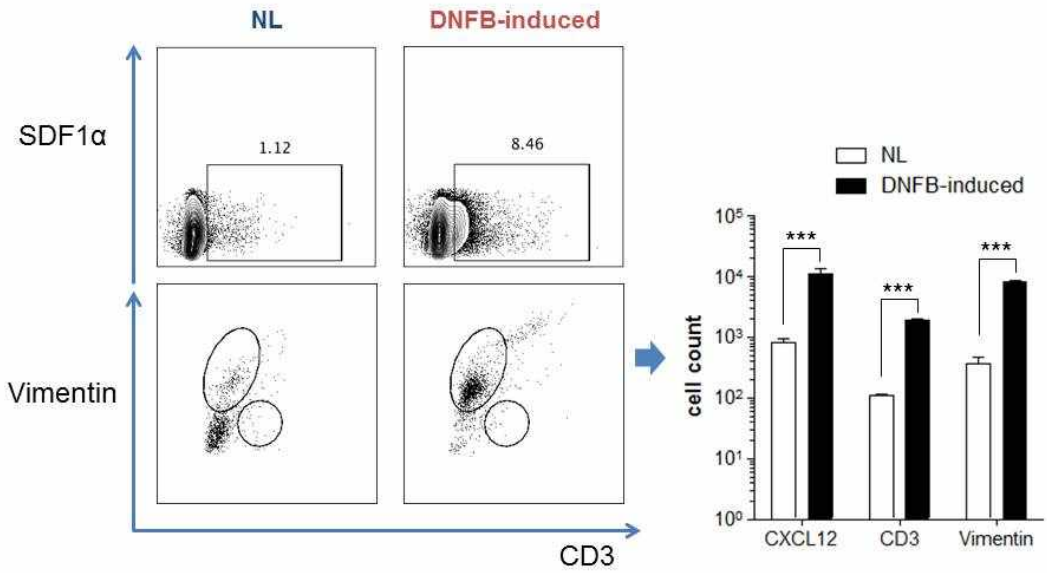
## Increased SDF1α expression in the lesional skin of DNFB-induced mouse

