Immune-Mediated Mechanisms of Atherosclerosis

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Inserm U970

TOLERAGE
Effect of Subendothelial Retention of Atherogenic Lipoproteins on Atherosclerosis


Wild type-control LDL

Proteoglycan-binding-defective LDL

Crucial role of monocytes/macrophages in atherosclerosis

M-CSF deficiency inhibits plaque formation (Smith, *PNAS* 1995)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Weight, g</th>
<th>Total cholesterol, mg/dl</th>
<th>Monocyte, % of leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>op0/E0</td>
<td>20.3 ± 5.3 (6)</td>
<td>21.3 ± 3.8 (10)</td>
<td>20.9 ± 4.3* (16)</td>
</tr>
<tr>
<td>op2/E0</td>
<td>29.9 ± 3.6 (10)</td>
<td>26.1 ± 2.9 (16)</td>
<td>27.5 ± 3.6* (26)</td>
</tr>
</tbody>
</table>

(Smith et al, *PNAS*, 1995)
Monocyte subsets

HUMAN

Classical

CD14⁺ CD16⁻  85 - 95%

Nonclassical

CD14Lo CD16⁺  5 - 15%

CD115⁺ Gr-1hi (Ly6C hi)  50%

CX₃CR1

CCR2

CD62L

MOUSE

CD115⁺ CD11c⁺ Gr-1lo (Ly6C lo)  50%
Woollard KJ, Nature Rev Cardiol 2010
The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C⁻ monocytes

Hannah RN, Nat Immunol 2011
Human CD14<sup>dim</sup> Monocytes Patrol and Sense Nucleic Acids and Viruses via TLR7 and TLR8 Receptors

Cros J et al, Immunity 2010
Nr4a1-Dependent Ly6C\textsuperscript{low} Monocytes Monitor Endothelial Cells and Orchestrate Their Disposal

Carlin LM et al, Cell 2013
Additive roles for monocyte subsets in atherosclerosis

Classical monocytes

$\text{CCR2}^{\text{hi}}$
$\text{CX3CR1}^{\text{lo}}$
$\text{CCR5}^+$

Non classical monocytes

$\text{CCR2}^{\text{lo}}$
$\text{CX3CR1}^{\text{hi}}$
$\text{CCR5}^+$

Lesion size in aortic sinus (10^3 μm^2)

$\text{Ccl2}$
$\text{Cx3cr1}$

$\text{+/+}$
$\text{+/+}$

$\text{-/-}$
$\text{-/-}$

$\text{+/+}$
$\text{-/-}$

$\text{-/-}$
$\text{-/-}$

Anti-CCR5

(Combadière et al, Circulation 2008)
Chemokines and Atherosclerosis

Control of Monocyte Number in Blood and Bone Marrow

Apoe<sup>−/−</sup> Apoe<sup>−/−</sup>/Ccl2<sup>−/−</sup>/ Cx3cr1<sup>−/−</sup>

Ly-6G

CCL2/CCR2

CX3CL1/CX3CR1

CCL2

CCR2+

Ly6C(hi)

CX3CR1+

Ly6C(lo)

Ly-6G

Macrophage

Dendritic Cell

Macrophage

Dendritic Cell
Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2 (Serbina MV, Nat Immunol 2006)
Local Macrophage Proliferation, Rather than Recruitment from the Blood, Is a Signature of TH2 Inflammation

Stephen J. Jenkins, Dominik Ruckerl, Peter C. Cook, Lucy H. Jones, Fred D. Finkelstein, Nico van Rooijen, Andrew S. MacDonald, Judith E. Allen

C: Thioglycollate injection
Number of Neutrophils, Gr-1<sup>+</sup>/Ly-6C<sup>high</sup> Monocytes, and Macrophages

D: L. sigmodontis infection
Number of Neutrophils, Gr-1<sup>+</sup>/Ly-6C<sup>high</sup> Monocytes, and Macrophages

A and B: Naive vs. Thio-glycolate infected mice

K<sub>67</sub> and BrdU staining

*** p < 0.001, ** p < 0.01, * p < 0.05
A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection

Regulated Accumulation of Desmosterol Integrates Macrophage Lipid Metabolism and Inflammatory Responses

Spann NJ et al. Cell 2012
Pro & Anti-Atherogenic Signaling Pathways

Tedgui & Mallat, Physiol Rev 2006
NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals

Pro & Anti-Atherogenic Signaling Pathways

Tedgui & Mallat, Physiol Rev 2006
Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an anti-inflammatory profile.

