GK1172
Rheumatoid Arthritis
Clinical Appearance and
Status of Current Research
Radeke 2009

structure

Radeke et al. 2009

- Epidemiology: Rheumatoid Arthritis
- Clinical features (disease burden)
- Immune pathological concepts:
  - from genes to autoimmunity
  - citrullinated proteins as autoantigens (MHC, PADs, PTPN)
  - inflammation: mechanisms of chronicity
  - role of chemokines
  - dendritic cells and tertiary lymphoid structures
  - regulatory T cells
- therapeutic options
- future targets

Rheumatoid Arthritis

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- One of the most common inflammatory disease (0.8% WHO)
- Prevalence 500-1000/100000 inhabitants (500.000 patients in Germany)
- Female / Male = 3:1 (onset often after pregnancy)
- Age of onset 20-45 years, up to now: life-long disease
- Joint damage and systemic inflammation results in average of 5-to 10-year decrease in life expectancy
- cause strong socio-economic consequences (disease costs 15.000 € per patient per year (without anti-TNF))
- at diagnosis already irreversible bone erosions

courtesy Dr. med. Andrea Himsel, Med. Klinik II.

Clinical features

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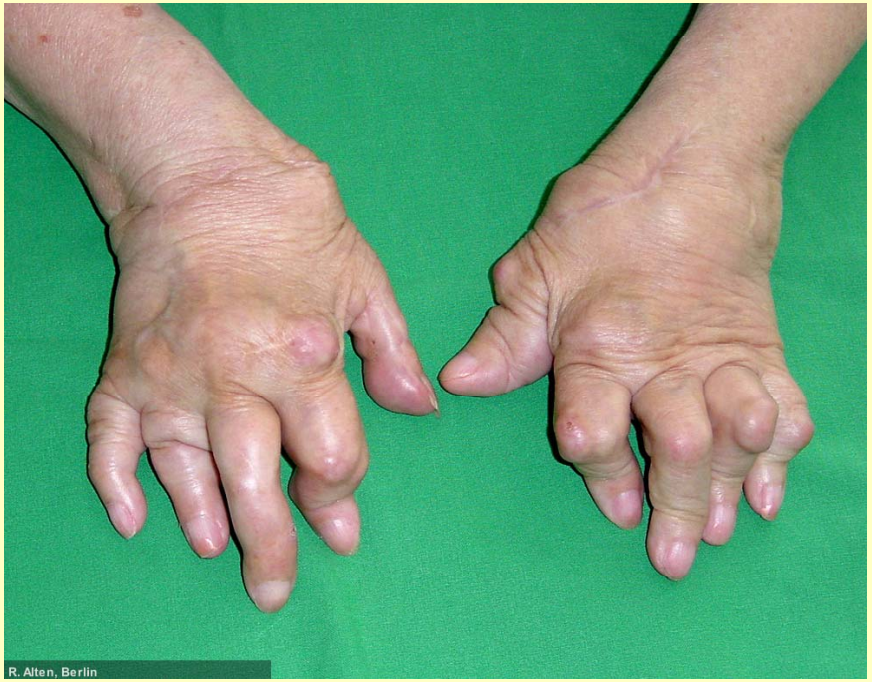
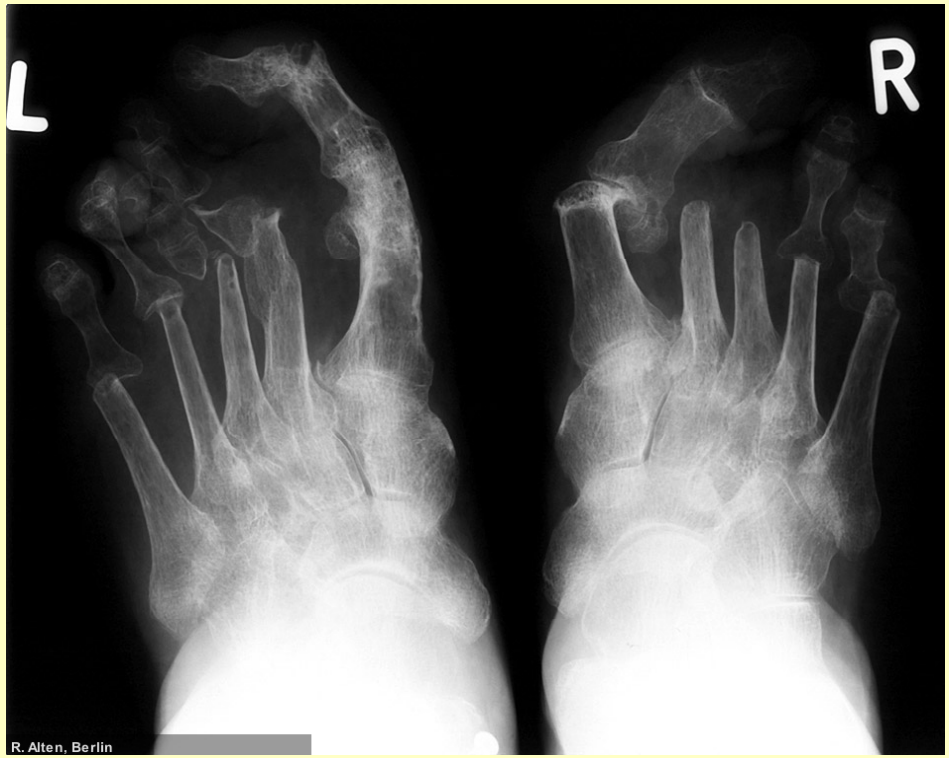


- **symmetrical swelling of joints**
- **tenderness**
- **luxation**
- **loss of function**

Osteoporosis

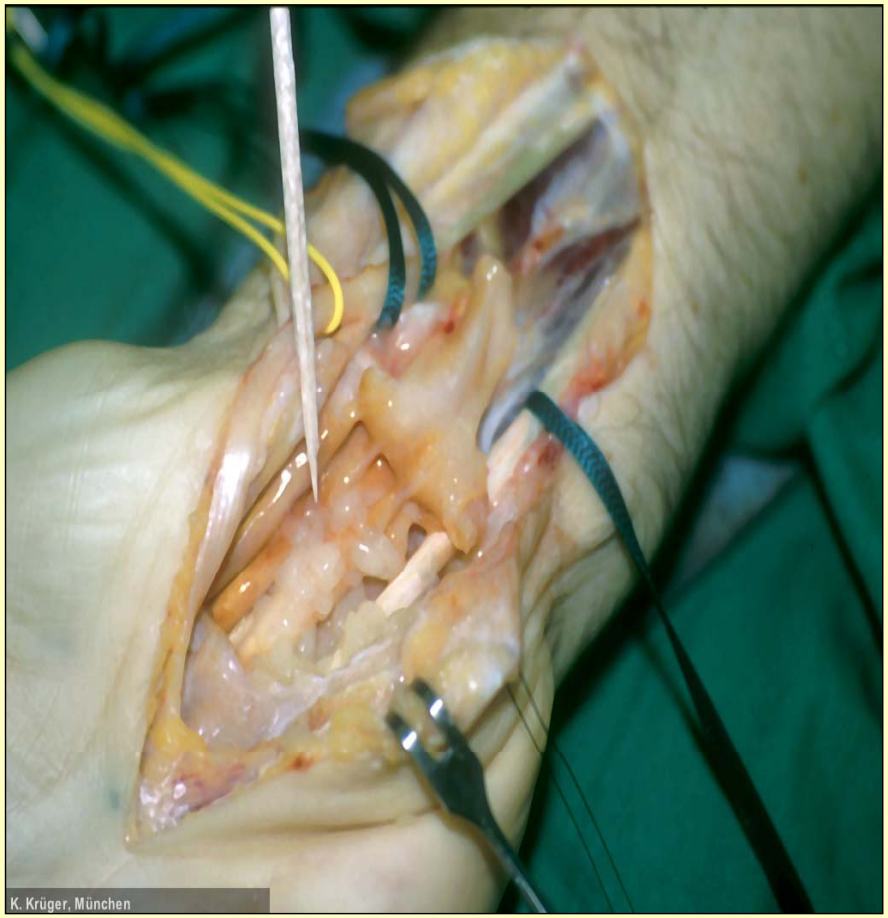
Bone erosion

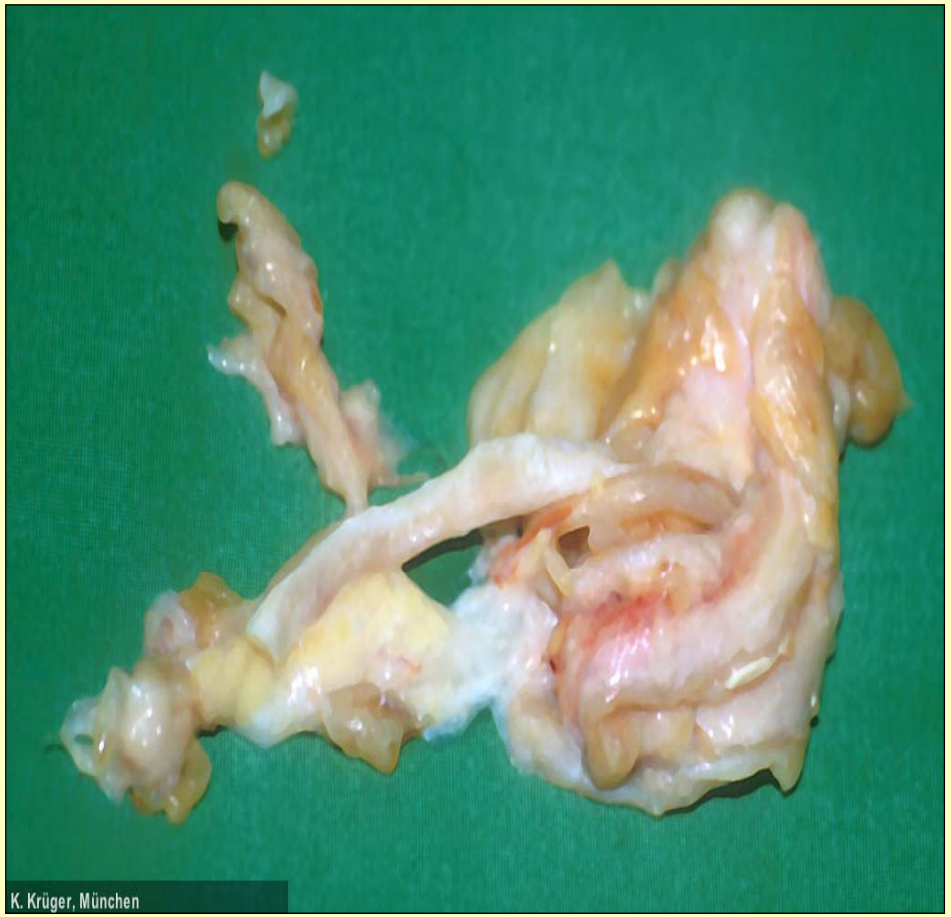
ankylosis



courtesy Dr. med. Andrea Himsel, Med. Klinik II.