- **SDF1α expressions in DNFB-induced SDF1α-DsRed mouse**

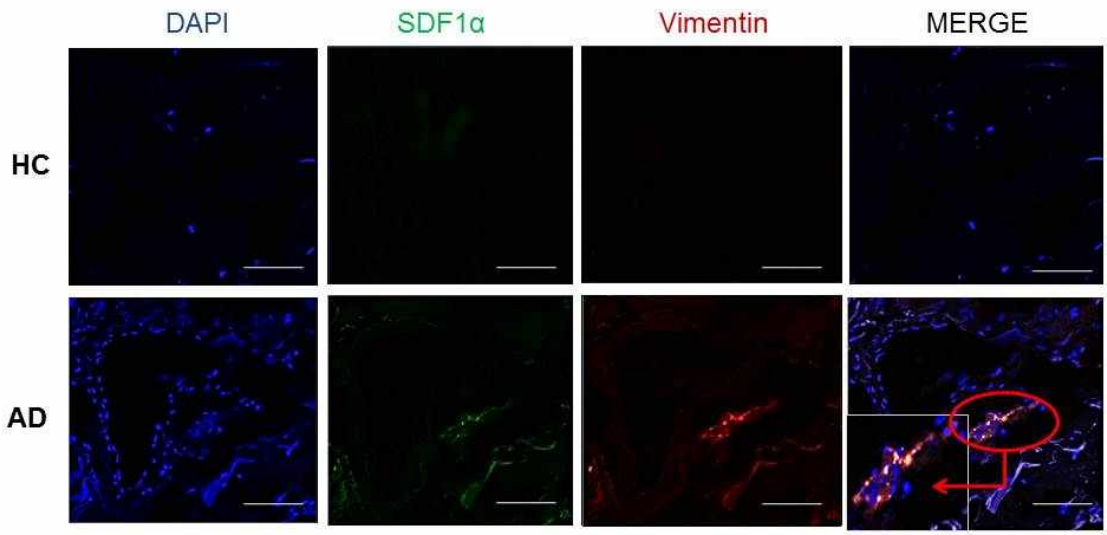



## Increased SDF1α expression in the lesional skin of DNFB-induced mouse

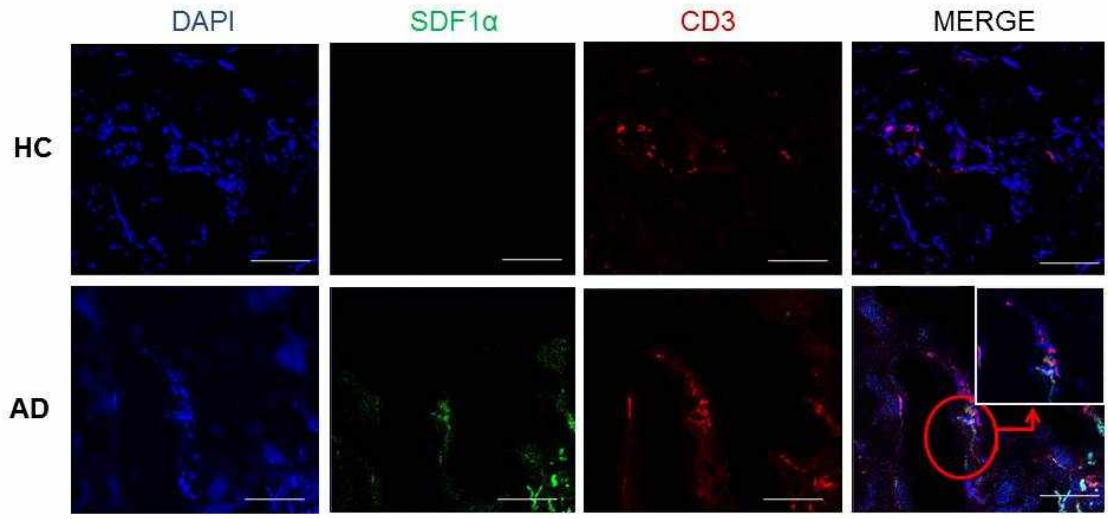
- **DNFB-induced SDF1α production in T cell and dermal fibroblasts**



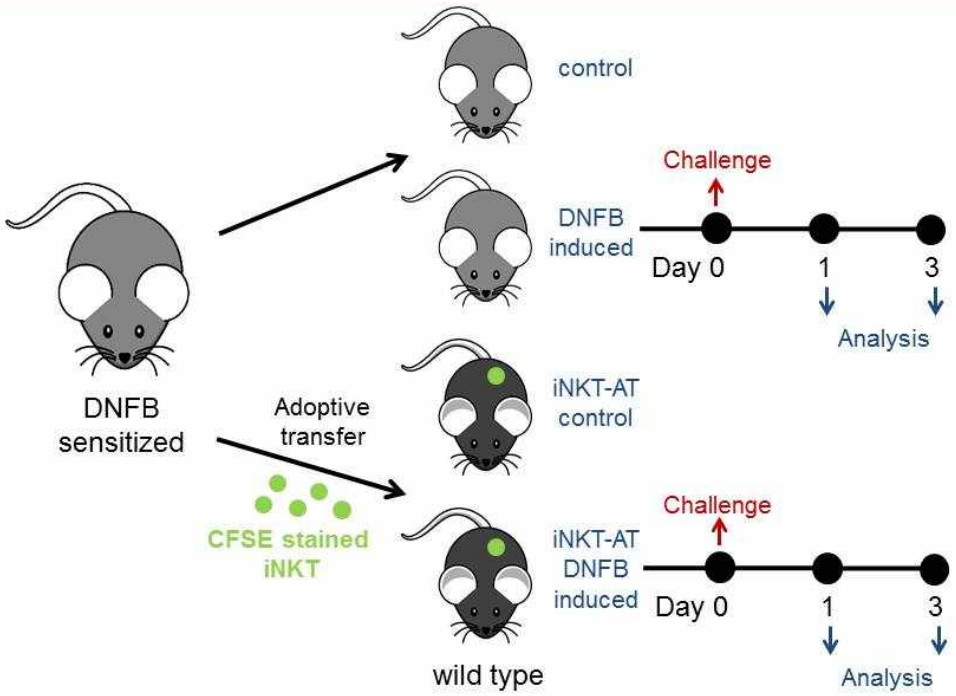
## Increased SDF1α expression in the lesional skin of AD patients




