The role of T-regulatory cells and TH17 effector cells in the development of fibrosis

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Beachte

Diese Präsentation ist für das medizinische Fachpublikum konzipiert.

Diagnostische und therapeutische Schlußfolgerungen obliegen dem Arztvorbehalt.

Wir bitte um adäquate Nennung bei der eventuellen Weiterverwendung der Informationsmaterialien.
## Faculty Disclosure

<table>
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<tr>
<th>Company</th>
<th>Nature of Affiliation</th>
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### Off-Label Product Usage

- None
1. The Immunology of Fibrosis
2. Immunological Approach to peri-SMI Fibrosis
3. Diagnostic Developments
4. Conclusions and Outlook
1. The Immunology of Fibrosis

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Major Causes of Fibrosis

(1) **Tissue damage**
   Postoperative, burns, liver cirrhosis, etc.

(2) **Inflammation**
   Infections, autoimmune disease, atherosclerosis, etc.

(3) **Foreign material**
   Silicone implants (passive, active), etc.

(4) “**Spontaneous**“
   Keloids, Dupuytren contracture, Peyronie disease, etc.

(5) **Tumors**
   Tumor stroma, Fibroma, etc.
G. Wick, C. Grundtman, Ch. Mayerl, TF Wimpissinger, J Feichtinger, B Zelger, R Sgonc and D Wolfram

The Immunology of Fibrosis

Annual Review of Immunology, Vol. 31: 107-135
(Volume publication date March 2013)
We formulated two major axioms

1. No fibrosis without prior inflammation

2. Irrespective of the underlying primary diseases, the final profibrotic molecular pathway is stereotypic
Immunohistochemistry panel

- Keloid CD25
- UCD Goldner
- ApOE –/+ Goldner
- SMI FN
- SMI HSP60 CD3
- S1 – band TGFβ1
- DP D45RA vWF DAPI
- Peyronie TGFβ1
- Fibrosarcoma CD3

Adaptive immune response in fibrosis

Primary causes:
- (Auto)antigens
- Chronic inflammation

Imbalance:
- Tregs
  - TGF-β, IL-10
- Th17
  - IL-17, IL-21, IL-22
- Th1
  - IFN-γ, TNF-α
- Th2
  - IL-4, IL-13, IL-6

Lymphocyte activation:
- CD4+ > CD8+ T cells

Final stage:
- Fibrosis
- ECM synthesis
- Myofibroblasts
- Migration, proliferation, transdifferentiation, EMT, and EndoMT

Sources:
Outline

1. The Immunology of Fibrosis

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4. Conclusions and Outlook
Local Side Effects

Constrictive Fibrosis

Infection

Rupture

Calcification
SMI: side effects

Systemic Side Effects

• Scleroderma
• Lupus Erythematodes
• Mixed Connective Tissue Disease
• Polyarthritis
• Rheumatoid Arthritis
• Polymyositis
• Siliconoma

Local Side Effects

• Constrictive Fibrosis
Capsule Fibrosis: the Major SIC
Overview Silicone Project

Silicone project

- Local immune reaction in the capsular tissue
  - Histological analyses
  - Phenotypical & functional analysis of intracapsular T-cells

- Systemic side effects of silicone mammary implants
  - Serological investigations
    - APA
    - sICAM-1
    - Procollagen type III
    - circulating immune complexes

- Proteomics
  - Identification of proteins with high affinity for silicone
    - SILISA-test
Silicone project

Local immune reaction in the capsular tissue
- Histological analyses …
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Proteomics
- Identification of proteins with high affinity for silicone
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Methods

21 explanted capsules and peripheral blood samples from each donor

Isolation and expansion of tissue derived T-cells and peripheral lymphocytes.

cultivated PBMCs & intracapsular cells

FACS analysis

Immunoscope: TCRβ analysis

CDR1 CDR2 CDR3

NH2 COOH

Cytokine detection from supernatant

CDR1 CDR2 CDR3
Immunoscope

Vβ1  Vβ5.1  Vβ7  Vβ12  Vβ22
Vβ2  Vβ5.3  Vβ8  Vβ14
Vβ3  Vβ6.1  Vβ9  Vβ20
Vβ4  Vβ6.2  Vβ11  Vβ21

periphery versus capsule
**Results (5 patients)**

- PBMC show a polyclonal T-cell receptor (TCR) repertoire

- Intracapsular T-cells possess predominantly an oligoclonal or monoclonal TCR-repertoire

**Restricted TCR-Repertoire in the capsular tissue**

(= Antigen driven immune response)
HSP 60 expression in a capsule with an implant duration of 14 months
Cytokines: Results

Purple = TH17/TH1
Turquoise = TH2
Blue = TH1
Capsular fibrosis is associated with a TH17/TH1 weighted immune response.

Immunosuppressive cytokines (IL-10) seem to play a minor part as compared to cell-cell contact for the function of Tregs.

Pro-fibrotic cytokines are abundant within capsules.
Double staining of capsular tissue with a-CD25/DAB and a-Foxp3/Fast Red.