Synovialitis





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courtesy Dr. med. Andrea Himsel, Med. Klinik II.

Immunopathology of RA

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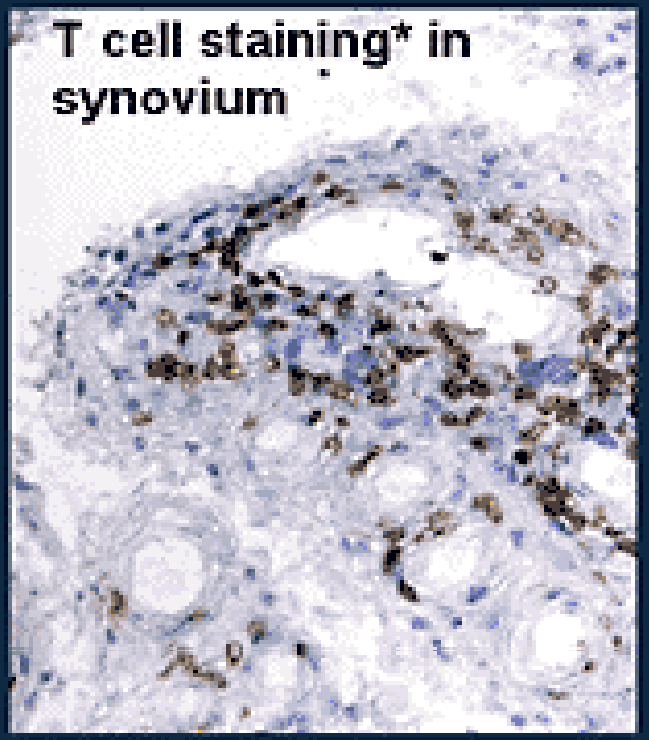
- Autoreactive CD4$^+$ T cells accumulate and are activated in the joint
- Inflammatory cell infiltrate
  - Activated B cells and T cells
  - Fibroblast-like synoviocytes
  - Macrophages
  - Neutrophils



*CD3-specific antibody staining (courtesy of Ann-Kristin Ulfgren).

Firestein. *Nature*. 2003;423:356.
Choy and Panayi. *N Engl J Med*. 2001;344:907.
Emery. *Expert Opin Investig Drugs*. 2003;12:673.

courtesy Dr. med. Andrea Himsel, Med. Klinik II.

Joint Tissue: Destructive Rheumatoid Arthritis

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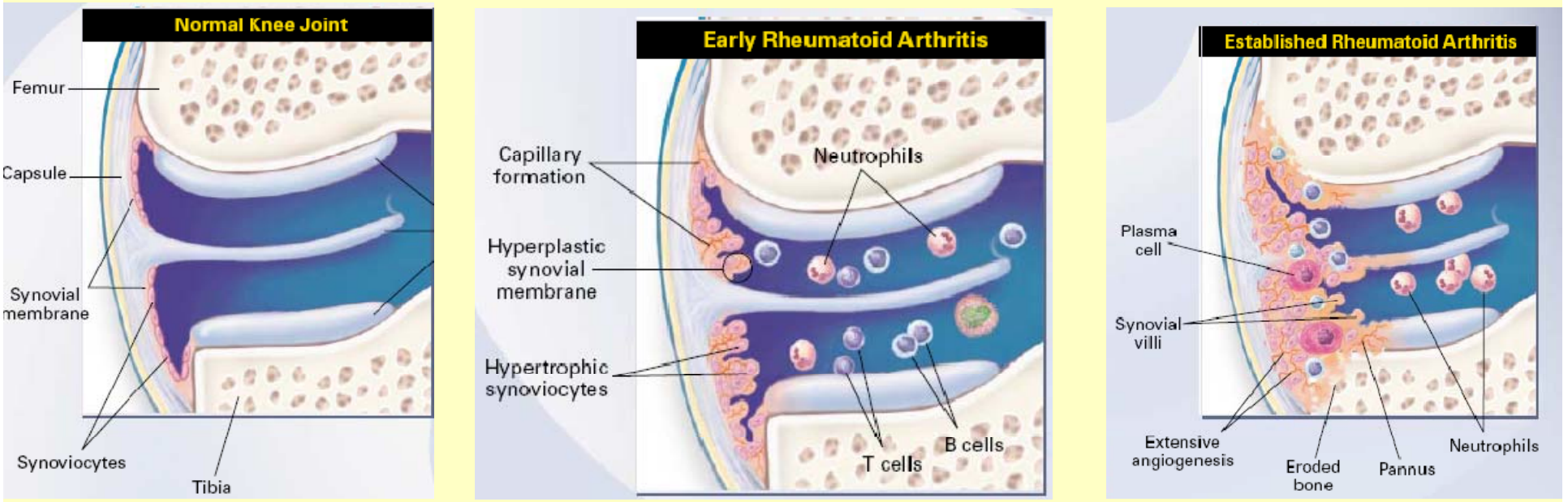
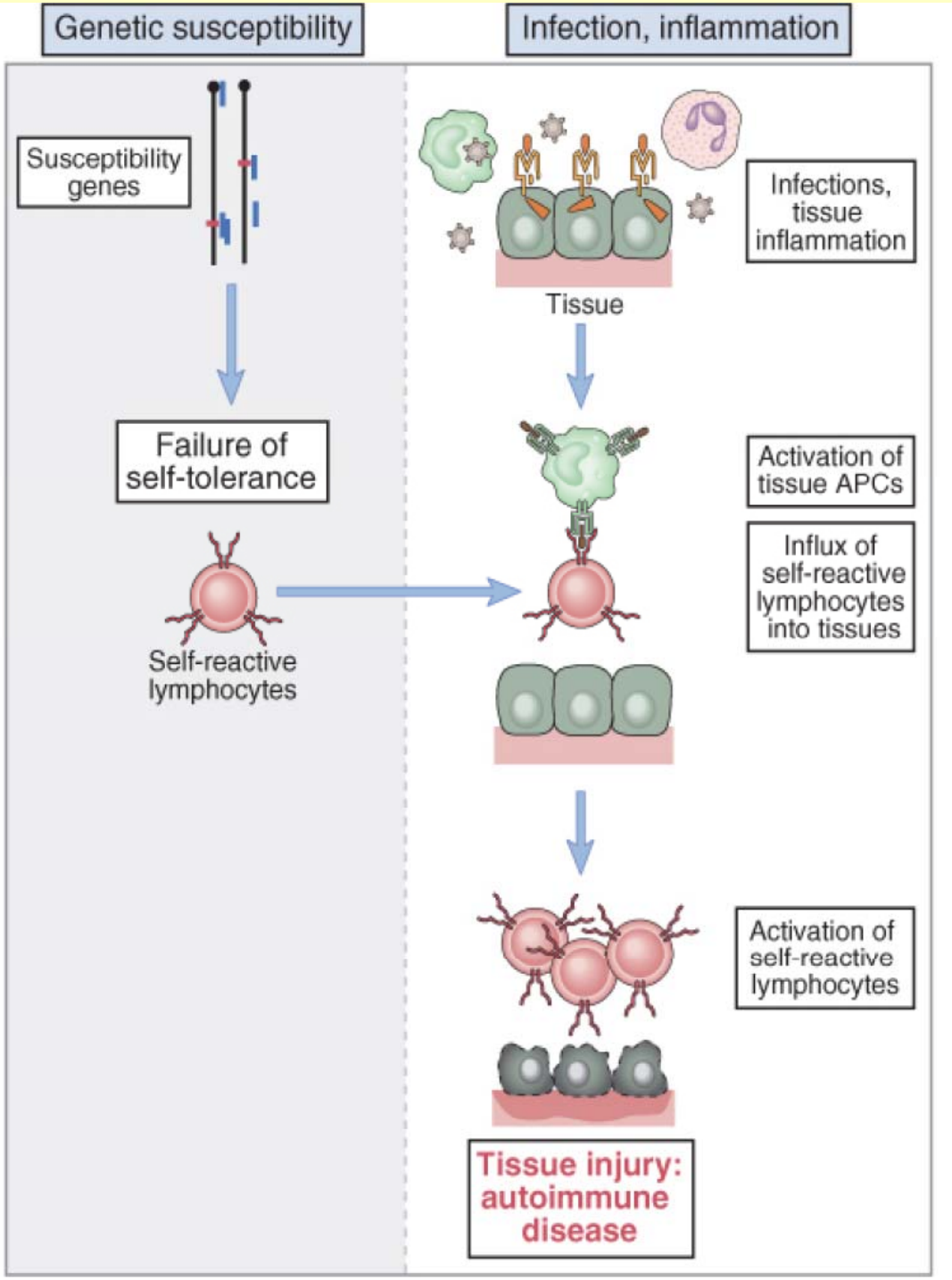