## Increased SDF1α expression in the lesional skin of AD patients

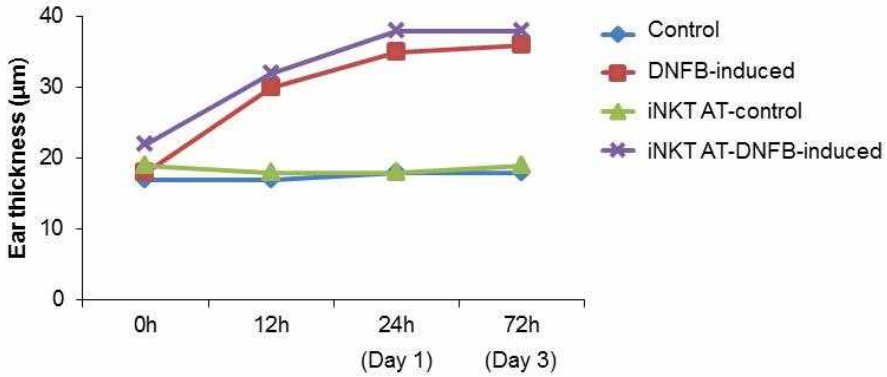


## Role of iNKT cell in DNFB induced mouse model



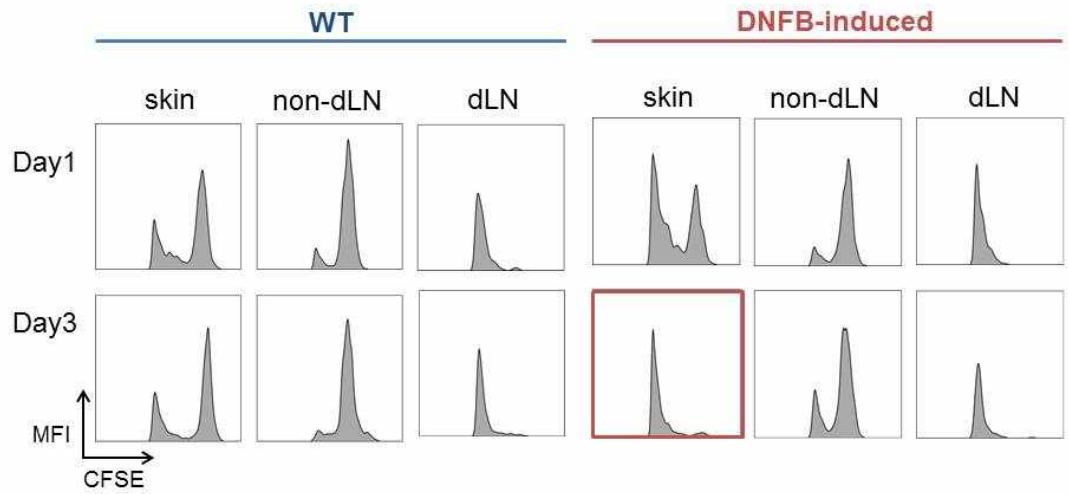

## Role of iNKT cell in DNFB-induced mouse model

- **Ear thickness was elevated in DNFB-induced mice, also in iNKT adoptive transfered DNFB-induced mice**



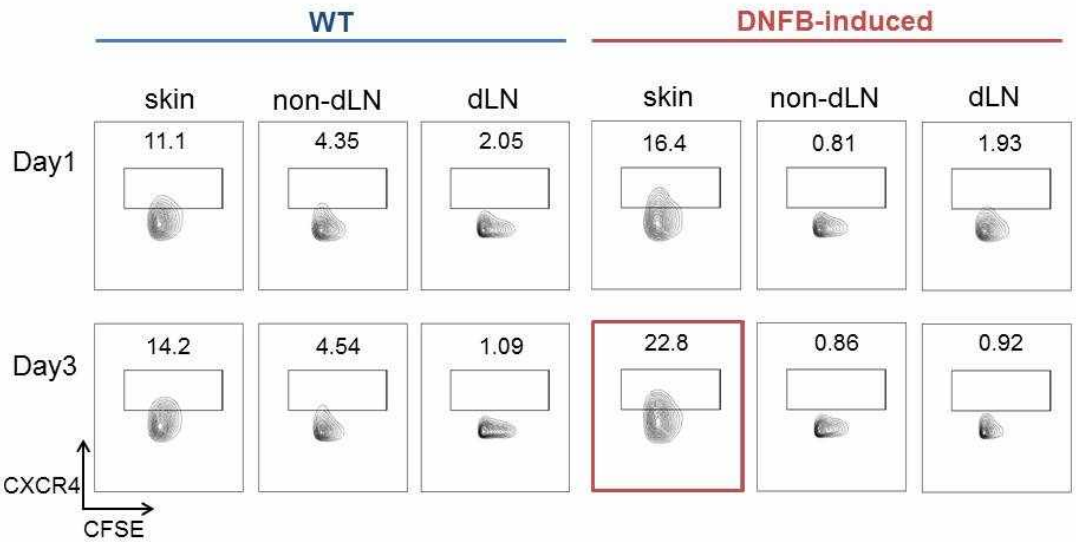
## Role of iNKT cell in DNFB-induced mouse model