Tregs are present within the capsular tissue.
FACS: T-regs

capsule vs. periphery

% within CD3 population

PBMCs
Capsules

n=11
n=14

FoxP3, CD25, CD4

***
FACS: T-reggs

B° I+II vs. B° III+IV

% within CD3 population

FoxP3, CD25, CD4

n=9

**

B° I+II vs. B° III+IV

n=5
FACS: T-regulatory cells

Patient S64:
T-regs in the left and right capsule
Cross-testing Suppression Assays: Intracapsular Tregs suppress peripheral blood Teff effectively, but intracapsular Teff are less suppressible.
### Suppression Assays

<table>
<thead>
<tr>
<th>Tregs</th>
<th>Teffs</th>
<th>Suppression</th>
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<tr>
<td>CAP</td>
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**Phenotype:**
(a) CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>  
(b) CD4<sup>+</sup>CD25<sup>-</sup>FoxP3<sup>-</sup>  
**Teff stimulation:** a-CD3/a-CD28
DEFICIENT IMMUNOREGULATION IN FIBROSIS

- Quantitative decrease of Tregs in CAPs
- CAP Teffs not suppressible (TH17?)
- CAP Tregs fully functional on peripheral Teffs
Proliferation, transdifferentiation

Myofibroblasts

ECM production

IL8 → angiogenesis, degradation of ECM, MMP production

TH1/17 immune reaction

TNFα, IL-6, TGFβ1, IL-17, IL-1, INFγ, FGF, CTGF

Lymphocyte activation

CD4+ T-Cells

Suppression via T-regs

Cryptic antigen complex: silicone + protein

Capsular fibrosis

Adhesion

Activated DC

DC

Protein

Peri–SMI Capsular Fibrosis

Silicone-filled implant

Saline-filled implant

SILICONE SHELL

Smooth round implant

Texture round implant
Methods: Testing different implants

Human PBMC (Peripheral Blood Mononuclear Cell)

Female donors (n=7)

Co-culture with different surface of implants available on the market

0 → +

4 days

End point: 1) cells harvesting from the cultures
2) FACS analysis (phenotype and proliferation)
3) Function: realtime PCR and cytokine assay
SILICONE surfaces preparation

SMOOTH

Smooth-Surface, ALLERGAN (US)

TEXTURE

SilkSurface®, MOTIVA (CENTRAL AMERICA)

VelvetSurface™, MOTIVA

Biocell, ALLERGAN

Polytech texture (POLYTECH) (EU)

Micropolyurethane Foam (POLYTECH)

Siltex, MENTOR (US)
Influence of silicone surface on adherent cells

- Smooth, Siltex and Polystyrene surfaces: higher average level of cell attachment
Cytokine profile from adherent cells (RNA)

IL-1β

mRNA fold regulation compared to SMOOTH

SilkSurface®
VelvetSurface®
Polytech texture
Micropolyurethane foam
Siltex
Polystyrene

TNF-α

mRNA fold regulation compared to SMOOTH

SilkSurface®
VelvetSurface®
Polytech texture
Micropolyurethane foam
Siltex
Polystyrene

IL-10

mRNA fold regulation compared to SMOOTH

SilkSurface®
VelvetSurface®
Polytech texture
Micropolyurethane foam
Siltex
Polystyrene

TGFβ-1

mRNA fold regulation compared to SMOOTH

SilkSurface®
VelvetSurface®
Polytech texture
Micropolyurethane foam
Siltex
Polystyrene

→ SilkSurface: lower degree of inflammation/fibrosis?
Cytokine profile in response to silicone breast implant surfaces (protein)

Polytech texture: IL-1β, IL-6, TNF-α ↑
Micropolyurethane foam: TNF-α ↑
IFN-γ not detectable on all surfaces
Cappellano et al., Immunophenotypic characterization of human T cells after in vitro exposure to different silicone breast implant surfaces. Plos One 2018
Immunophenotypic characterization of human T cells after *in vitro* exposure to different silicone breast implant surfaces


https://doi.org/10.1371/journal.pone.0192108 (2018)
1. The Immunology of Fibrosis
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1. Axioms
   - No fibrosis without prior inflammation
   - Irrespective of primary disease, final molecular fibrotic pathway stereotypie

2. Peri-SMI fibrosis a model for fibrosis development in healthy humans

3. Serum/wound bed fluid proteins deposited on SMI surface (adhesion!)

4. MNC infiltrate of peri-SMI capsules dominated by CD4 T cells and MØ

5. Pseudosynovial layer of MØ and fibroblasts at contact zone with SMI (HSP60 expression!)

6. Inverse correlation of number of intracapsular CD4CD25FoxP3+ Tregs with degree of fibrosis
7. Intracapsular Tregs suppress peripheral blood Teffs (TGFβ); but intracapsular Teffs resistant to suppression

8. IL-6 and TNFα most abundant cytokines in cultures of intracapsular Tcells

9. Development of Tregs hampered even under high IL-6 concentrations (mechanisms?)

10. TGFβ produced by non-Tregs (e.g. MØ) fuels fibrosis

11. Differences in Treg development on different SMI surfaces

12. Aims
   • Better prediction of risk for fibrosis (SILISA)
   • Better biocompatibility of SMIs
Fibrosis / Silicone

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Innate immune mechanisms in liver fibrosis