Choy & Panayi, N Engl J Med, 344(12) 2001

From defective genes to inflammation



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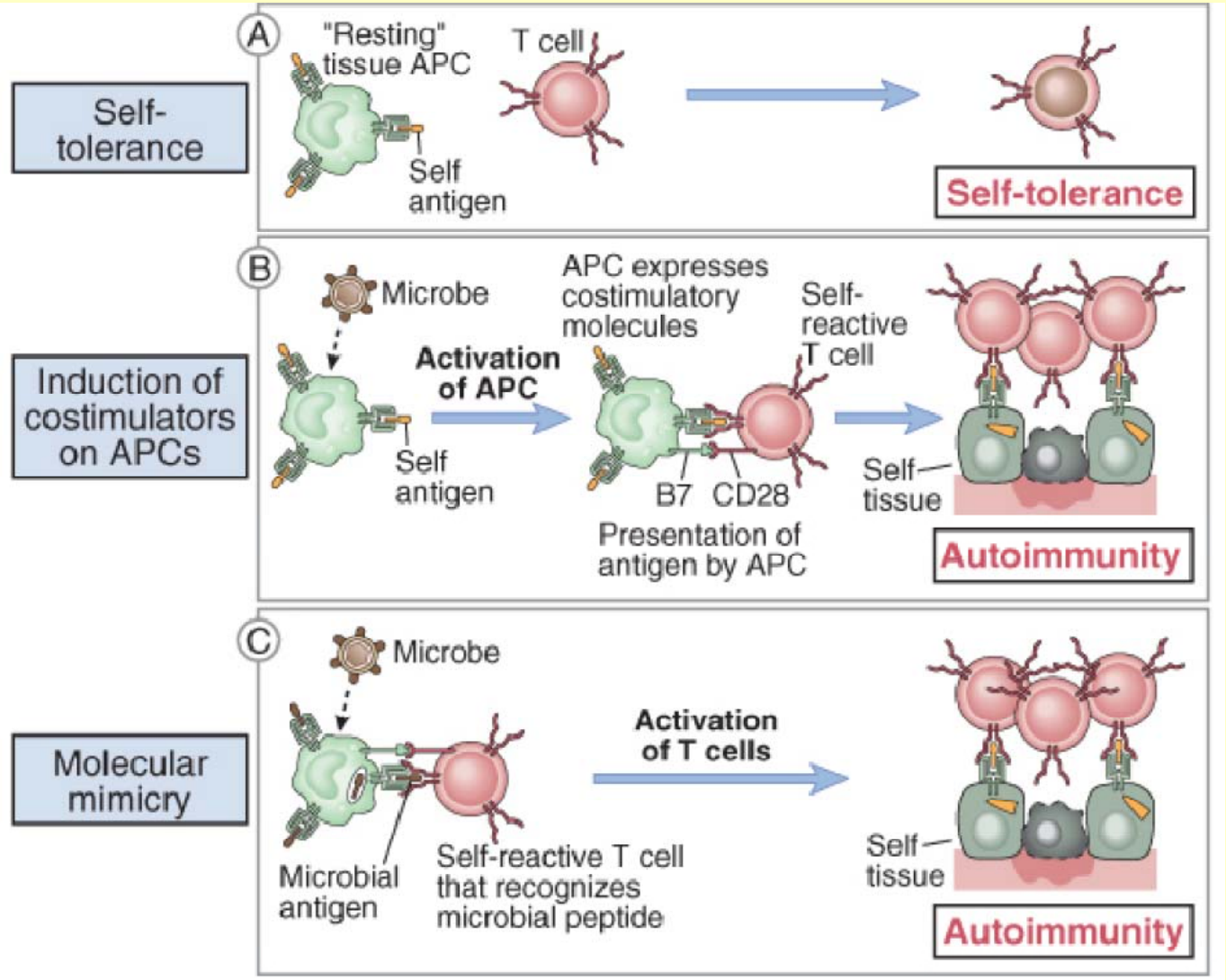
Breaking of Self-tolerance and Molecular Mimicry



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development of autoreactivity

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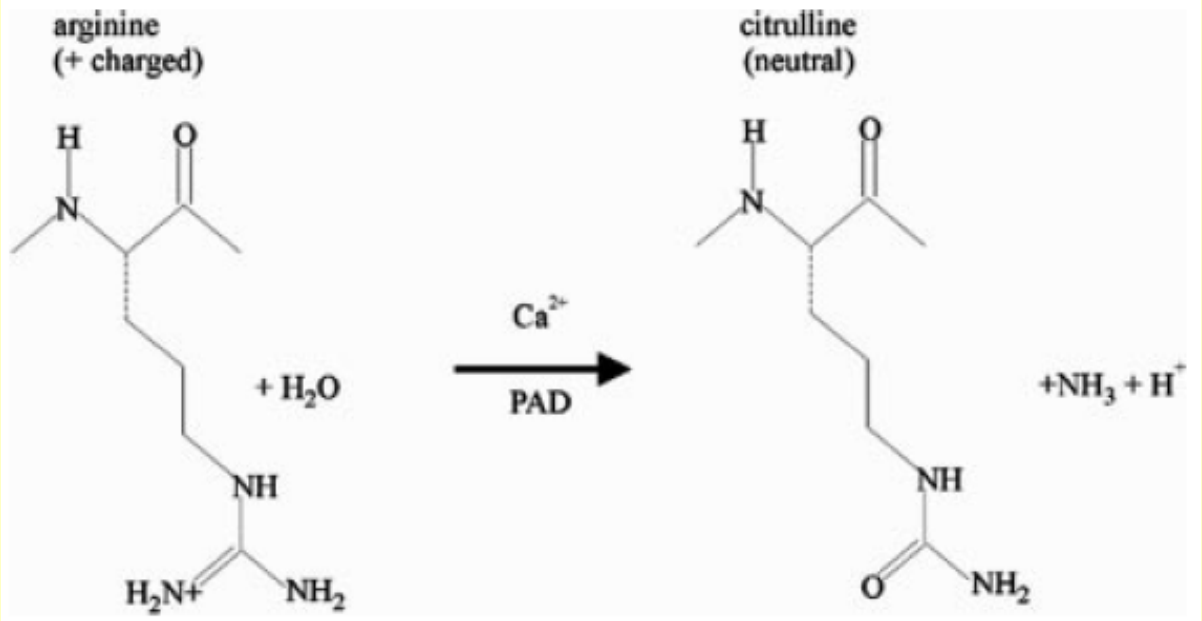

Enzymatic conversion of arginine to citrulline is catalyzed by PAD enzymes.
Five mammalian peptidylarginine deiminases (PADs),PAD1–4 and PAD6, are known

Developement of an anti-citrullinated protein autoimmunity in RA

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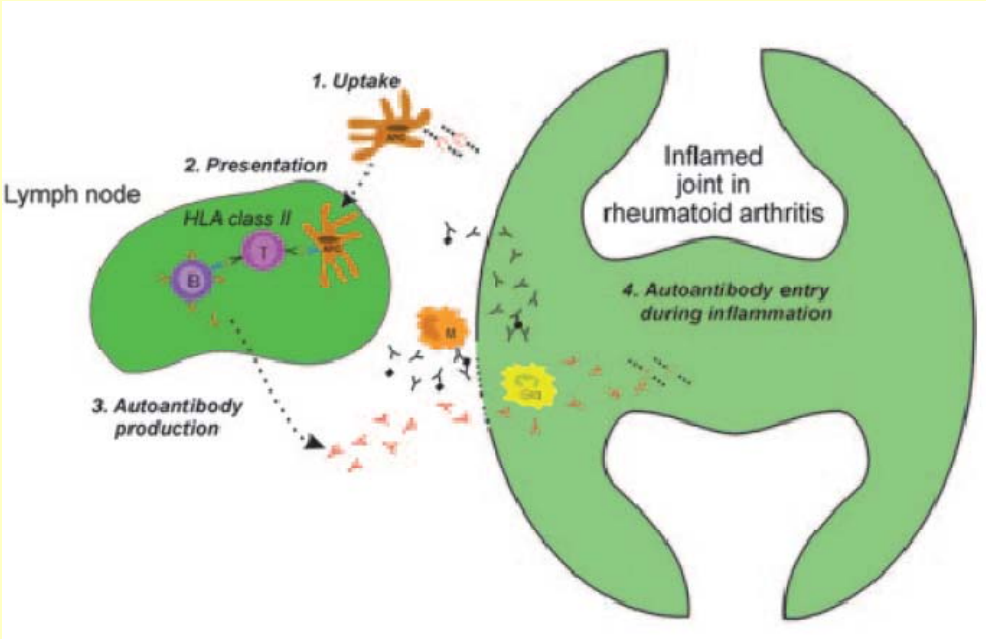

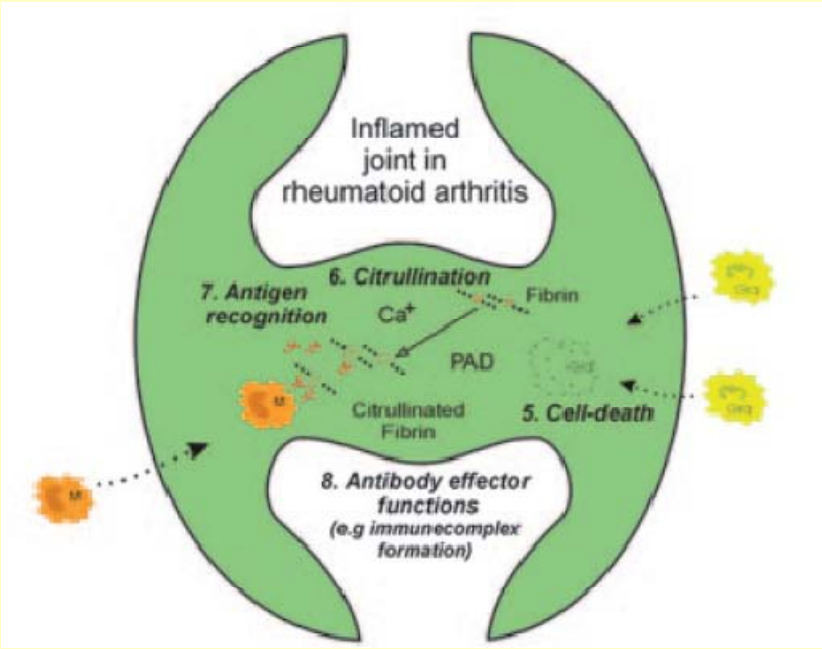

- **associated with HLA-DR genetics**
- **only with HLA-DR enhancement by smoking**
- **antibodies to citrullinated proteins found up to 9 years before onset of RA !!**

