Παθογένεια Ρευματοειδούς Αρθρίτιδας

ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
ΙΑΤΡΙΚΗ ΣΧΟΛΗ

Π. Σιδηρόπουλος
Ρευματολογία, Κλινική Ανοσολογία, Αλλεργιολογία
Ιατρική Σχολή, Παν. Κρήτης
www.rheumatology-uoc.gr
sidiropp@uoc.gr

ΠΑΝΕΠΙΣΤΗΜΙΑΚΟ
ΝΟΣΟΚΟΜΕΙΟ ΗΡΑΚΛΕΙΟΥ

References

- Immunity. 2017;46(2):183 Immunopathogenesis of Rheumatoid Arthritis
- Nat Rev Rheumatol. 2012 Oct;8(10):573-86. Pre-rheumatoid arthritis: predisposition and transition to clinical synovitis. Arend WP, Firestein GS.
- N Engl J Med. 2011 Dec 8;365(23):2205-19. doi: 10.1056/NEJMra1004965. The pathogenesis of rheumatoid arthritis. McInnes IB1, Schett G.
- Nat Rev Immunol. 2017 Jan;17(1):60-75.
  The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting.
  Malmström V, Catrina AI, Klareskog L

Outline

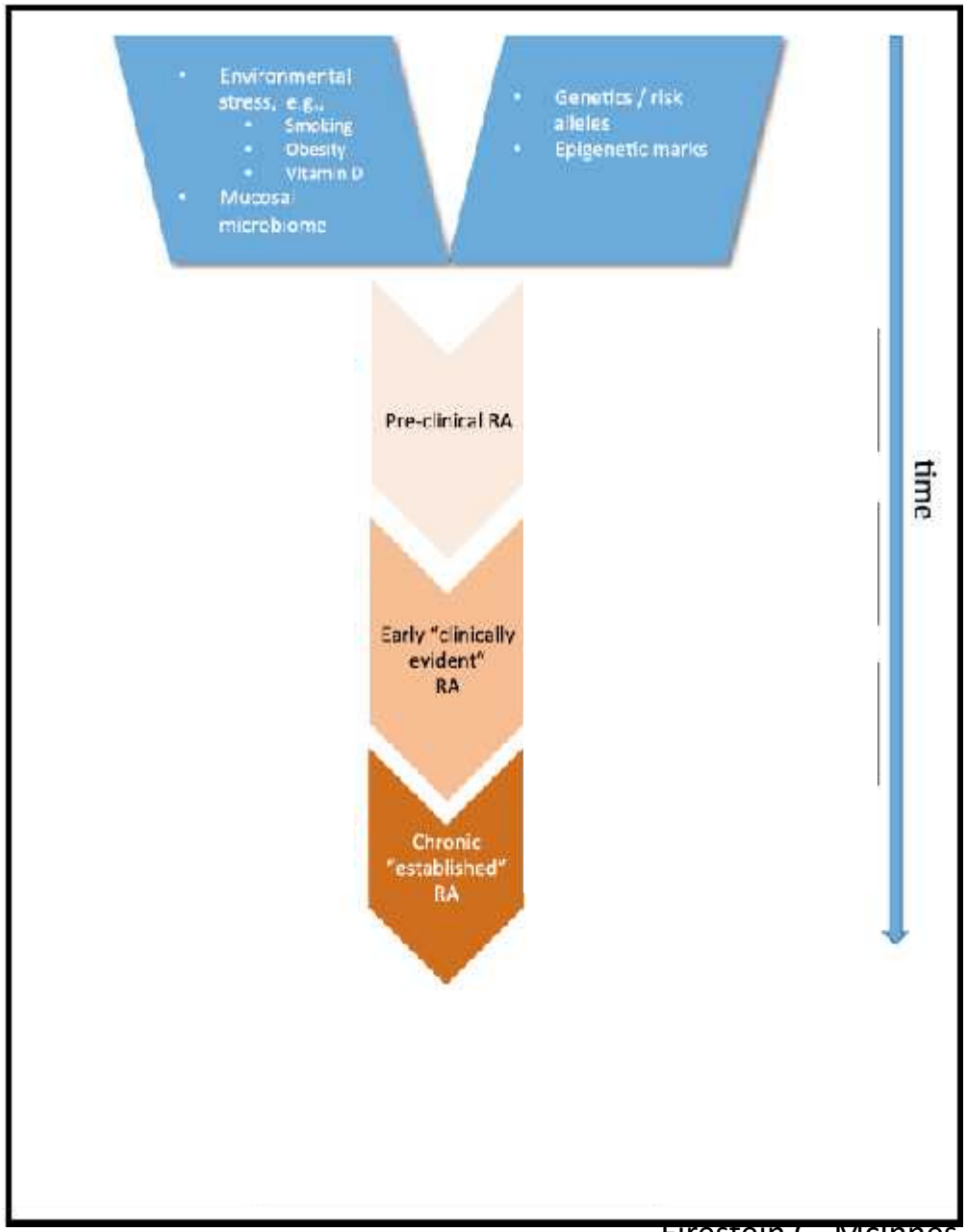
- Development of autoimmunity
  - Genes
  - Environment
  - Citrullination