- **skin iNKT cells are proliferated at day 3**



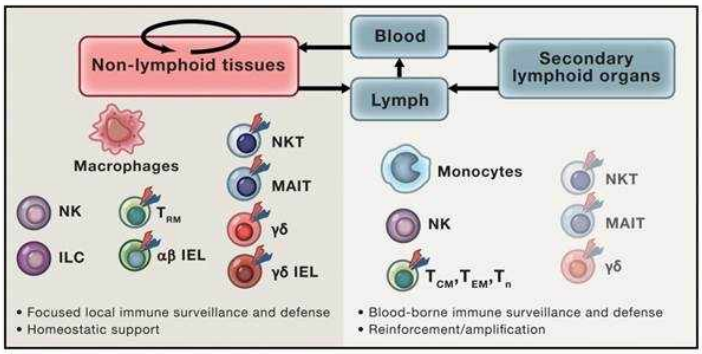

## Role of iNKT cell in DNFB-induced mouse model

- **CXCR4 expressed higher in skin iNKT cells than other lymphoid organs**



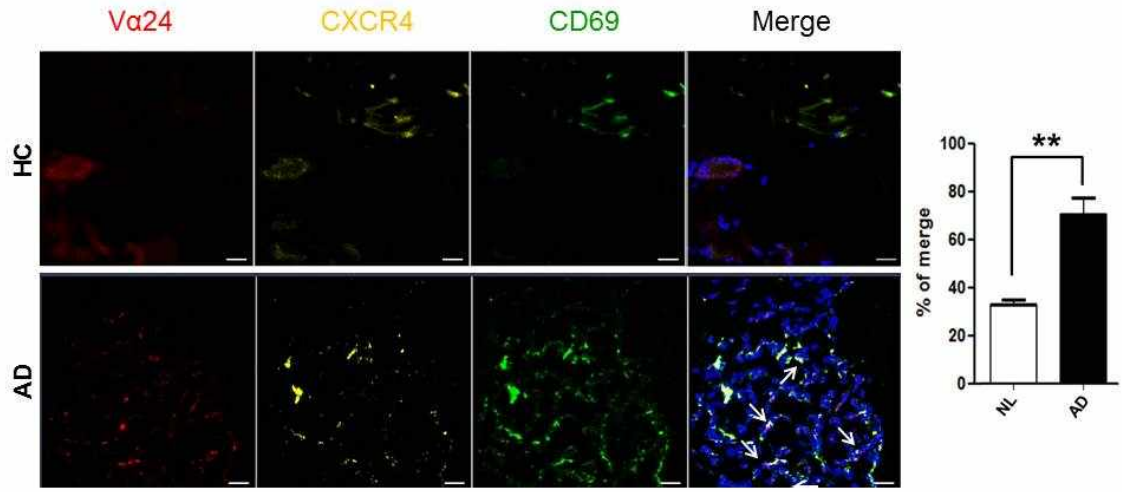
# Tissue-resident memory T ($T_{RM}$)

- Memory T cells give the host a highly effective immunity against previously encountered pathogens.
- Memory T cells are divided into central memory T ($T_{CM}$) and effector memory T ($T_{EM}$) and tissue-resident memory T ($T_{RM}$) cells.
- $T_{RM}$ cells are non-recirculating residents of non-lymphoid tissues and organs such as gastrointestinal tract, gut, and skin.