Multiple Hit Model for RA

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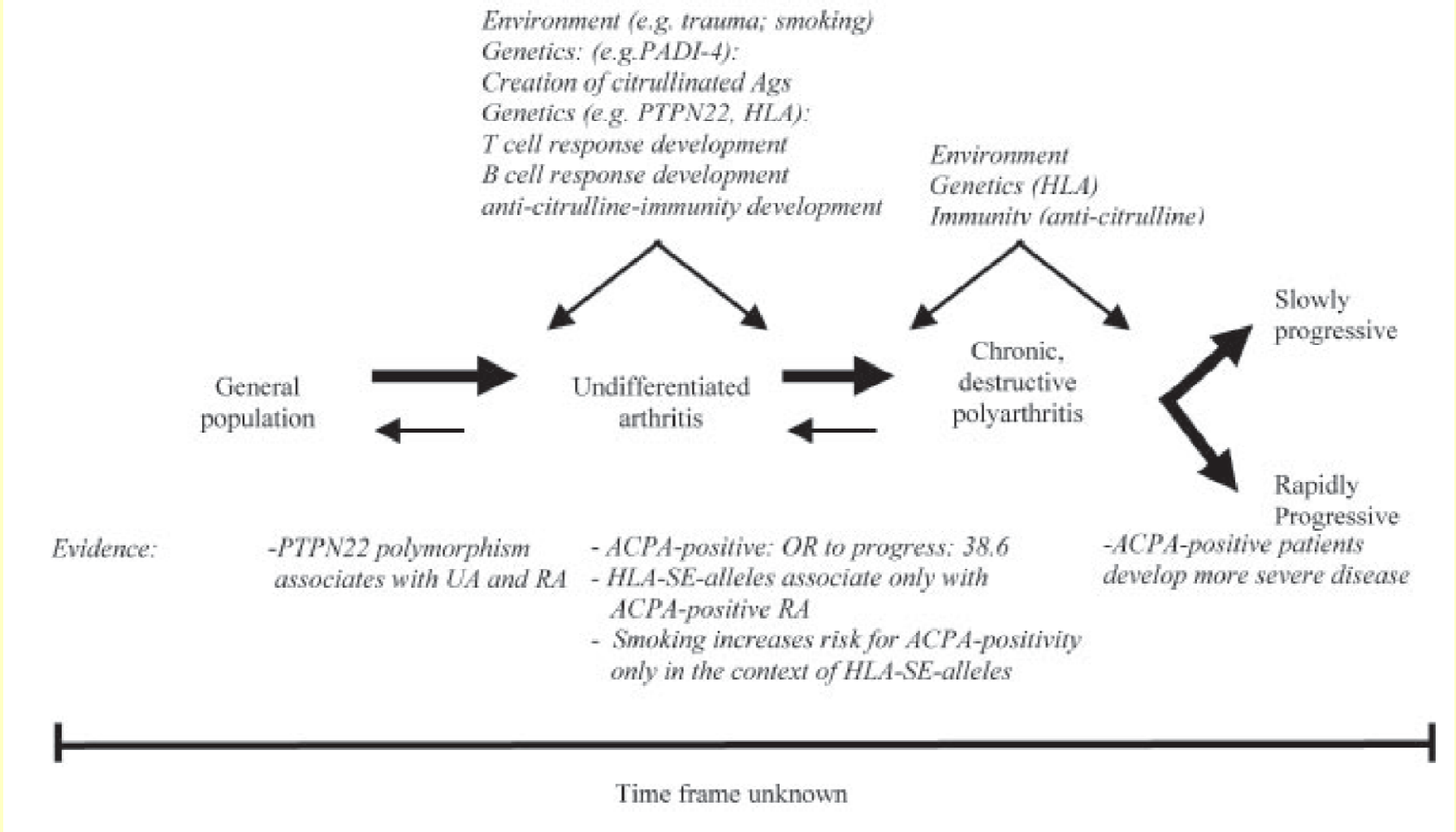

Concepts of Chronic Inflammation

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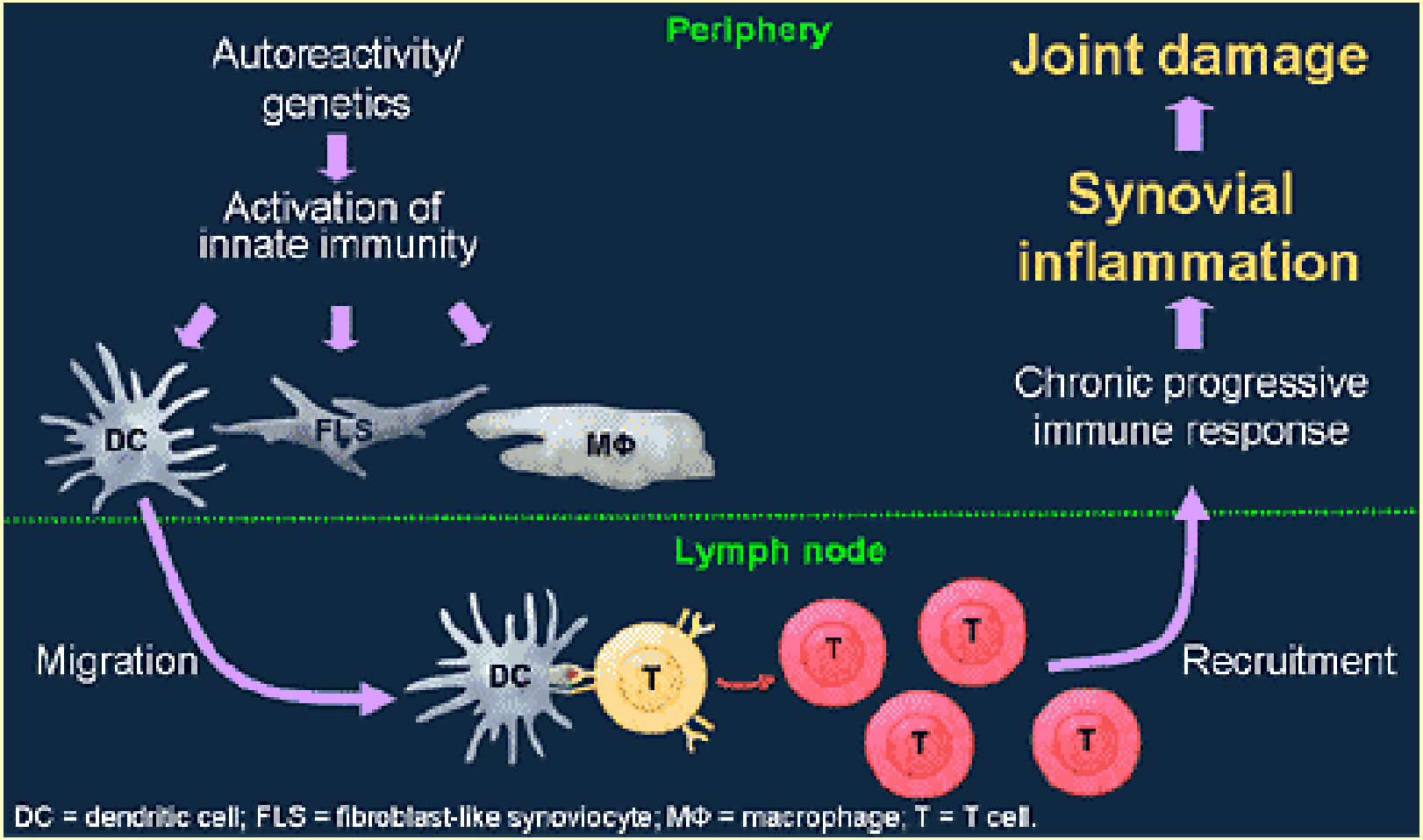
Why does inflammation persist: a dominant role for the stromal microenvironment?

Michael R. Douglas, Karen E. Morrison, Michael Salmon and Christopher D. Buckley

Inflammatory responses occur within tissue microenvironments, with functional contributions from both haematopoietic (lymphocytic) cells and stromal cells (including macrophages and fibroblasts). These environments are complex – a compound of many different cell types at different stages of activation and differentiation. Traditional models of inflammatory disease highlight the role of antigen-specific lymphocyte responses and attempt to identify causative agents. However, recent studies have indicated the importance of tissue microenvironments and the innate immune response in perpetuating the inflammatory process. The prominent role of stromal cells in the generation and maintenance of these environments has begun to challenge the primacy of the lymphocyte in regulating chronic inflammatory processes. Sensible enquiries into factors regulating the persistence of inflammatory disease necessitate an understanding of the mechanisms regulating tissue homeostasis

Model for the Etiology of RA



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courtesy Dr. med. Andrea Himsel, Med. Klinik II.

Detection of Th1-attracting, pro-inflammatory chemokines in RA

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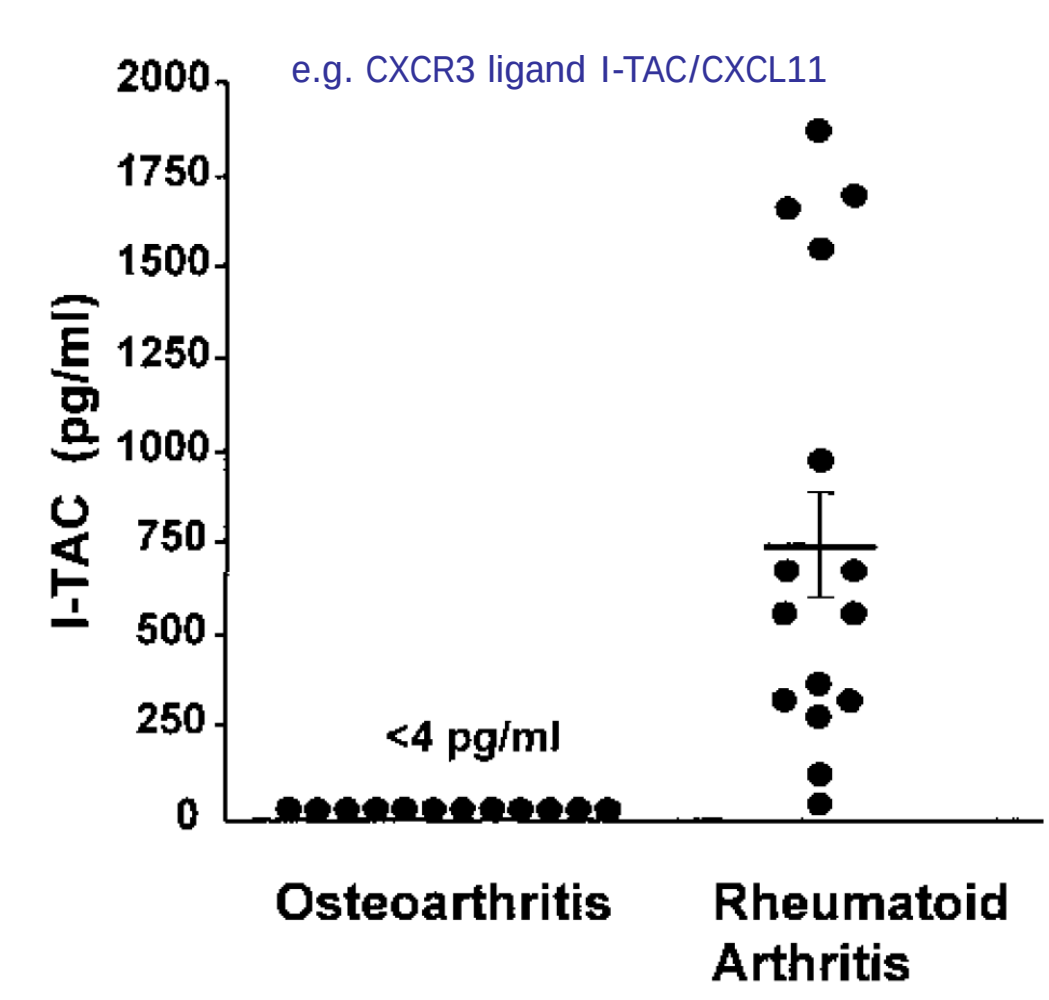

from Mohan et al., J. Immunol. 168:6420-6428, 2002

important other

CCL2 / MCP-1
CCL3 / MIP-1α
CCL5 / RANTES

CXCL9 / MIG
CXCL10 / IP-10

Chemokine receptors and ligands in Rheumatoid Arthritis (meta-analysis)