- Synovial pathology
  - Lymphocyte/Macrophages
  - Cytokines

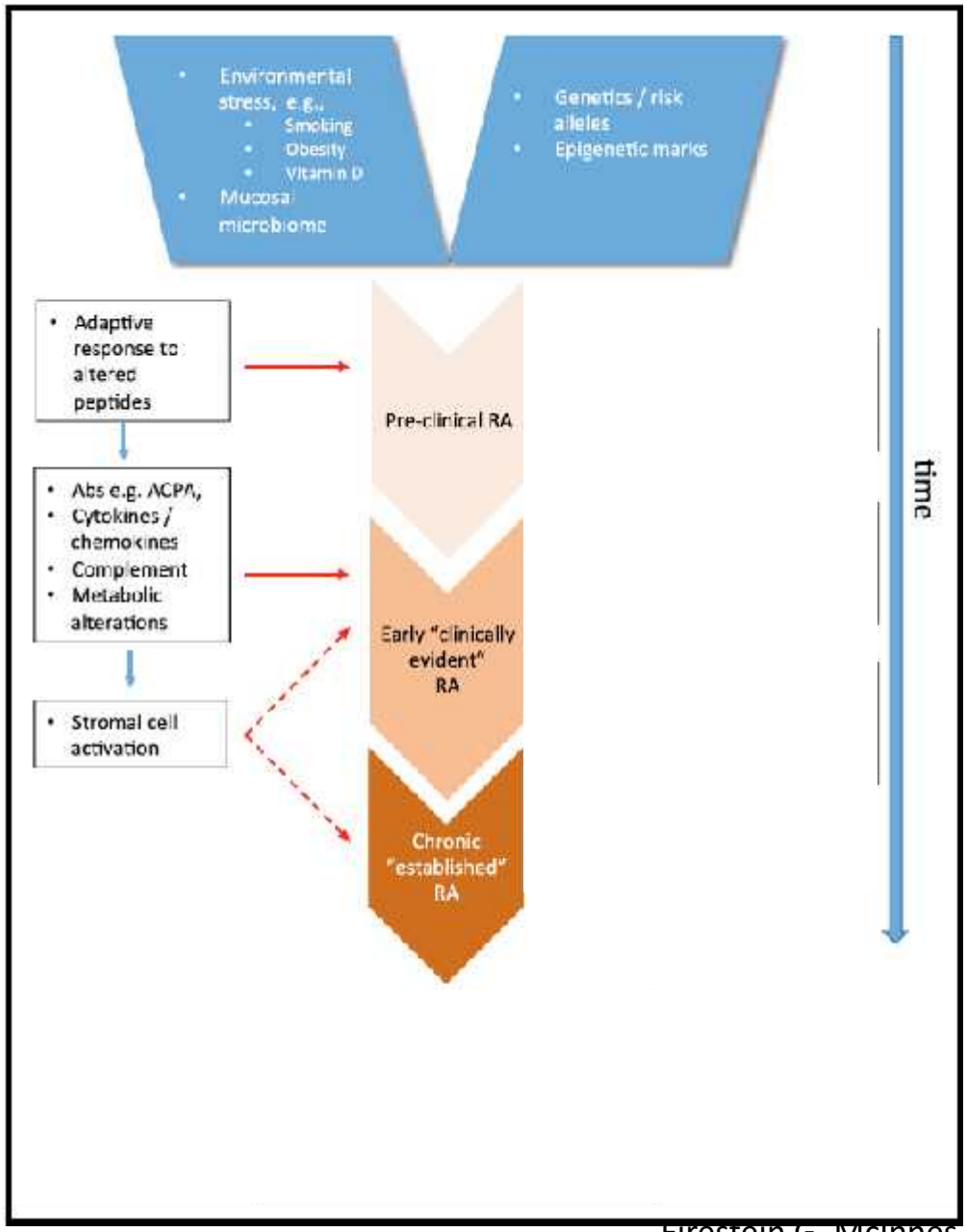
General concept for RA pathogenesis

"RA is characterized by evidence of **disordered innate immunity**, including immune complex-mediated complement activation, **adaptive immune** responses against ''self''-antigens comprising predominantly **post-translationally** modified proteins, dysregulated **cytokine** networks, **osteoclast** and chondrocyte activation, and imprinting of resident stromal cells that in turn develop semi-autonomous features that support disease progressions"

Firestein G, McInnes I. Immunity. 2017;46(2):183



Firestein G, McInnes I. Immunity. 2017;46(2):183



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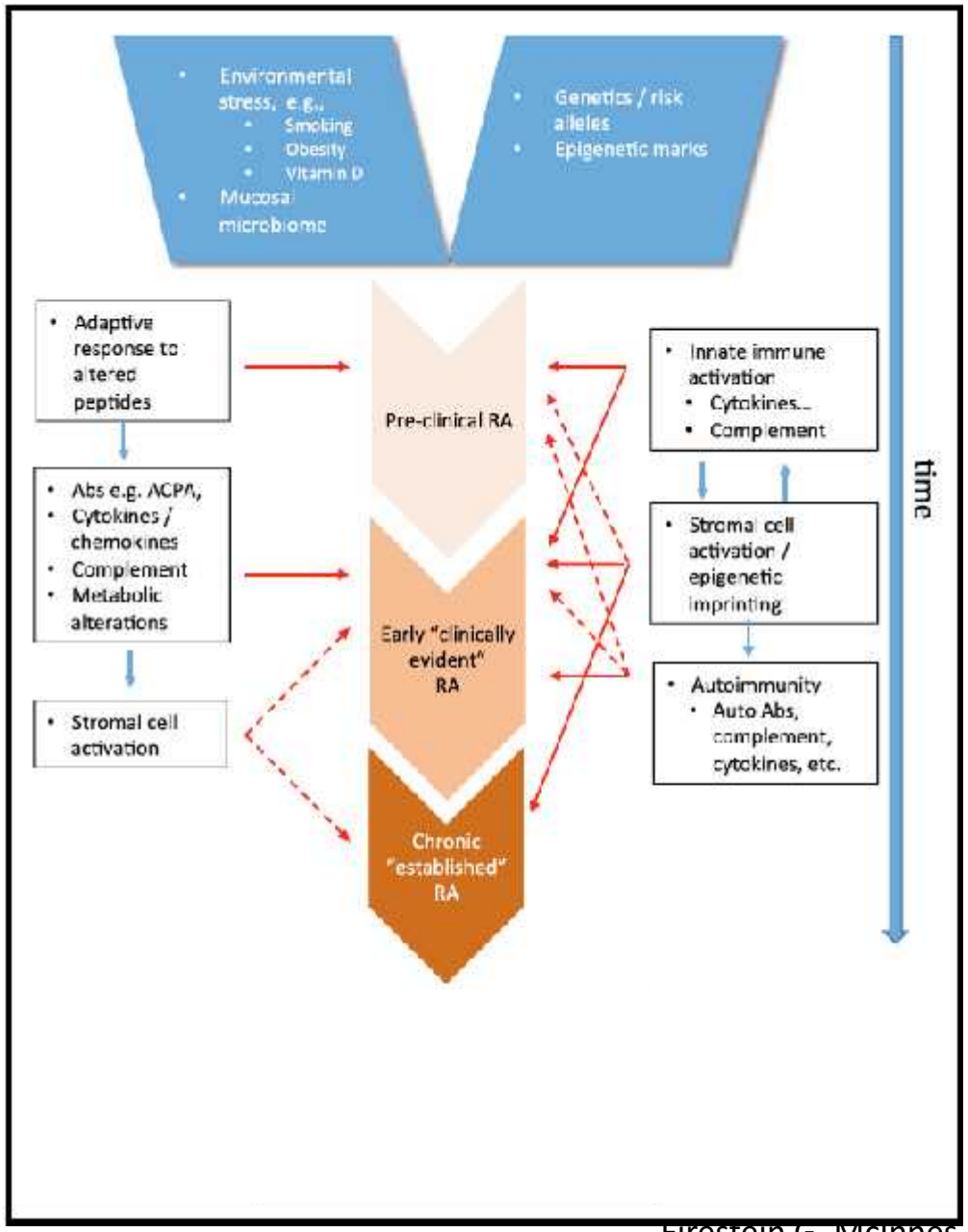
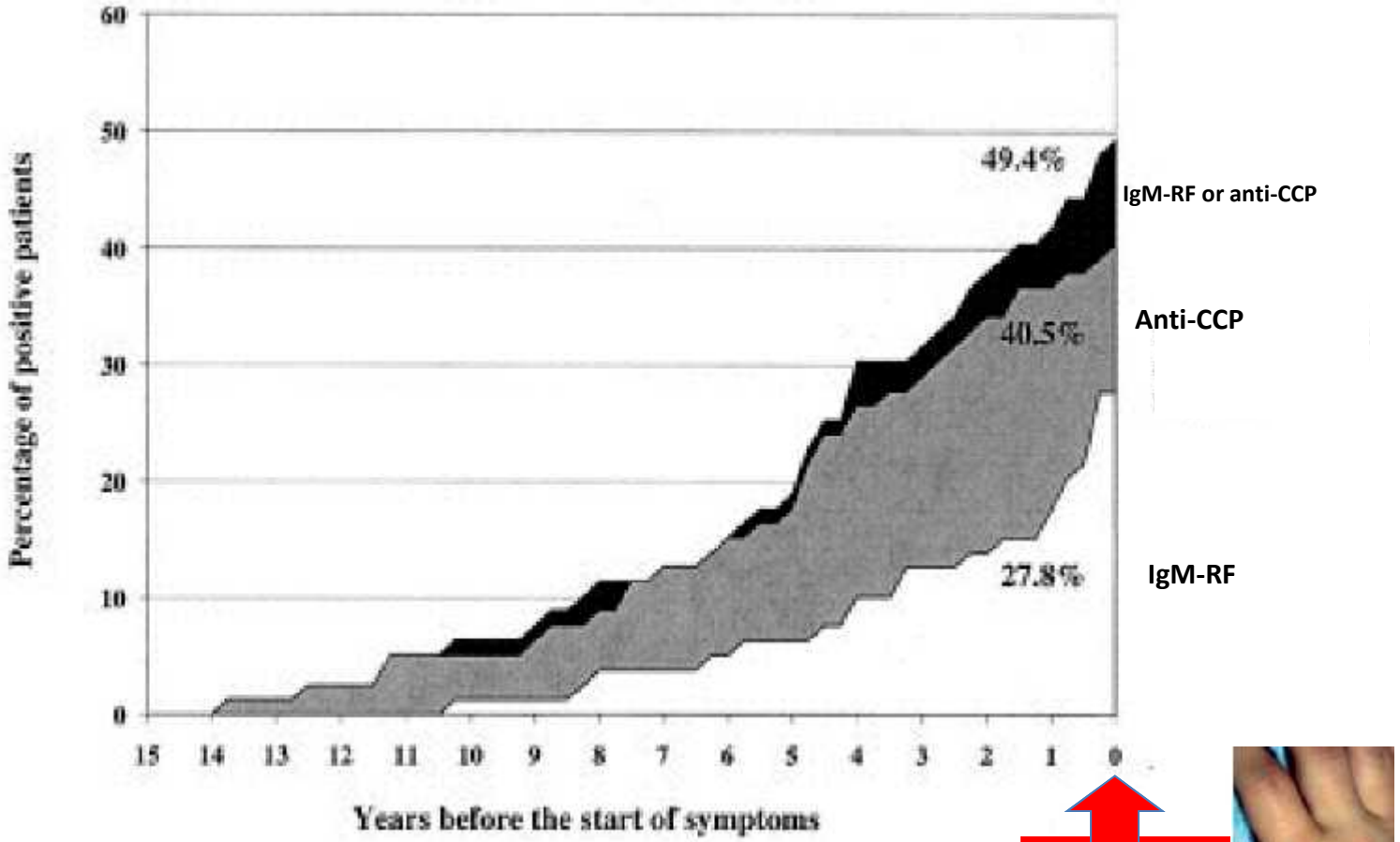