Lutgens E et al, J Exp Med 2010
Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis

Jenny Jongstra-Bilen,1,2 Mehran Haidari,1,3 Su-Ning Zhu,1,3 Mian Chen,1,3 Daipayan Guha,1 and Myron I. Cybulsky1,3
GM-CSF regulates intimal cell proliferation in nascent atherosclerotic lesions

Su-Ning Zhu,¹ Mian Chen,¹ Jenny Jongstra-Bilen,¹,²,³ and Myron I. Cybulsky¹,²

![Image of cell proliferation](image_url)

- **Number of BrdU-labeled nuclei**
  - Time after BrdU injection: 2 h, 24 h

- **Ldlr**
  - Ldlr⁺/⁺
  - Ldlr⁻⁻⁻

- **BrdU analysis**
  - Time before BrdU (h): -22, -46
  - ± PTx
  - Control, PTx

- **Significance**
  - *: p < 0.05
  - **: p < 0.01
Resident Intimal Dendritic Cells Accumulate Lipid and Contribute to the Initiation of Atherosclerosis

Kim E. Paulson, Su-Ning Zhu, Mian Chen, Sabrina Nurmohamed, Jenny Jongstra-Bilen, Myron I. Cybulsky

A

CD11c

lipid

merge

B

PBS or DT

BrdU

Analysis

-72
0
2
24 h

BrdU* cells

Pretreatment: PBS, DT

Time of analysis after BrdU: 2 h, 24 h

Area positive for Nile red (percent of ascending aorta)

Undepleted

DC-depleted

*
Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent

Elena Galkina,1,4 Alexandra Kadl,4 John Sanders,3,4 Danielle Varughese,4 Ian J. Sarembock,3,4 and Klaus Ley1,2,4
Identification of antigen-presenting dendritic cells in mouse aorta and cardiac valves

Choi JH et al., 2006
Dynamic T cell–APC interactions sustain chronic inflammation in atherosclerosis

Koltsova EK et al., JCI 2012
Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis

Andreas Hermansson,¹ Daniel F.J. Ketelhuth,¹ Daniela Strothoff,¹ Marion Wurm,¹ Emil M. Hansson,² Antonino Nicoletti,³ Gabrielle Paulsson-Berne,¹ and Göran K. Hansson¹
Recruitment and Activation of Th1 in Atherosclerosis

[Diagram showing the interaction between T cells, CD4+ T cells, endothelial cells, and APCs with cytokines such as IL-12, IL-15, and IL-18, and the role of IFNγ and TNF in the process.]
Potential Role of Th2 Cells in Atherosclerosis

Mature DC → Th2

IL-25, IL-13, IL-6

MHC-II, TCR, OX40L, OX40

STAT6, GATA-3

IL-4, IL-5, IL-13, IL-33

Pro-atherogenic

endothelial activation

VSMC

B1 cell

oxLDL-IgM

Athero-protective

Cardilo-Reis L et al., EMBO Mol Med 2012

Binder CJ et al., JCI 2004

Miller AM et al., JEM 2008

Lahoute C & Mallat Z, 2011
Pro & Anti-Atherogenic Signaling Pathways

Tedgui & Mallat, Physiol Rev 2006
Regulatory T cells Control Atherosclerosis

Mallat et al. 2003
Tr1 cells: IL-10

Ait-Oufella et al. 2006
nTreg and TGF-β

Hanson GK.
N Engl J Med 2005
### Types of regulatory T cell: origin, phenotype and function