• CXCR1/ CXCR2[1,3,6,23]	CXCL1[28], CXCL5[1], CXCL6[1], CXCL8[1]	
• **CCR1**[1,2,9,12]	CCL3[1,14,25], CCL5[1,8,9,28], CCL7[1,9], CCL8[1,9], CCL15[1,9]	
• CCR2[1,2,13]	CCL2[1,8], CCL7[1,9], CCL8[1,9]	
• **CCR5**[1,2,4,6,9,13,14,15,24,25]	CCL3[1,14,25], CCL4[1,25], CCL5[1,8,9], CCL8[1,9],	
• **CXCR3**[1,2,3,4,6,11,13,24,25]	CXCL9[1,3,11,25,28], CXCL10[1,3,7,11,19,25], CXCL11[1,20]	
• **CCR6**[5,6,13,16,17,18]	CCL20[5,16,17,18]	(homing)
• CCR7[2,5,6]	CCL19[5], CCL21[5,10]	(homing)
• **CXCR4**[2,6,9,18,22,26,27]	CXCL12[2,8,18,21,22,26]	(homing)
• CXCR5[2,6]	CXCL13[2,10]	(homing)

1) Ambrosio et al., J Immunol Methods, 273:3–13, 2003
2) Buckley, Rheumatology, 42:1–12, 2003
3) Ruschpler et al., Arthritis Res. Ther, 5:R241-R251, 2003
4) Qin et al., J. Clin. Invest. 101:746–754, 1998
5) Page et al., J. Immunol. 168:5333-5341, 2002
6) Nanki & Lipsky, Arthritis Res. 2:415–423, 2000
7) Hanaoka et al. Arthritis Res. Ther. 5:R74-R81, 2003
8) Shadidi et al., Scand. J. Immunol. 57:192-198, 2003
9) Haringman et al., Arthritis Res. & Ther. 5(S1):S12, 2003
10) Manzo et al., Arthritis Res. & Ther. 5(S1):S32, 2003
11) Krystufkova et al., Arthritis Res. & Ther. 4(S1):S22, 2002
12) Haringman et al., Arthritis Res. & Ther. 4(S1):S34, 2002
13) Ruth et al., Arthr.& Rheum. 44(12):2750–2760, 2001
14) Wang & Liu, Clin. Exp. Immunol. 132:371–378, 2003
15) Gomez-Reino et al., Arthr.& Rheum. 42(5):989–992, 1999
16) Ruth et al., Lab. Invest. 83:579–588, 2003
17) Matsui et al., Clin. Exp. Immunol. 125:155-161, 2001
18) Cravens & Lipsky, Immunol. Cell Biology 80:497–505, 2002
19) Salomon et al., J. Immunol. 169:2685–2693, 2002 (rat)
20) Mohan et al., J. Immunol. 168:6420–6428, 2002
21) Pablos et al., J. Immunol. 170:2147–2152, 2003
22) Blades et al., Arthr.& Rheum. 46(3):824–836, 2002
23) Podolin et al., J. Immunol. 169:6435–6444, 2002 (rabbit)
24) Wedderburn et al., Arthr.& Rheum. 43(4):765–774, 2000
25) Patel et al., Clin. Immunol. 98(1):39–45, 2001
26) Buckley et al., J. Immunol. 165: 3423–3429, 2000
27) Matthys et al., J. Immunol. 167: 4686–4692, 2001 (mouse)
28) Konig et al., Virchow Arch. 436:449–458, 2000

Chemokines and cytokines define the cellular composition of lymphatic organs (normal)

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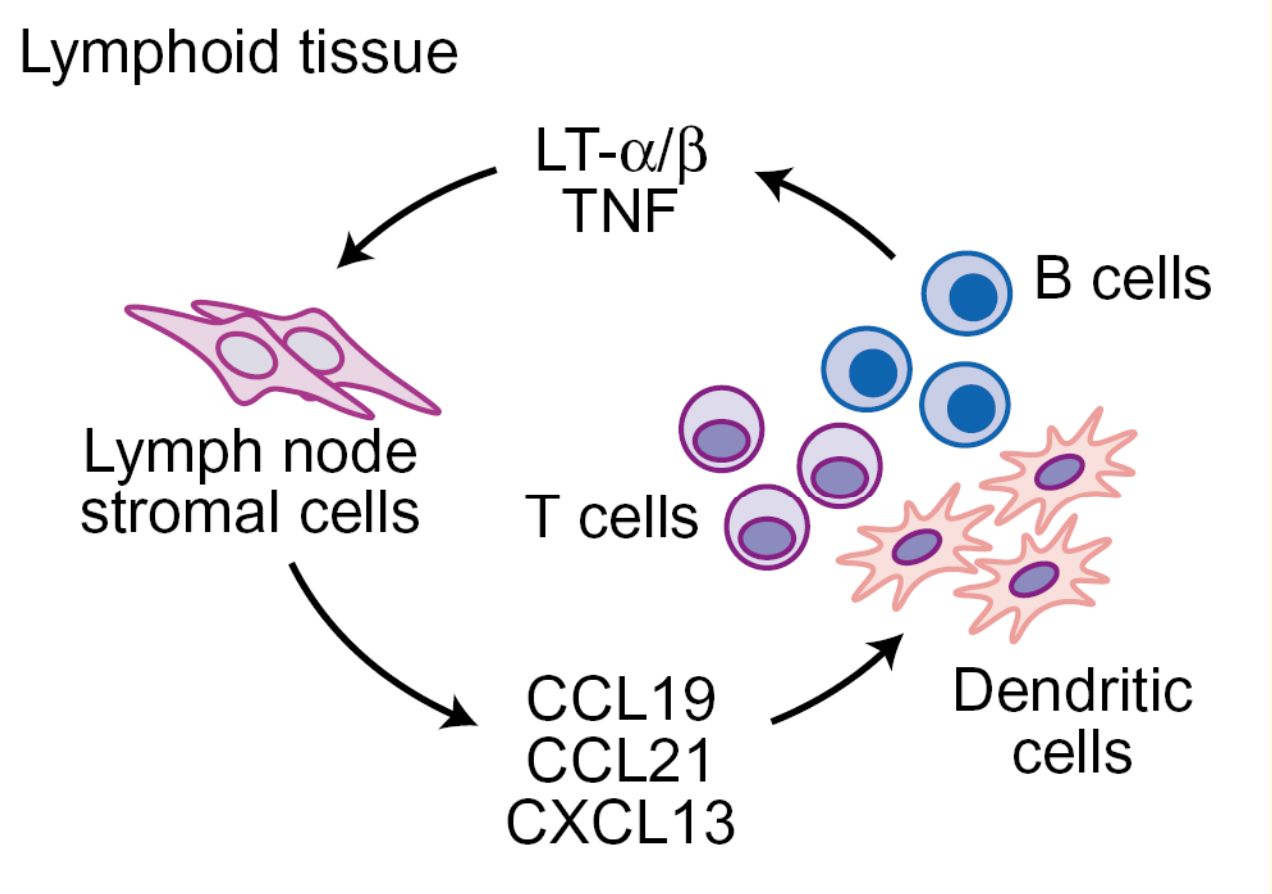

Modifiied from CD Buckley. Why does inflammation persist? expert reviews in molecular medicine 2002

Chemokines and cytokines also define the cellular composition of pathological lymph nodes

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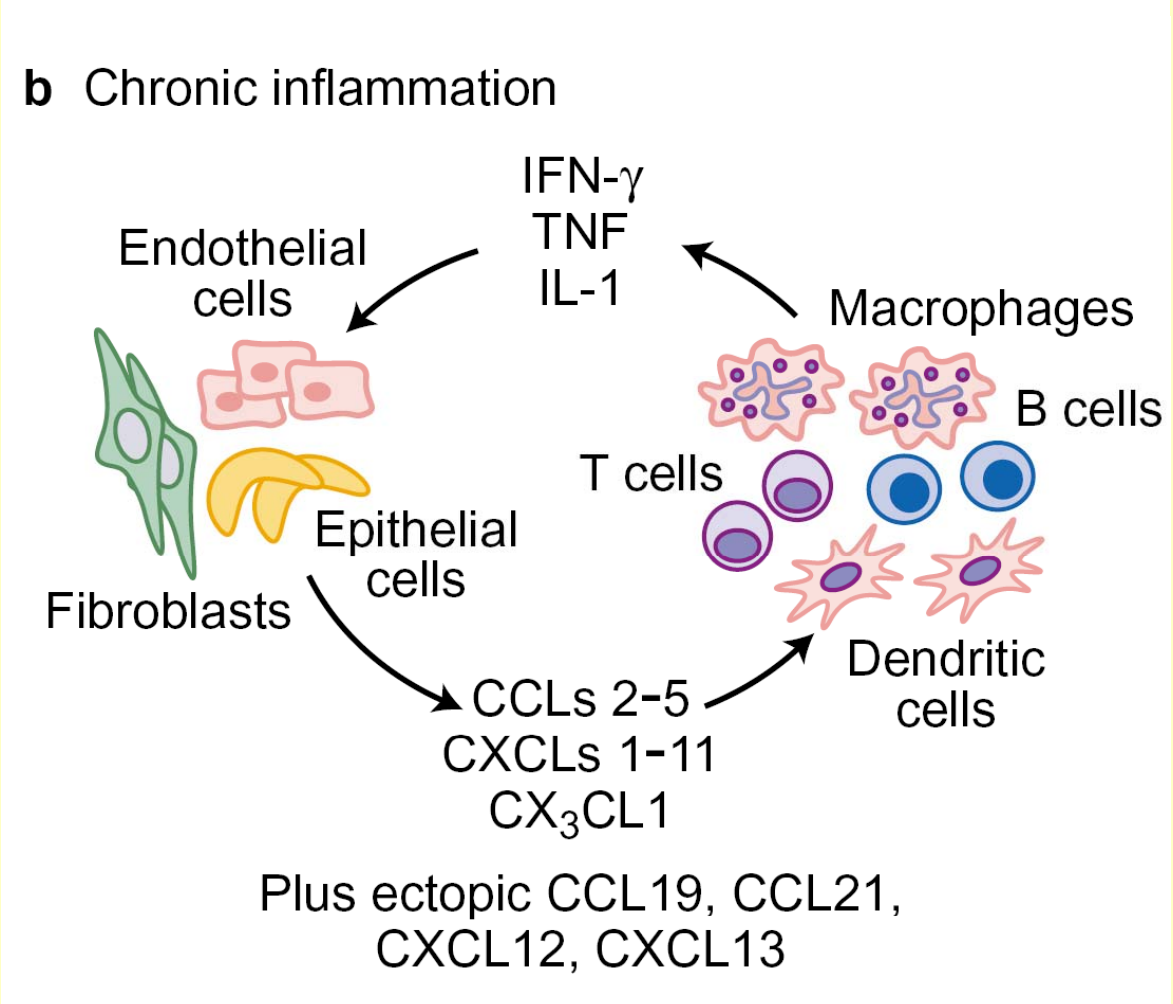