Firestein G, McInnes I. Immunity. 2017;46(2):183

RA pathogenesis:

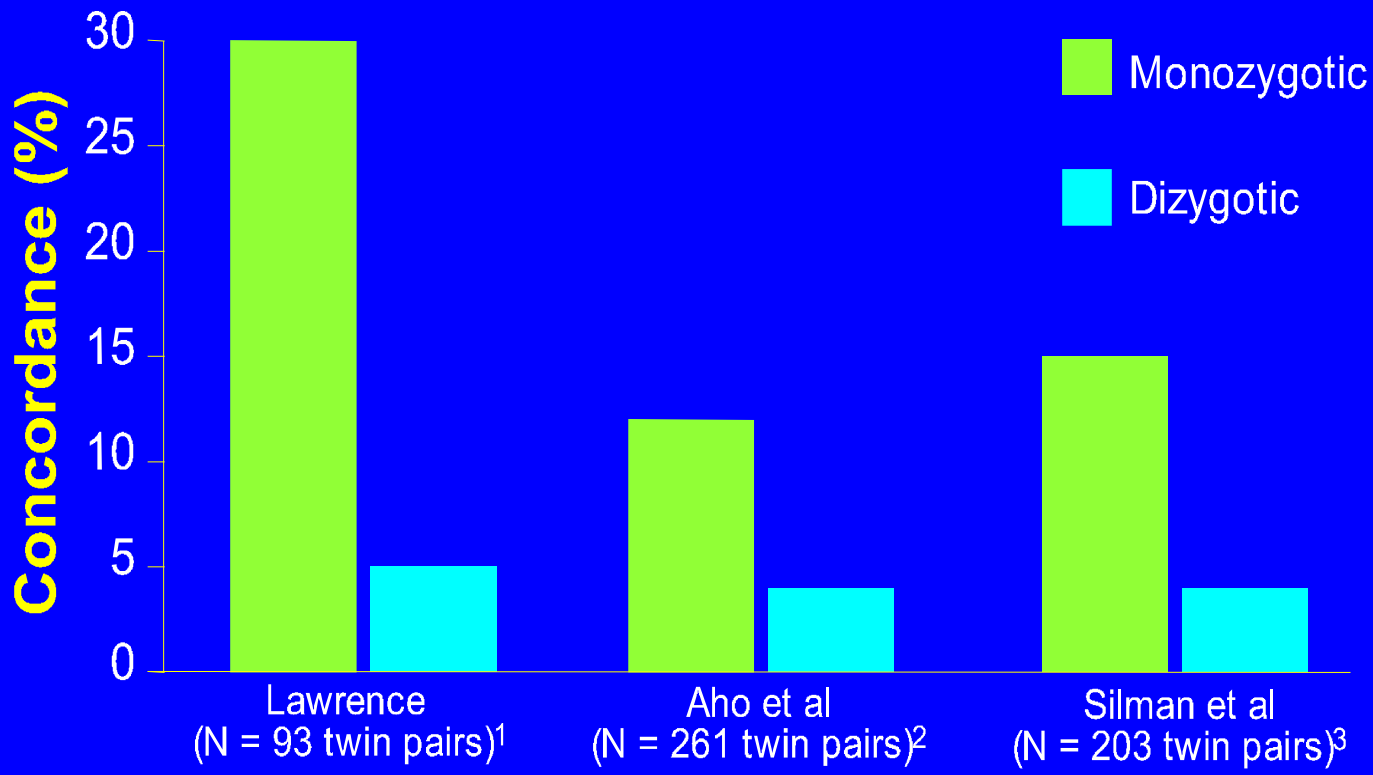
- ✓ **Autoimmunity starts long before symptoms and remotely from the joints!**
  - ✓ **ACPA earlier and in more prevalent vs RFs!**



Nielen M et al, A&R50; 2004, 380

Genetic factors in RA pathogenesis

Twin Studies in Rheumatoid Arthritis



1. Lawrence JS. *Ann Rheum Dis.* 1970;28:357-379.
2. Aho K et al. *J Rheumatol*. 1986;13:899-902.
3. Silman AJ et al. *Br J Rheumatol.* 1993;32:903-907.