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Phenotype*</th>
<th>Mechanism*</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural T(_{\text{Reg}}) cells(^+)</td>
<td>FOXP3(^+) CTLA4(^+) GITR(^+) CD45RB(^{\text{low}}) CD62L(^{+/\text{low}}) CD127(^{\text{low}})</td>
<td>Contact dependent, IL-10, TGFβ, CTLA4 and GITR</td>
<td>Thymus</td>
</tr>
<tr>
<td>Natural T(_{\text{Reg}}) cells (expanded)</td>
<td>FOXP3(^+) HLA-DR(^+) CD62L(^{+/\text{low}}) CD69(^+)</td>
<td>Contact dependent</td>
<td>Expansion of natural T(_{\text{Reg}}) cells</td>
</tr>
<tr>
<td>Induced T(_{\text{Reg}}) cells</td>
<td>FOXP3(^+) CTLA4(^+) GITR(^+) CD45RB(^{\text{low}})</td>
<td>Contact dependent and in some cases TGFβ</td>
<td>Conversion and/or expansion of non-regulatory CD4(^+) T cells</td>
</tr>
<tr>
<td>T(_{\text{H}}) 3 cells</td>
<td>FOXP3(^{+/\text{not well defined}})</td>
<td>TGFβ and/or IL-10</td>
<td>Periphery</td>
</tr>
<tr>
<td>T(_{\text{R}}) 1 cells</td>
<td>FOXP3(^+)</td>
<td>IL-10</td>
<td>Periphery</td>
</tr>
<tr>
<td>CD8(^+) T cells</td>
<td>CD28(^{+/\text{low}})</td>
<td>Cell contact, ILT3 and ILT4</td>
<td>Periphery</td>
</tr>
</tbody>
</table>

Akbar AN et al., *Nat Rev Immunol*, 2007
Natural regulatory T cells control the development of atherosclerosis in mice  

Ait-Oufella et al. *Nat Med* 2006
Flt3 Signaling-Dependent Dendritic Cells Protect against Atherosclerosis

Choi J-H et al., Immunity 2011
Flt3 Signaling-Dependent Dendritic Cells Protect against Atherosclerosis

Choi J-H et al., Immunity 2011
CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice

Webber C et al., JCI 2011
Maintenance of tolerance

Th1

Th2

Treg

Mac.

IL10

TGFβ

Atherosclerotic plaque

Lymph node

Tolerogenic DC

Compromised Treg induction/survival in inflammatory site

Treg

Mfge8, Mertk dependent clearance

Aortic debris

Regulation of inflammation

Th2

Th1

IL10

TGFβ

oxLDL, HSP...

Pathogenic DC

Pathogenic immunity

SMC

EC

SMC

Mallat et al., Physiol Rev 2006; J Lipid Res 2008
Lactadherin Deficiency Leads to Apoptotic Cell Accumulation and Accelerated Atherosclerosis in Mice

Ait-Oufella et al., Circulation 2007

**a**

Mfge8^{+/+} vs Mfge8^{-/-}

**b**

Mfge8^{+/+} vs Mfge8^{-/-}

**c**

Caspase-3

**d**

Percent acellular core

**e**

Microparticle level (x10^3/μl plasma)
Mfge8-dependent clearance

TGF-β

IL-10

APC

Tolerogenic APC

Treg cell

Atherosclerosis

Th1

Th2

Th17

IL-6

IL-12

IL-17

IFNγ

IL-4

Free Cholesterol

No Mfge8 (defective clearance)

αvβ3

APC

PRR

( Toll4…)

Pathogenic T cell

Mature APC

PRR

Mature APC

αvβ3

TGF-β

IL-10

Apoptotic bodies

No Mfge8 (defective clearance)

Free Cholesterol
Mfge8 Inhibits inflammasome-induced IL1β production

Deroide N, Li X et al., J Clin Invest 2013
STAT3 Signaling and the Hyper-IgE Syndrome

Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome

Yoshiyuki Minegishi, Masako Saito, Shigeru Tsuchiya, Ikuya Tsuge, Hidetoshi Takada, Toshiro Hara, Nobuaki Kawamura, Tadashi Ariga, Srdjan Pasic, Oliver Stojkovic, Ayse Metin & Hajime Karasuyama

STAT3 Mutations in the Hyper-IgE Syndrome

Steven M. Holland, M.D., Frank R. DeLeo, Ph.D., Houda Z. Elloumi, Ph.D., Amy P. Hsu, B.A., Gulbu Uzel, M.D., Nina Brodsky, B.S., Alexandra F. Freeman, M.D., Andrew Demidowich, B.A., Joie Davis, A.P.R.N.