Modifiied from CD Buckley. Why does inflammation persist? expert reviews in molecular medicine 2002

Why leukocytes accumulate in synovial tissue…

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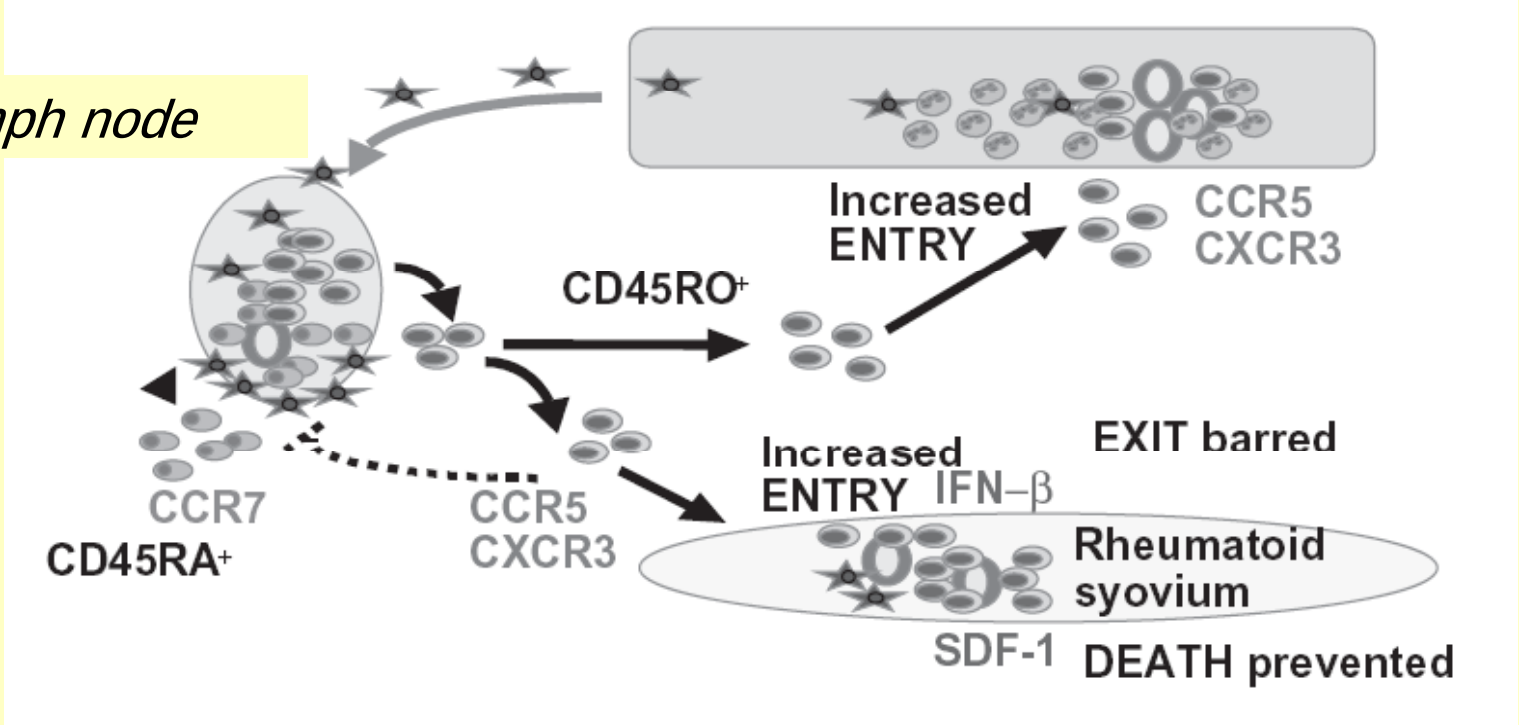
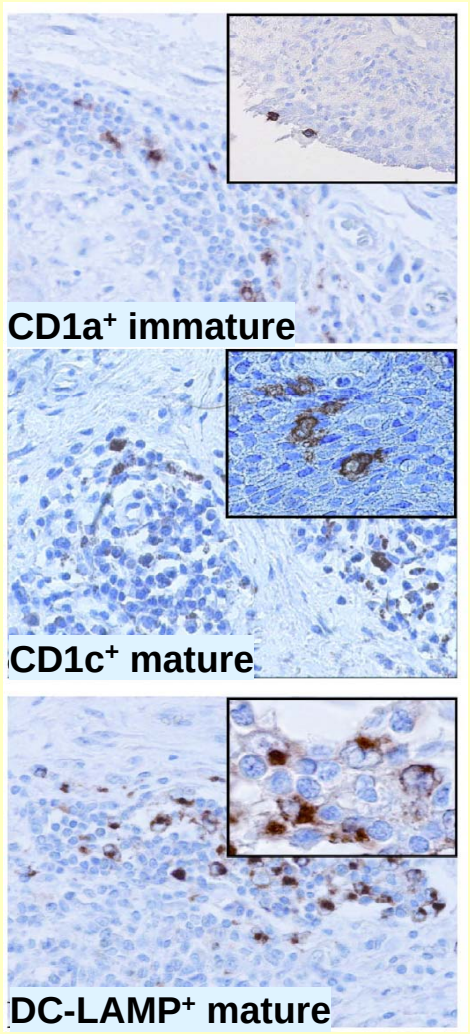
inflammatory infiltrate



RA: tertiary lymph follicle

Dendritic cells and chemokines in different tertiary lymph follicles of RA synovial tissue

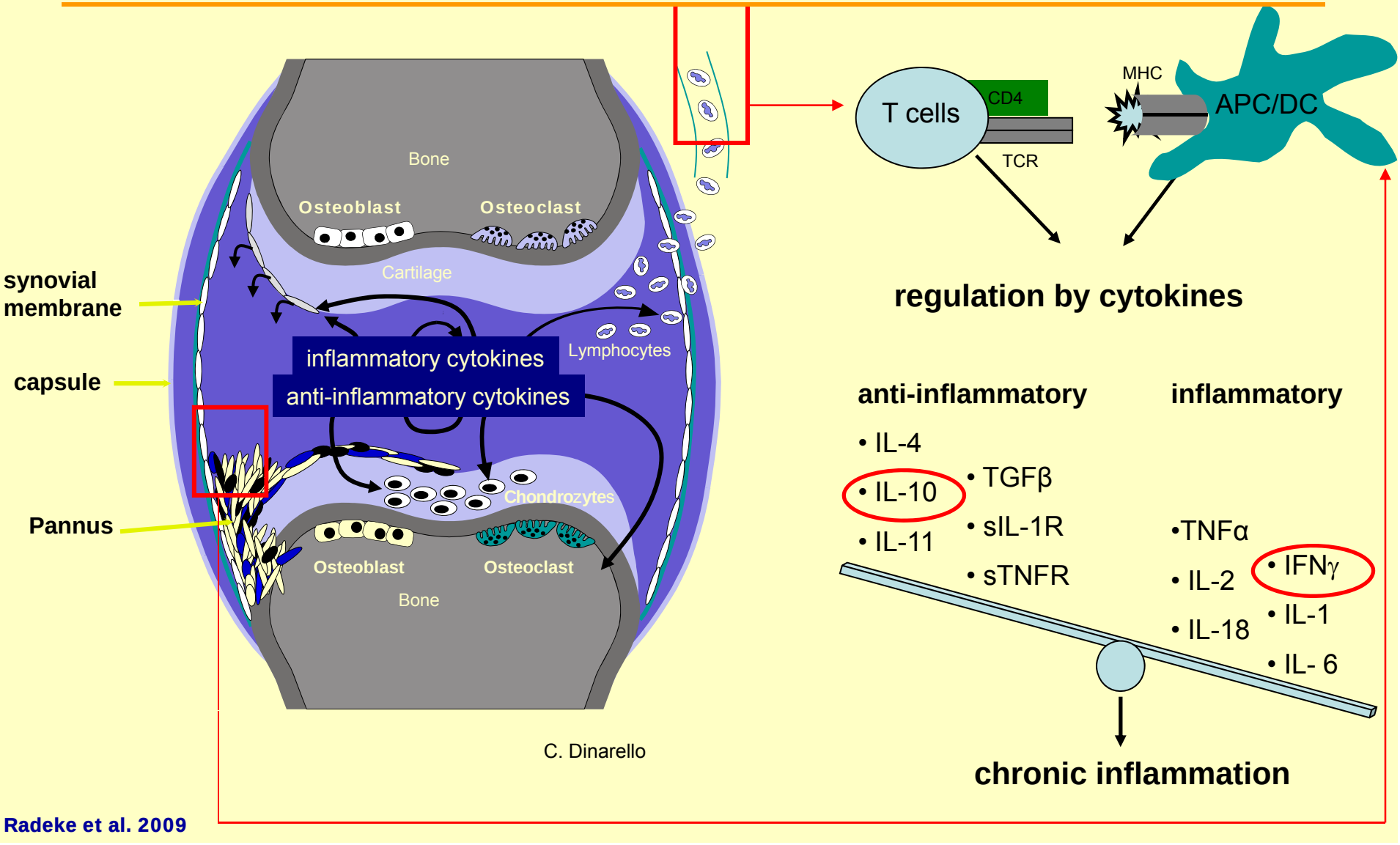
1) immature DC	**MIP-3α / CCL20** [1] naïve T helper
2) mature myeloid DC	**SDF-1 / CXCL12** [2] **SLC / CCL21** [1] Th1 memory homing lympho-, myelopoiesis
3) follicular DC	**BCA-1 / CXCL13** [3] B cell homing/activation



1) u.a. Page et al. J. Immunol. 168:5333-5341, 2002
2) u.a. Blades et al., Arthr.& Rheum. 46(3):824–836, 2002
3) u.a. Buckley, Rheumatology, 42:1–12, 2003

1) Modifiziert nach Page et al. J. Immunol. 168:5333-5341, 2002

Rheumatoid Arthritis: immunological activity in three compartments



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Regulatory T cells in Rheumatoid Arthritis

Behrens F, Himsel A, ...Radeke HH. Annals of the Rheumatic Diseases, 66:1151-1156, **2007**