RA:
Multigenic disease

- Most autoimmune diseases are multigenic, with multiple susceptibility genes working in concert to produce the abnormal phenotype

- In general, the polymorphisms also occur in normal people and are compatible with normal immune function. Only when present with other susceptibility genes do they contribute to autoimmunity

MHC II and RA

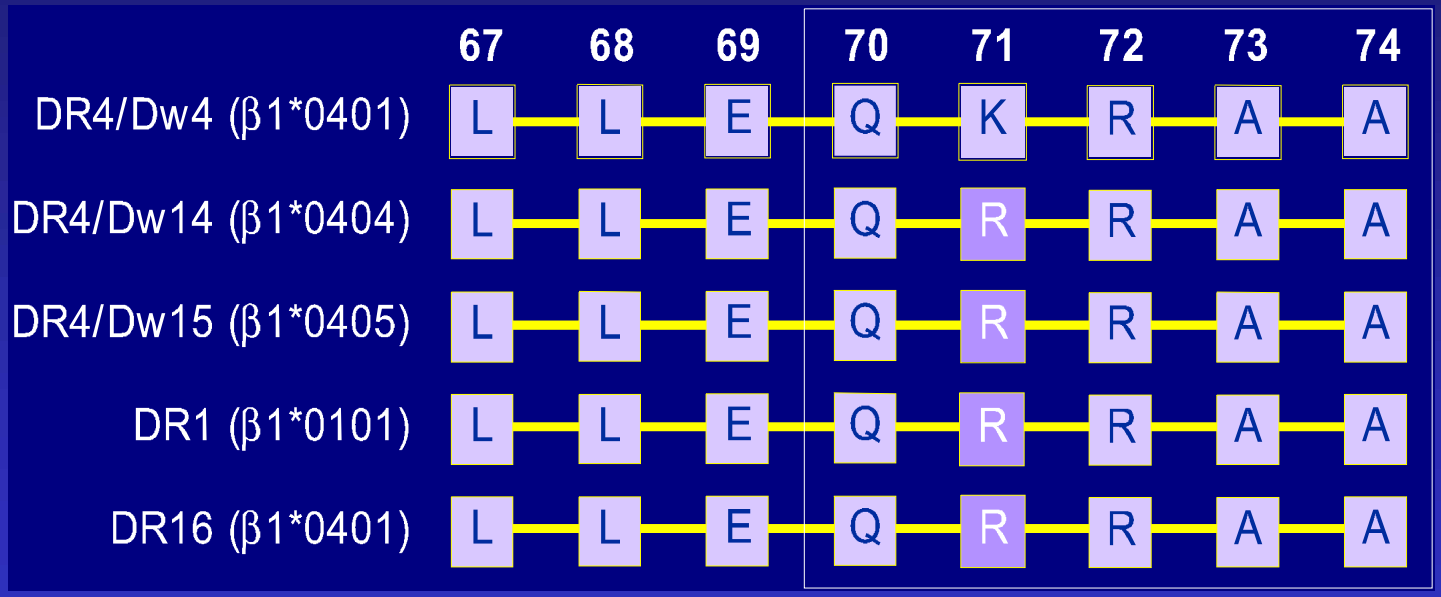
Genetic risk for RA in numbers

- Studies in twins ➔genetic contribution to RA accounts **for ~60%** of the variation in liability to disease.
- The most important genetic risk : class **MHC class II** region (HLA-DR).
- MHC contribute **18–37%** of the total genetic susceptibility to RA, increasing disease liability **4–6-fold**.

Nat Rev Rheumatol. 2012 Oct;8(10):573-86
Nat Genet. 2012 Jan 29;44(3):291-6

Shared Epitope Hypothesis

Alleles Associated with Rheumatoid Arthritis



Lipsky PE. In: *Harrison's Principles of Internal Medicine*. 1994:1648

Shared epitope hypothesis

All *HLADRB1* alleles associated with RA risk encode a conserved sequence of 5 amino acids (positions 70–74) **that surrounds the peptide-binding pocket** of the antigen-presenting molecule.

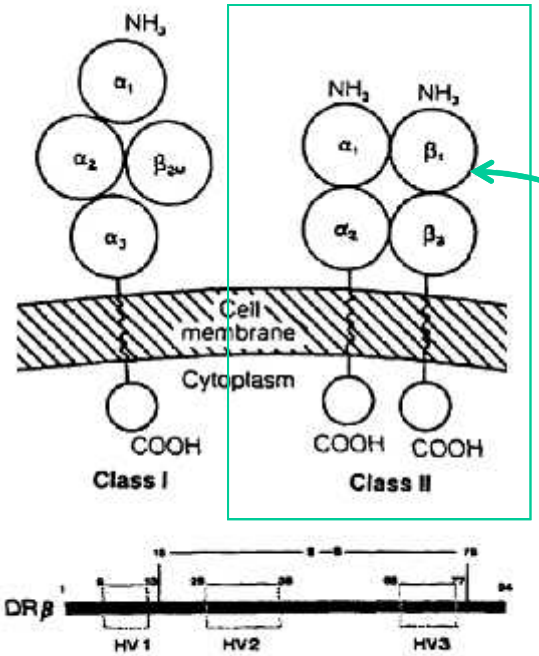


Figure 2. Top. Schematic diagram comparing the structural features of class II molecules at the cell surface with those of class I molecules. Bottom. The first domain, which contains regions of variability that alternate with invariant regions.