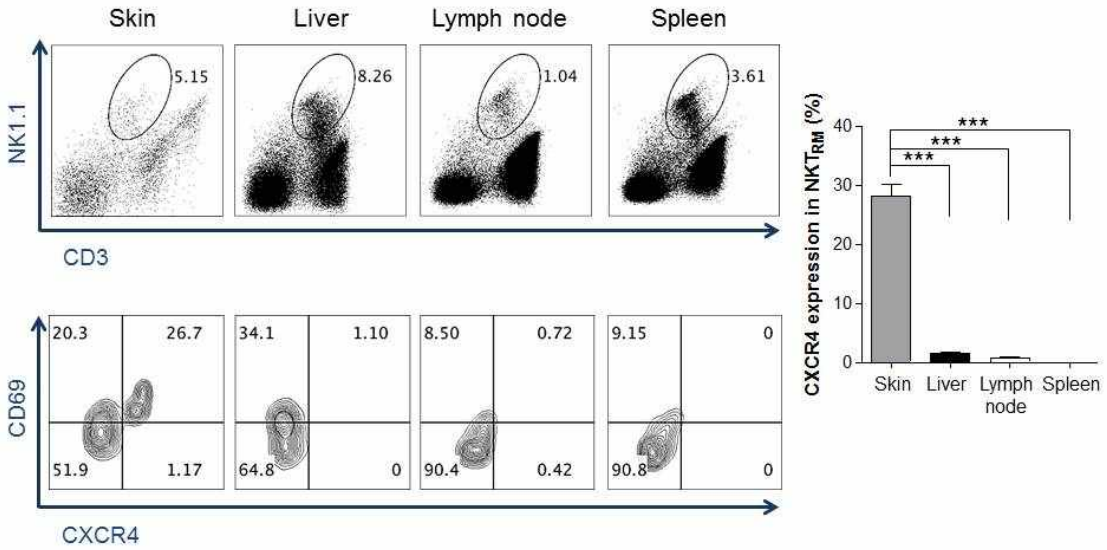



Fan X, Rudensky AY, **Cell Rev.**, 2016

## Expression of CXCR4 in Vα24+ $iNKT_{RM}$ cells in the human skin of healthy controls and AD patients

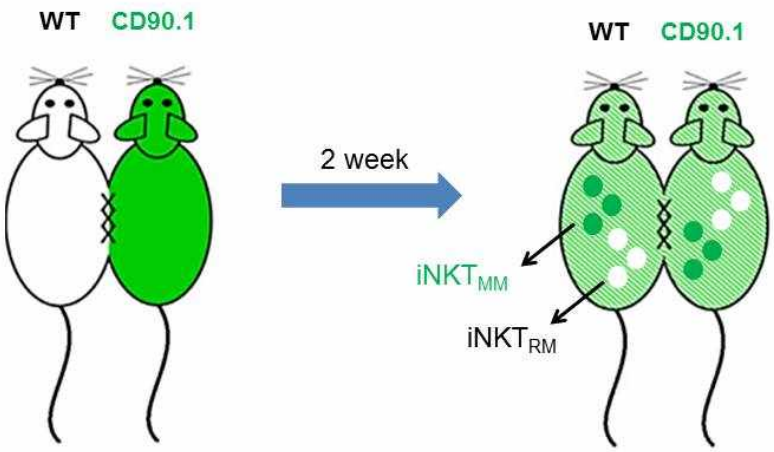


## Different CXCR4 expression of iNKT$_{RM}$ cells in mouse skin, liver, lymph node, and spleen

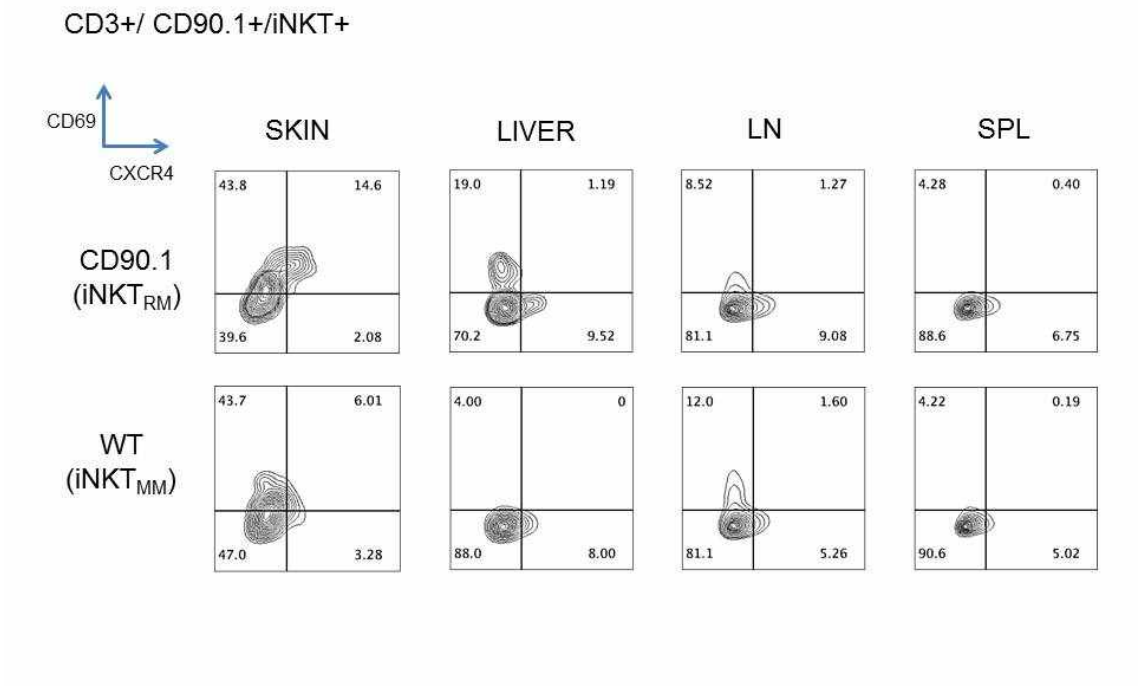


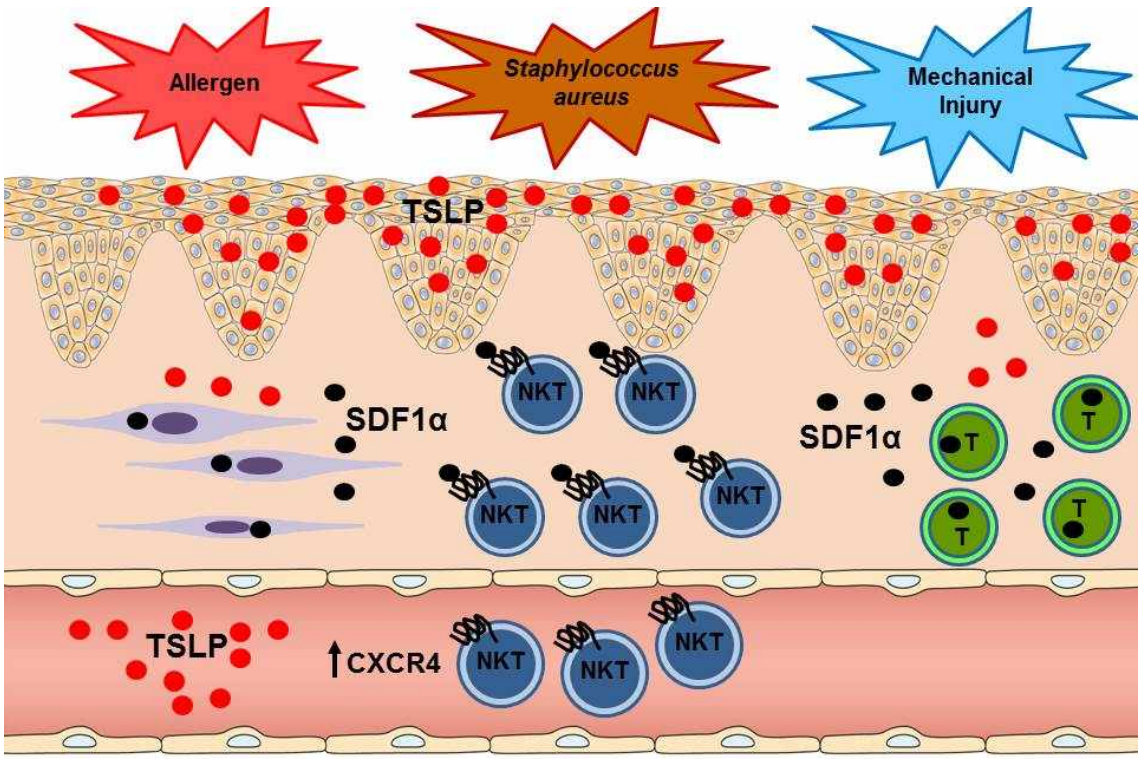

## CXCR4 expression of iNKT$_{RM}$ and iNKT$_{MM}$ cells in mouse skin, liver, lymph node, and spleen

- Parabiosis



# CXCR4 expression of iNKT$_{RM}$ and iNKT$_{MM}$ cells in mouse skin, liver, lymph node, and spleen



# Summary

- CXCR4 and SDF1α were significantly infiltrated into AD skin compared to normal skin.
- CXCR4+ iNKT cells was also increased in AD skin.
- Dermal fibroblasts and T cells in AD skin produced SDF1α compared to those in normal skin.
- iNKT cells in skin specifically expressed CXCR4, rather than those in liver, lymph node, and spleen.
- We suggest CXCR4/SDF1α axis of skin iNKT cells as a novel therapeutic strategy for AD.