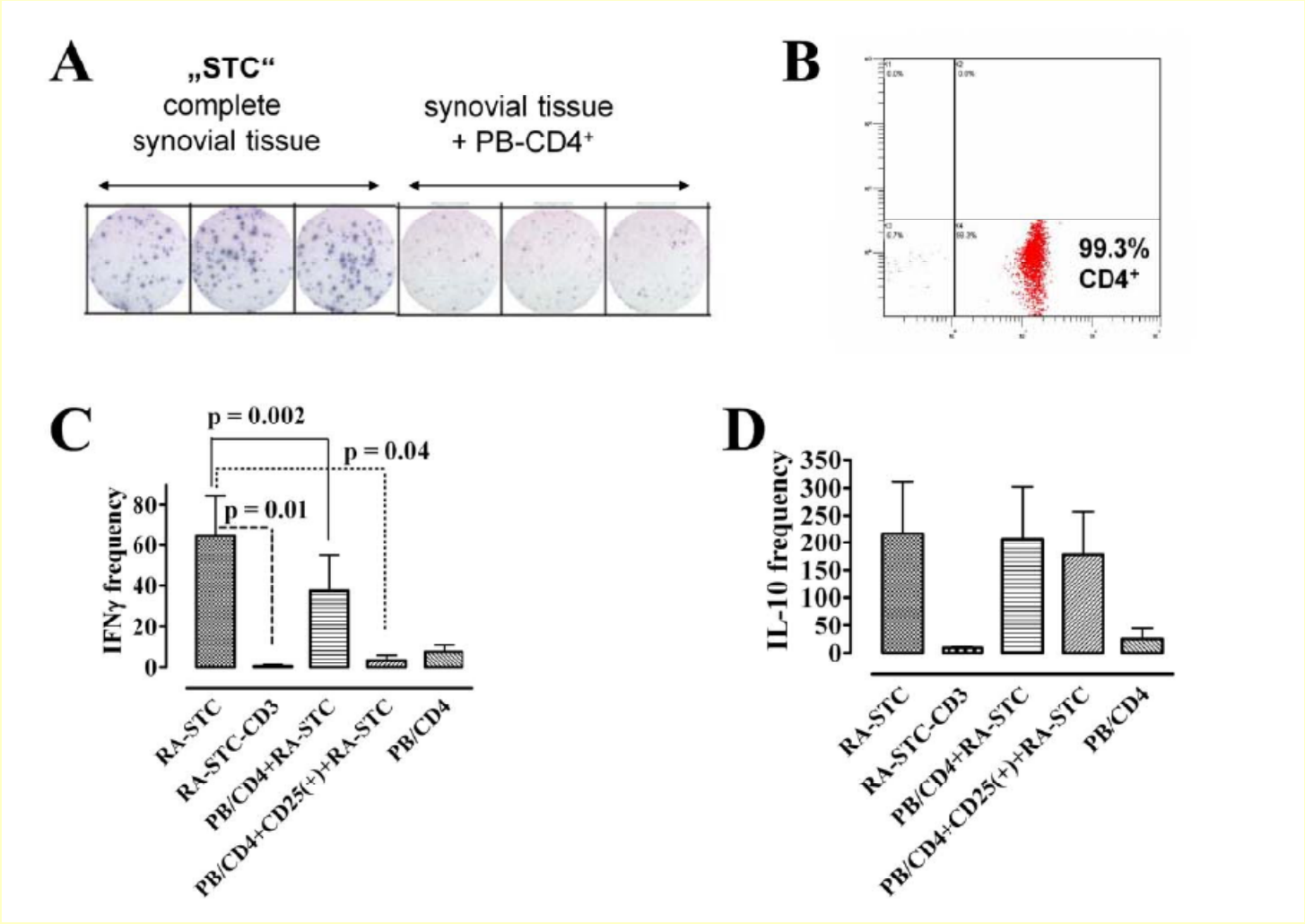
- controversy exists regarding to the frequency of Treg cells in the peripheral circulation of patients with RA
- elevated numbers of Treg cells are present in the synovial fluid of patients with RA as compared with the peripheral circulation
- However, little known about regulatory T cells in situ, i.e. in the synovial tissue

Radeke et al. 2009

courtesy Dr. med. Andrea Himsel, Med. Klinik II.

High Th1 inflammatory activity in synovial tissue (ST) and inhibition by autologous Tregs

Behrens F, Himsel A, ...Radeke HH. Annals of the Rheumatic Diseases, 66:1151-1156, **2007**



???

Behrens F, Himsel A, ...Radeke HH. Annals of the Rheumatic Diseases, 66:1151-1156, **2007**

- If Treg are present in principle and
  are able to reduce immune responses

Why don`t they suppress arthritis?

Possible Reasons:

- can`t invade into the synovial membrane ?
- impaired function ?

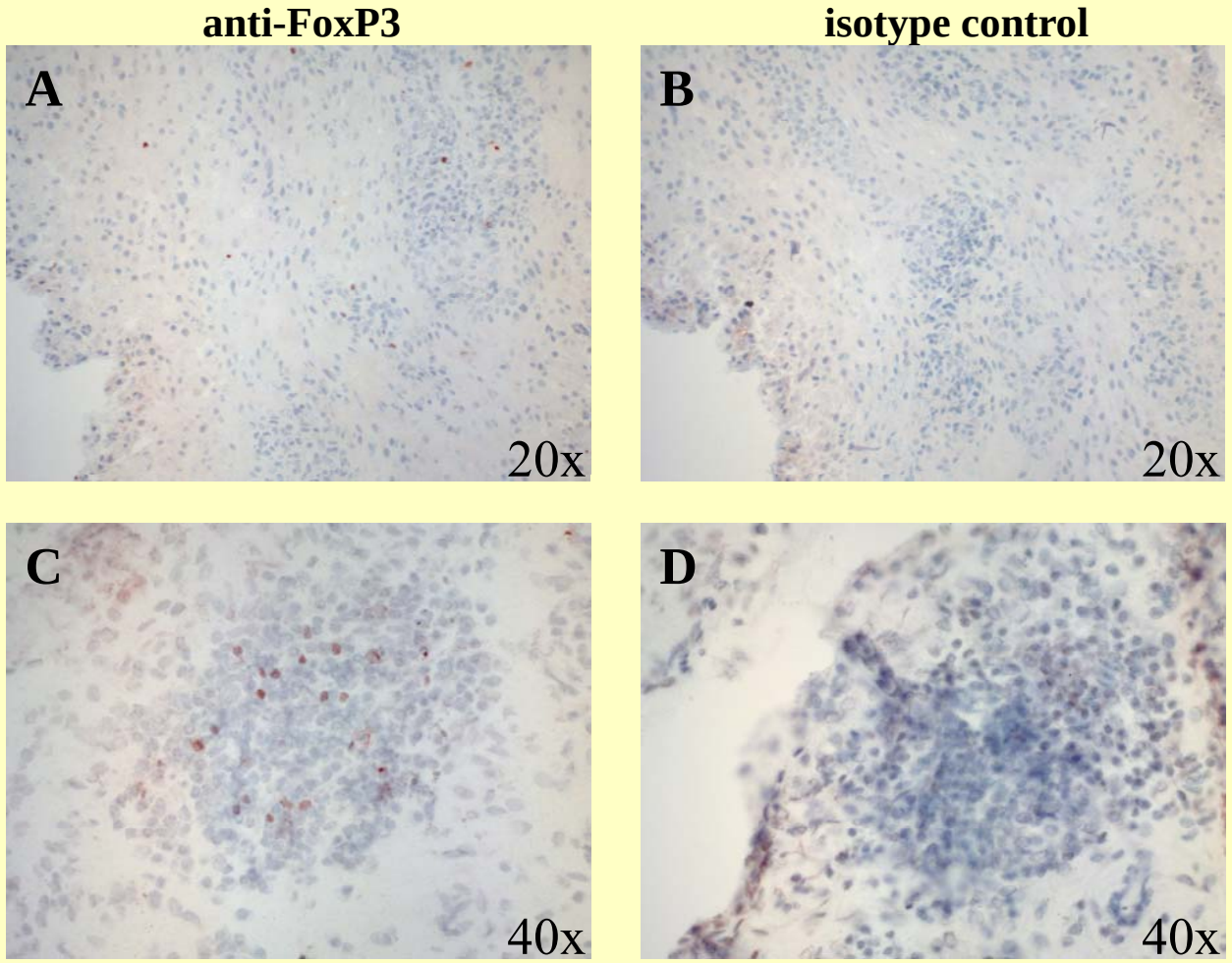
Initial questions

Behrens F, Himsel A, ...Radeke HH. Annals of the Rheumatic Diseases, 66:1151-1156, **2007**

- Can we identify specific markers for Treg (Foxp3) vs. Th1-lympocytes (T-bet) at transcription level in the target organ of RA - synovial tissue- in situ (ex vivo) ?
- how is the distribution of Foxp3 and T-bet between the different compartments of RA-joint ?

FoxP3-positive Tregs in synovial tissue

Behrens F, Himsel A, ...Radeke HH. Annals of the Rheumatic Diseases, 66:1151-1156, **2007**



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How can we normalize the patient samples from different tissues?

Behrens F, Himsel A, ...Radeke HH. Annals of the Rheumatic Diseases, 66:1151-1156, **2007**

1.

18S rRNA is the **most** stable housekeeping gene (compared to GAPDH and β-actin) for normalization of mRNA expression levels in human T lymphocytes

(A. Bas et al.; Scandinavian Journal of Immunology 59, 566–573)