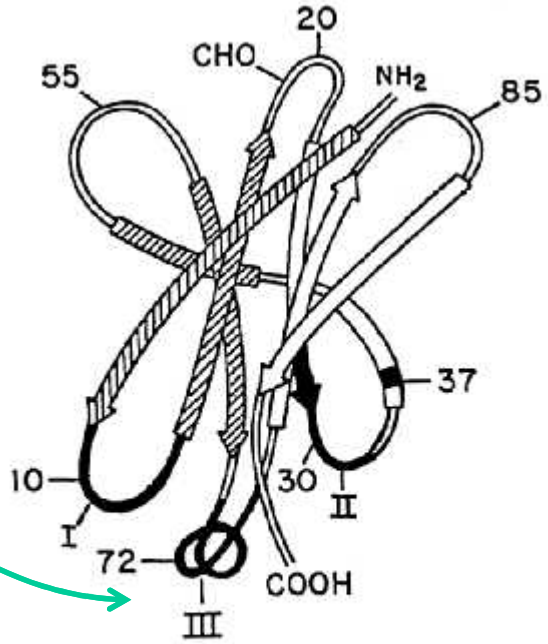
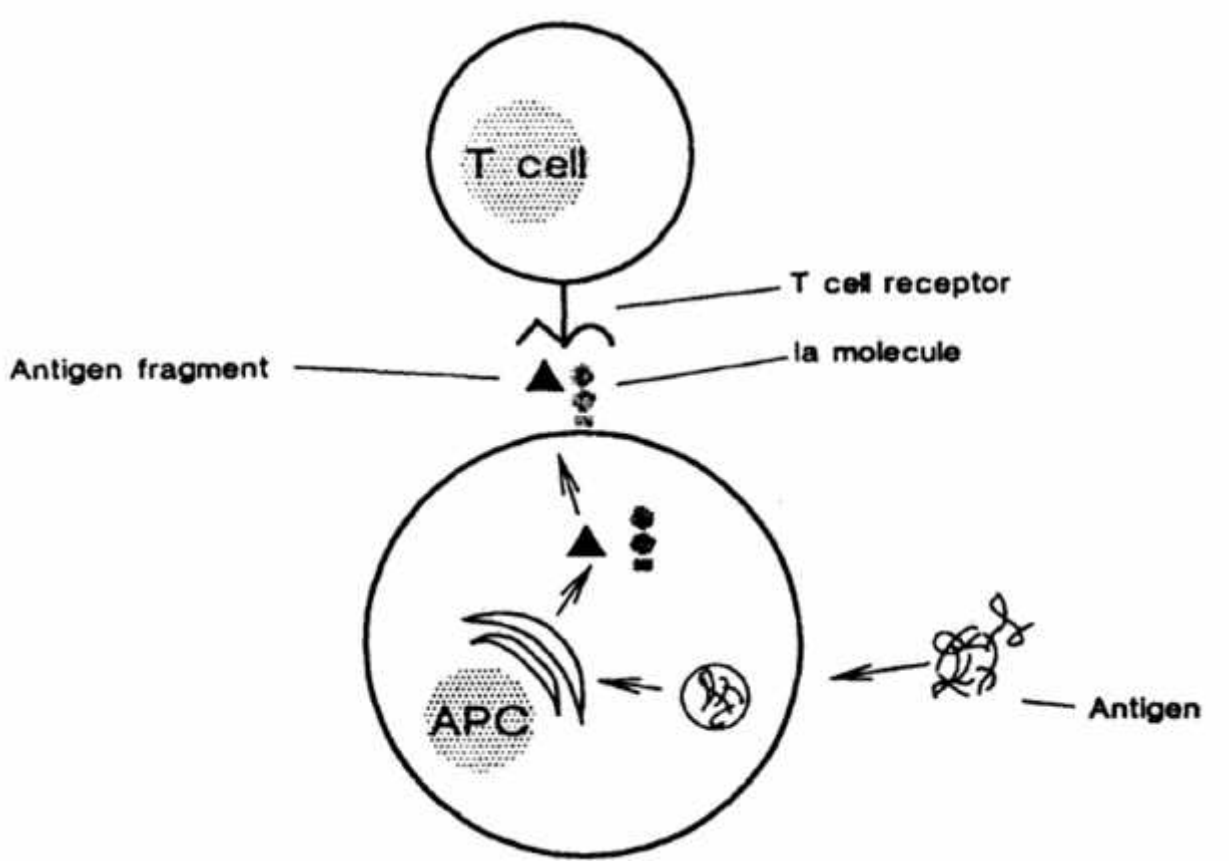


Figure 4. The predicted 3-dimensional structure of a class II β chain. Note that the third hypervariable region around position 70 is predicted to contain a region of α helical structure. It is this region which differs among the DR4 subtypes. Adapted from the *Scandinavian Journal of Immunology* (Norcross and Kanehisa [4]), copyright 1985, and used with the permission of Blackwell Scientific Publications.

Arthritis and Rheumatism, 1987, Vol. 30, No. 11

Shared epitope hypothesis

The presence of this shared epitope suggests that the molecules containing it might bind the same antigen, induce altered T-cell–antigen presenting cell interactions, and/or shape the T-cell repertoire participating in broader adaptive immune responses.



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MHC II & I and RA

Genetic risk for RA in numbers

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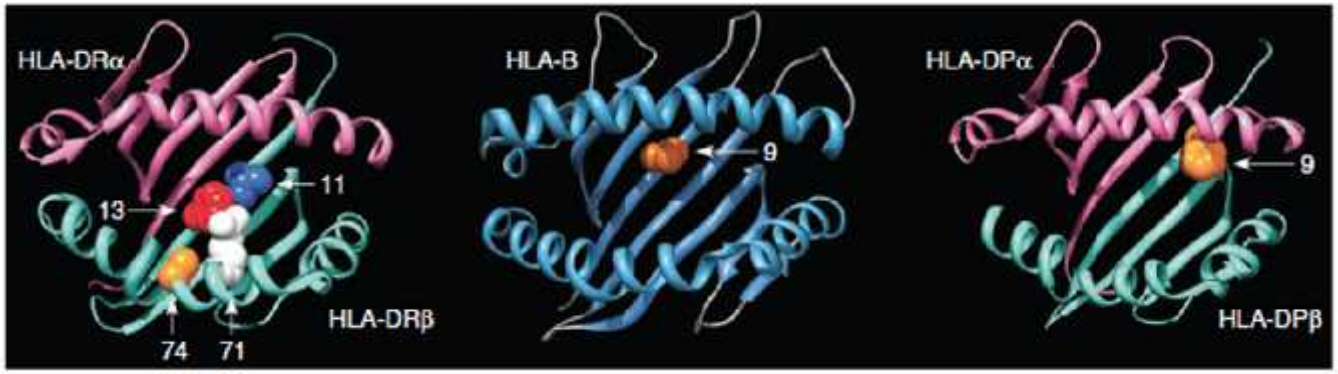
- The most important genetic risk : class **MHC class II** region (HLA-DR).
- MHC contribute **18–37%** of the total genetic susceptibility to RA, increasing disease liability **4–6-fold**.