Impaired $T_\text{H}17$ cell differentiation in subjects with autosomal dominant hyper-IgE syndrome


Mutations in STAT3 and IL12RB1 impair the development of human IL-17–producing T cells

Ludovic de Beaucoudrey, Anne Puel, Orchidée Filipe-Santos, Aurélie Cobat, Pegah Ghandil, Maya Chrabiieh, Jacqueline Feinberg, Horst von Bernuth, Arina Samarina, Lucile Jannière, Claire Fieschi

Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3

Cindy S. Ma, Gary Y.J. Chew, Nicholas Simpson, Archana Priyadarshi, Melanie Wong, Bodo Grimbacher, David A. Fulcher, Stuart G. Tangye, and Matthew C. Cook

Levy DE & Loomis CA, NEJM 2007
Frequent and Widespread Vascular Abnormalities in Human Signal Transducer and Activator of Transcription 3 Deficiency

Marie-Olivia Chandesris, MD; Arshid Azarine, MD, MSc; Kim-Thanh Ong, MD, PhD; Soraya Taleb, PhD; Pierre Boutouyrie, MD, PhD; Elie Mousseaux, MD, PhD; Mélissa Romain, MSc; Erwan Bozec, PhD; Stéphane Laurent, MD, PhD; Nathalie Boddaert, MD, PhD; Caroline Thumerelle, MD, PhD; Isabelle Tillie-Leblond, MD, PhD; Cyrille Hoarau, MD, PhD; Yvon Lebranchu, MD, PhD; Nathalie Aladjidi, MD; François Tron, MD, PhD; Vincent Barlogis, MD; Gérard Body, MD; Marine Munzer, MD; Roland Jaussaud, MD, PhD; Felipe Suarez, MD, PhD; Olivier Clement, MD, PhD; Olivier Hermine, MD, PhD; Alain Tedgui, PhD; Olivier Lortholary, MD, PhD; Capucine Picard, MD, PhD; Ziad Mallat, MD, PhD; Alain Fischer, MD, PhD

Background—Signal transducer and activator of transcription 3 (STAT3) deficiency is responsible for autosomal dominant hyperimmunoglobulin E syndrome, characterized by recurrent bacterial and fungal infections, connective tissue abnormalities, hyperimmunoglobulin E, and Th17 lymphopenia. Although vascular abnormalities have been reported in some patients, the prevalence, characteristics, and etiology of these features have yet to be described.

Methods and Results—We prospectively screened 21 adult patients with STAT3 deficiency (median age, 26 years; range, 17 to 44) for vascular abnormalities. We explored the carotid arteries with whole-body magnetic resonance imaging angiography, coronary multislice computed tomography, and echo-tracking–based imaging. We also assayed for serum biomarkers of inflammation and endothelial dysfunction. Finally, we studied murine models of aortic aneurysm in the presence and absence of inhibitors of STAT3-dependent signaling. Ninety-five percent of patients showed brain abnormalities (white matter hyperintensities, lacunar lesions suggestive of ischemic infarcts, and atrophy). We reported peripheral and brain artery abnormalities in 84% of the patients and detected coronary artery abnormalities in 50% of the patients. The most frequent vascular abnormalities were ectasia and aneurysm. The carotid intima-media thickness was markedly decreased, with a substantial increase in circumferential wall stress, indicating the occurrence of hypotrophic arterial remodeling in this STAT3-deficient population. Systemic inflammatory biomarker levels correlated poorly with the vascular phenotype. In vivo inhibition of STAT3 signaling or blockade of IL-17A resulted in a marked increase in aneurysm severity and fatal rupture in mouse models.

Conclusions—Vascular abnormalities are highly prevalent in patients with STAT3 deficiency. This feature is consistent with the greater susceptibility to vascular aneurysm observed after inhibition of STAT3-dependent signaling in mouse models. (Circ Cardiovasc Genet. 2012;5:00-00.)
Loss of SOCS3 expression in T cells reveals a regulatory role for interleukin-17 in atherosclerosis

Neutralization of IL17 Abrogates the Athero-Protective Effect of T Cell-Specific SOCS3 Deletion

IL-17 reduces atherosclerosis in LDLr-/- mice
Circulating levels of interleukin-17 and cardiovascular outcomes in patients with acute myocardial infarction

Tabassome Simon¹*, Soraya Taleb², Nicolas Danchin³, Ludivine Laurans², Benoit Rousseau¹, Simon Cattan⁴, Jean-Michel Montely⁵, Olivier Dubourg⁶, Alain Tedgui², Salma Kotti¹, and Ziad Mallat²,⁷*