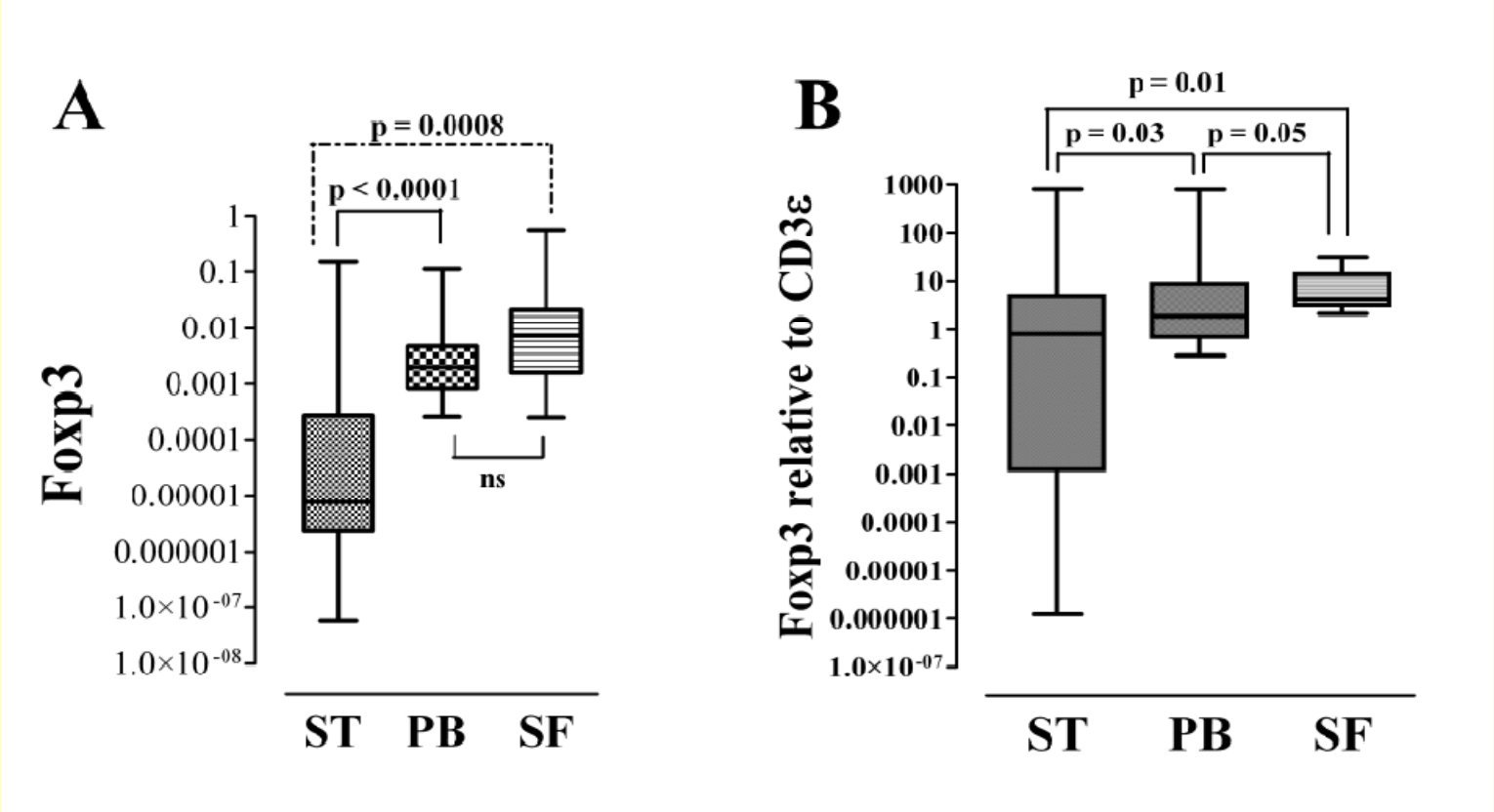
2.

CD3ε is constitutively expressed in T lymphocytes and a single-mutation in the CD3ε change cause immunodeficiency (SCID)

(G. de Saint Basile et al.; J Clin Invest. 114,1512-1517)

relative lack of Tregs in synovial tissue

Behrens F, Himsel A, ...Radeke HH. Annals of the Rheumatic Diseases, 66:1151-1156, **2007**



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The ins and outs of Ly in RA: cell sorting is a pathomechanism…

Behrens F, Himsel A, …Radeke HH. Annals of the Rheumatic Diseases, 66:1151-1156, **2007**

T-bet mRNA:

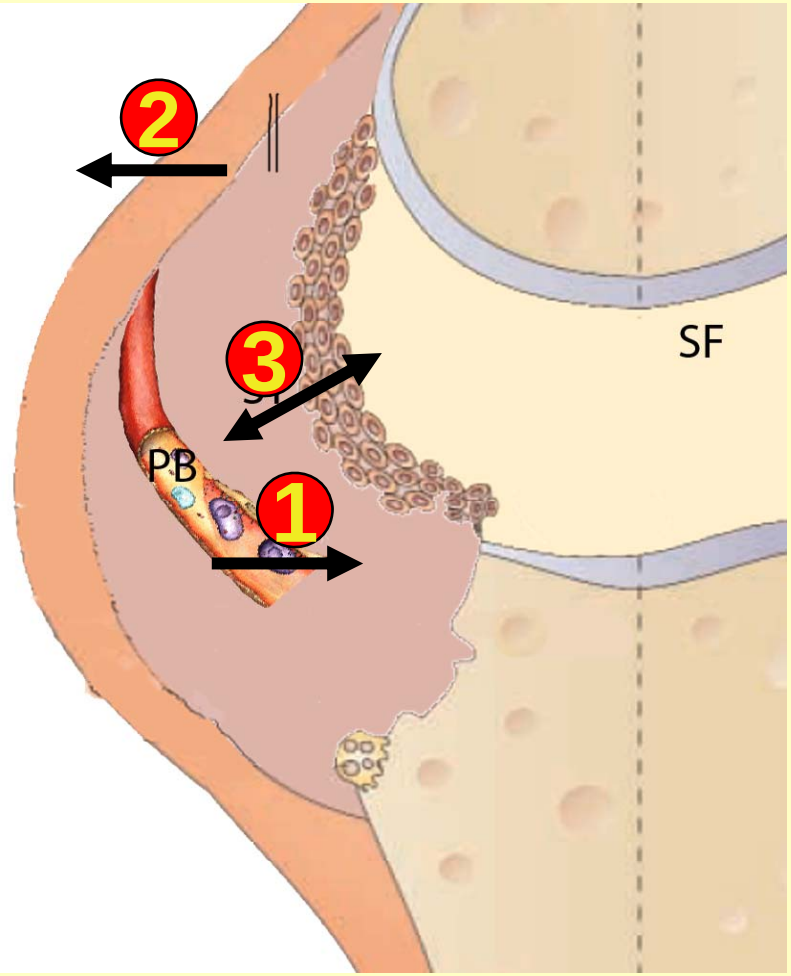
ST > PB > SF

FoxP3/T-bet mRNA

ST << PB < SF

disease activity correlates with:

DAS 28 ≙ T-bet/FoxP3 in ST



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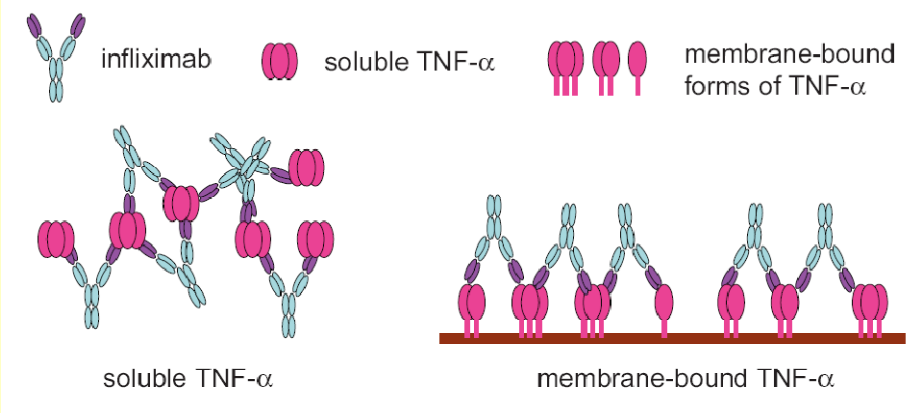
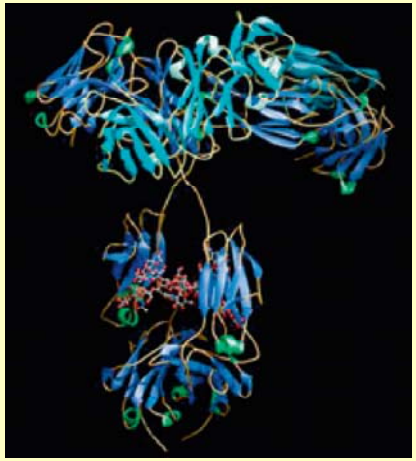
Therapeutic success and serious side effects of the TNF-alpha blockade with Etanercept, Infliximab, and Adalimumab

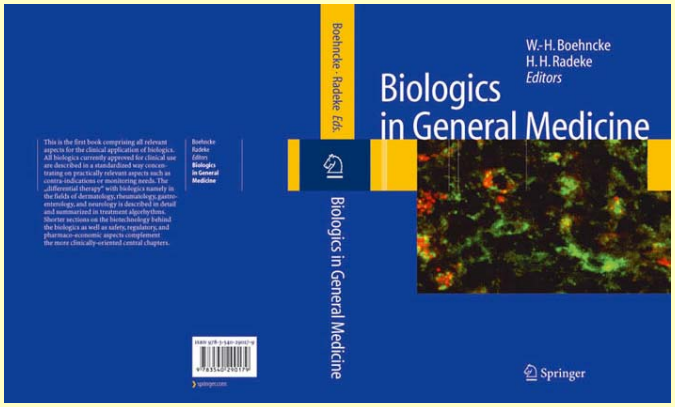


Infliximab



Adalimumab

Serious Adverse Events:
- **reactivation of latent tuberculosis**
- **increased risk of lymphoma**
- **anti-nuclear antibodies (SLE)**
- **demyelinating disease (MS)**
- **infection rate increased**



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Discussion – new targets – possible GK1172 projects

- Coop. BMBF grant application H. Burckhart / H. Radeke to investigate lymphocyte extravasation, migration and differentiation in situ in human synovial tissue symples with the MelTec technology (3D proteomics)
- New targets? **IL-6** (IL-6R Ab), **IL-12**, IL-15, **IL-17** IL-18, **IL-21**, **IL-23**, B lymphocytes (**CD20/Rituximab**), collagenases, co-stimulatory (**CTLA4-Ig** (Abatacept, binding CD80/86), PD-1) and adhesion molecules (**VLA-4, LFA-1** alpha subunit CD11a (Natalizumab; Efalizumab, CD2 – a LFA-3/Ig fusion protein binding to **CD2 (Alefacept**)) and chemokines (CCR2-, CXCR3 antagonists), TLRs (TLR3, etc.)
- Conny is working on a completely new target - **ncf1** (Rikard Holmdahl, Lund – Karolinska): a NADPH-oxidase component p47phox whose genetic defect „paradoxically“ leads to enhanced frequency of CIA in rats and mice. Classically it is deduced that a lack of NADPH oxidase components might improve inflammatory conditions (see therapeutic concepts involving the toxicity of radicals), however in this case the opposite is happening …. OR NOT…?!
- Clinical Trials running: IL-15Ab, IL-18RA, CTLA4-Ig, CD11a, IL12p40AB … etc. pp.

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Summary

- Rheumatoid Arthritis is a multifactor autoimmune and chronic inflammatory disease with high prevalence, social and individual costs and reduced life expectancy
- genetic and environmental mechanisms of Th1 autoimmunity can be detected
- Chronicity may be based on
  - deterioation of local synoviocytes,
  - chemokine-induced tertiary lymph nodes and/or a
  - pathological distribution of regulatory T cell activity
- modern „**biologics**“ for the first time may deserve the label as DMARDs: „**disease modifying anti-rheumatic drugs**“

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