- For **ACPA +ve RA: HLA-DRβ1** and two additional amino acid positions in **HLA-B** and **HLA-DP** in conferring risk to anti-CCP–positive rheumatoid arthritis.
  - These variants account for **12.7%** of the phenotypic variance of seropositive RA risk
  - Common validated alleles outside the MHC explain **~4%** of this variance



Nat Rev Rheumatol. 2012 Oct;8(10):573-86
Nat Genet. 2012 Jan 29;44(3):291-6

MHC II & I are associated to RA (CCP +ve)

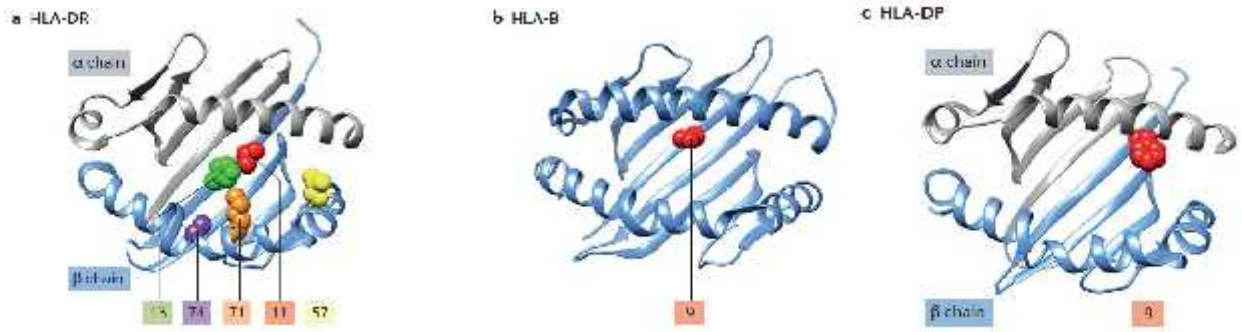
Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis

Soumya Raychaudhuri[1–4], Cynthia Sandor[1,4], Eli A Stahl[1,2,5], Jan Freudenberg[5], Hye-Soon Lee[6], Xiaoming Jia[1,4,7], Lars Alfredsson[8], Leonid Padyukov[9], Lars Klareskog[9], Jane Worthington[10], Katherine A Siminovitch[11], Sang-Cheol Bae[6], Robert M Plenge[1,2,4], Peter K Gregersen[5] & Paul I W de Bakker[1,4,12,13]

- Conditional and haplotype analyses identified that amino-acids at the peptide-binding grooves almost completely explain the MHC association to rheumatoid arthritis risk
  - HLA-DRβ1: 3 amino acid (positions 11, 71 and 74)
  - single–amino-acid polymorphisms in HLA-B (9) and HLA-DPβ1 (9)

- Location of these positions **within the peptide-binding grooves** implies **functional impact** on antigenic **peptide presentation** to T cells, **either during early thymic development or during peripheral immune responses**

- These results could facilitate the **evaluation of specific citrullinated polypeptides** with molecular modeling and binding assays to guide our **understanding of how HLA risk alleles influence the immune repertoire and disease susceptibility**.



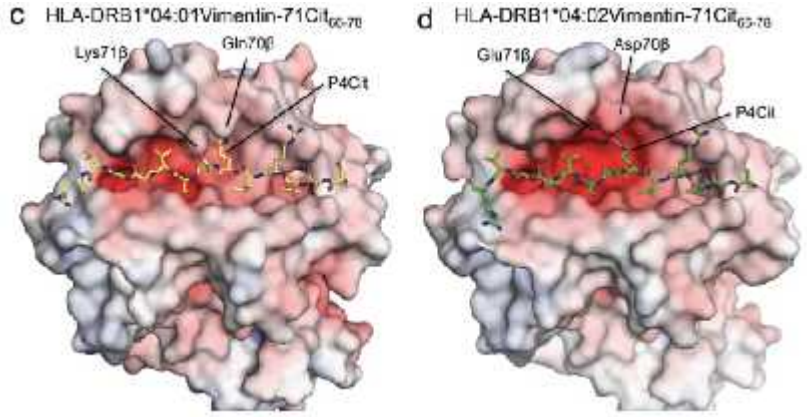
Raychaudhuri S, Nat Genet. 2012;44(3):291

“Mechanism” of the HLA-DR alleles and citrullinated proteins interaction

- The P4 pocket of HLA-DRB1*04:01 is highly suited to **preferentially accommodate citrulline over the Arg**, with Lys71β of the SE playing a key discriminatory role

- Citrullination has a double-edged effect:
  - facilitates binding of autoantigenic epitopes to RA-associated HLA allotypes
  - alters protease cleavage patterns protecting regions of the antigen normally degraded in APCs

“Interaction of “SE” HLA-II with Cit Ag leads to the presentation of peptides that can interact with the corresponding autoreactive T cell repertoire to increase selection and/or expansion of autoreactive CD4+ T cells.”



Comparison of the interactions between citrulline and arginine in the P4 pocket of HLA-DRB1*04:02.

J. Exp. Med. 2013 Vol. 210 No. 12 2562

Genes → Autoimmunity → RA phenotype

Genes ➔ Autoimmunity ➔ RA phenotype

Shared Epitope alleles (HLA-DRB1) are associated ONLY with anti-CCP +ve RA (and RF +ve RA)

Table 1. Distribution of SE and anti-CCP positivity*

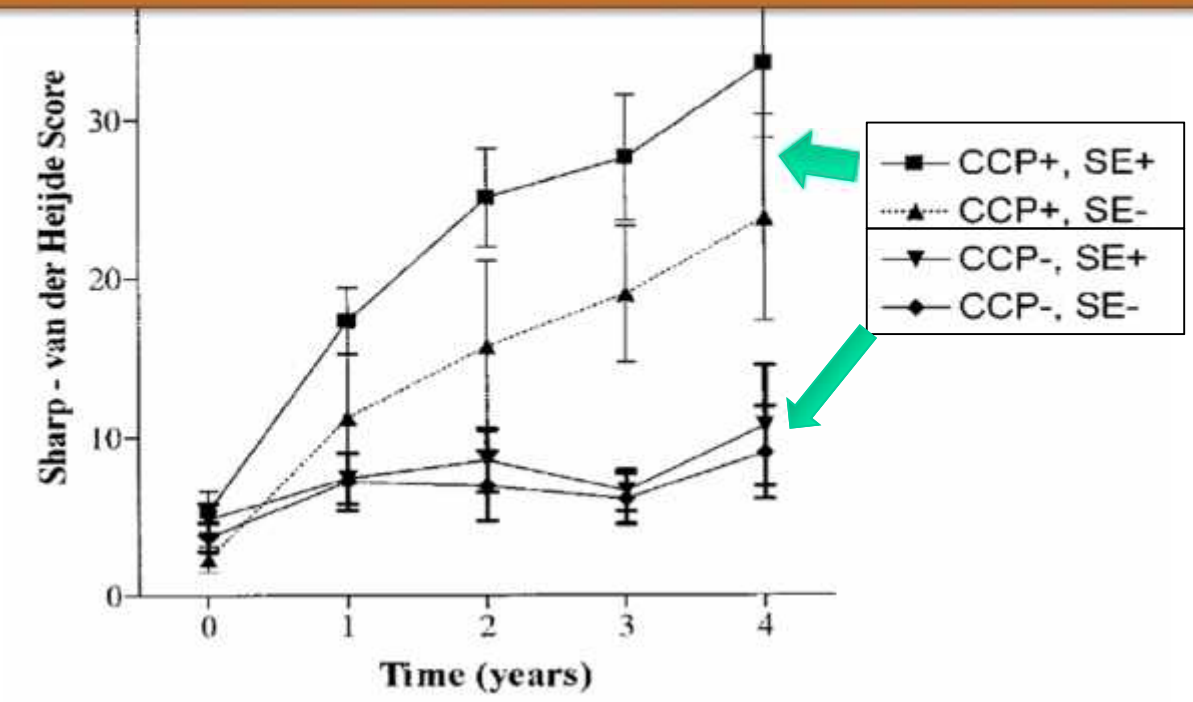
SE	Dutch controls (n = 423), no. (%)	Dutch EAC RA patients: Anti-CCP positive (n = 195) No. (%)	Anti-CCP positive (n = 195) OR (95% CI)	Anti-CCP negative (n = 213) No. (%)	Anti-CCP negative (n = 213) OR (95% CI)
+/+	26 (6)	49 (25)	11.79 (6.58–21.13)	16 (8)	1.38 (0.71–2.67)
+/−	153 (36)	107 (55)	4.37 (2.88–6.65)	88 (41)	1.29 (0.91–1.82)
−/−	244 (58)	39 (20)	1.0	109 (51)	1.0

* The following alleles were classified as shared epitope (SE) positive: DRB1*0101, *0102, *0104, *0401, *0404, *0405, *0408, *0413, *0416, *1001, and *1402 (4). EAC = Early Arthritis Clinic; RA = rheumatoid arthritis; CCP = cyclic citrullinated peptide; OR = odds ratio; 95% CI = 95% confidence interval.

AR, Vol. 52, No. 11, 2005, pp 3433–3438

Genes ➔ Autoimmunity ➔ RA phenotype

Shared Epitope alleles (HLA-DRB1) are associated ONLY with anti-CCP +ve RA (and RF +ve RA)



- 2 phenotypes (CCP+ & -) exhibit different genetic associations suggest the presence of distinct pathways underlying disease induction/progression in anti-CCP–positive and anti-CCP–negative RA

AR, Vol. 52, No. 11, 2005, pp 3433–3438

General concept for RA pathogenesis

"Together these data support the notion that HLA-DR risk for RA is based at least in part on the **increased efficiency of antigen presentation for altered peptides rather than native proteins.**

Citrullination of peptides in the presence of environmental stress is ubiquitous in mammalian cells and is not a unique feature of RA.

Instead, **production of antibodies recognizing citrullinated peptides differentiates individuals at risk.**

The emergence of **numerous other post-translationally modified protein targets**, (via carbamylation or acetylation), recognized by autoantibodies in RA is consistent with the notion of altered presentation of post-translationally modified peptides; other families of altered peptides could be implicated in discrete subsets of patients."

Firestein G, McInnes I. Immunity. 2017;46(2):183

RA - Genetic susceptibility of non-HLA genes

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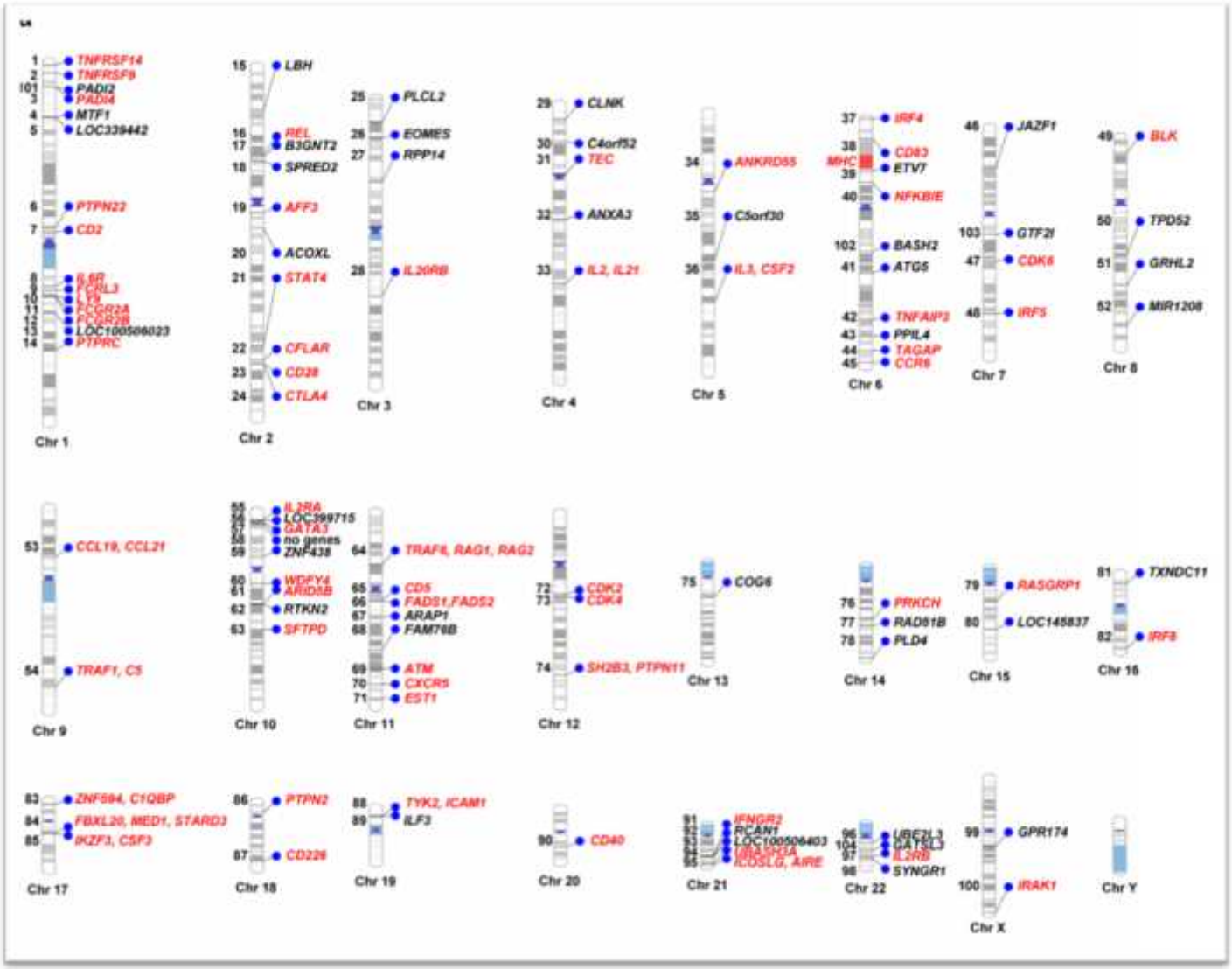
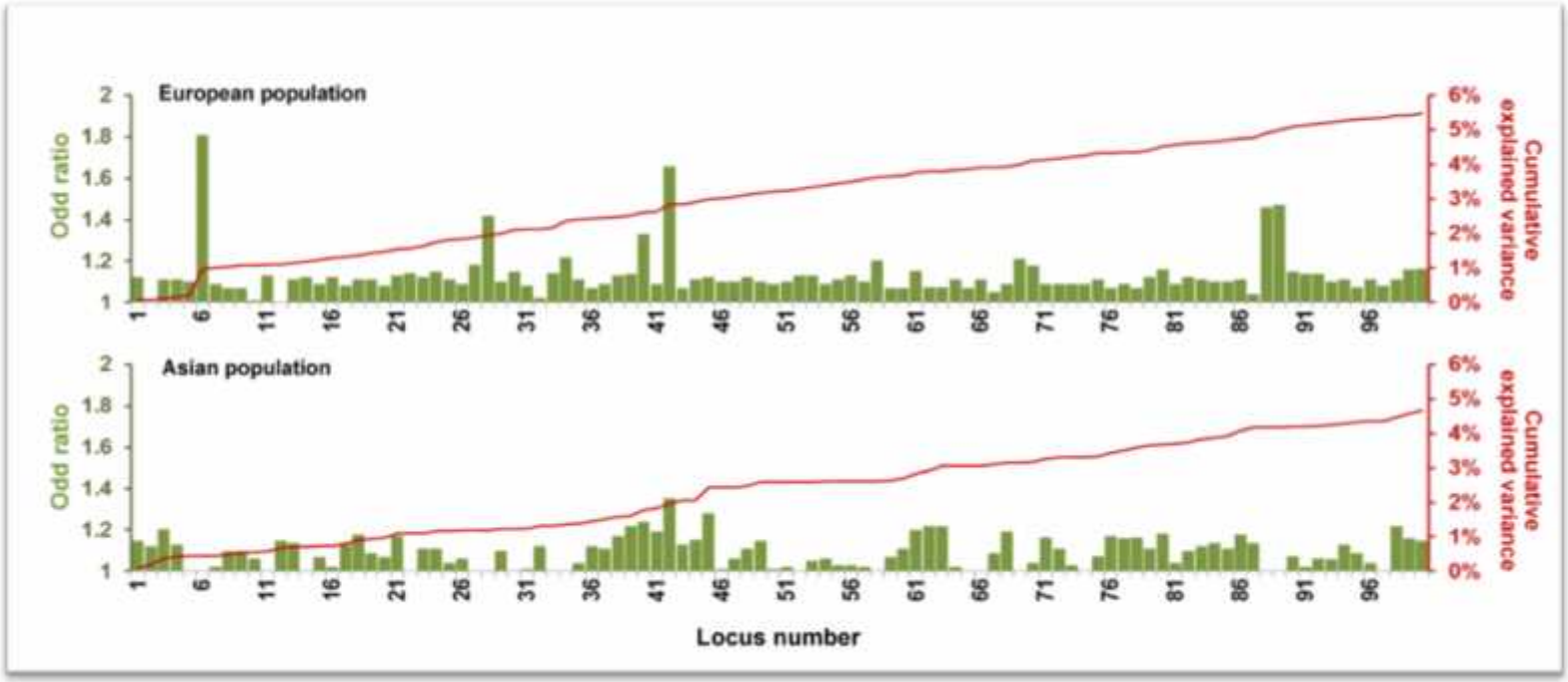


Okada Y, Nature 506, 376–381 (2014).

Kim K, Nat Rev Rheumatol. 2017 Jan;13(1):13

RA - Genetic susceptibility of non-HLA genes

- ✓ 80% of risk loci are characterized by noncoding variants alone, accounting for ~70% of the heritability explained by the non-MHC RA-risk loci
- ✓ These associations explain 5.5% of the heritability of RA in European populations
- ✓ Individual SNPs usually provide modest contribution to risk with odds ratios typically in the 1.05- to 1.2-fold range, though this need not infer low functional impact.
- ✓ Combinations of these genes can potentially interact to increase risk: HLA-DR, PTPN22, and TRAF1-C5 SNPs increases risk more than 40-fold.



Okada Y, Nature 506, 376–381 (2014).
Kim K, Nat Rev Rheumatol. 2017 Jan;13(1):13

RA - Genetic susceptibility of non-HLA genes

MHC: makes up 12.7% of total genetic variance
Non-MHC: makes up ~4% of total genetic variance

Table 1. Candidate Genes with Single-Nucleotide Polymorphisms (SNPs) Linked to Rheumatoid Arthritis and Their Potential Function in Pathogenesis.*

Candidate Gene and Pathway	SNP Locus	Function Relevant to Pathogenesis
T-cell activation		
HLA-DRB1†	6p21	HLA DRB1 allele (also known as the shared epitope) involved in MHC molecule–based antigen presentation and responsible for self-peptide selection and T-cell repertoire; first discovered and still by far the strongest genetic link to rheumatoid arthritis
PTPN22	1p13.2	Lymphocyte-specific nonreceptor tyrosine phosphatase involved in regulation of activation threshold of lymphocytes; second genetic link described in rheumatoid arthritis
AFF3	2q11.2	Transcription factor for lymphoid development
CD28	2q33.2	Costimulatory molecule for T-cell activation
CD40	20q13.12	Costimulatory molecule that enhances interactions between T and B cells and increases auto-antibody production
CTLA4	2q33.2	Costimulation suppressor that regulates interactions between T cells and antigen-presenting cells
IL2RA	10p15.1	High-affinity receptor for interleukin-2 on lymphocyte subsets
IL2	4q27	Cytokine that regulates activation of T cells, particularly regulatory T cells
IL-21	4q27	Cytokine that regulates differentiation of T cells, particularly Th17, and activation of B cells
PRKCQ	10p15.1	Member of the protein kinase C family that regulates T-cell and macrophage activation
STAT4	2q32.3	Transducer of cytokine signals that regulate proliferation, survival, and differentiation of lymphocytes
TAGAP	6q25.3	Rho-GTPase enzyme involved in T-cell activation

N Engl J Med 2011;365:2205

RA - Genetic susceptibility of non-HLA genes

Table 1. Candidate Genes with Single-Nucleotide Polymorphisms (SNPs) Linked to Rheumatoid Arthritis and Their Potential Function in Pathogenesis.*

Candidate Gene and Pathway	SNP Locus	Function Relevant to Pathogenesis
NF-κB pathway		
REL	2p16.1	Proto-oncogene member of the NF-κB family that regulates leukocyte activation and survival
TNFAIP3	6q23.3	Signaling protein and negative regulator of TNF-α–induced NF-κB activation
TRAF1	9q33.1	Regulator of TNF-α–receptor superfamily signaling (e.g., to NF-κB and JNK)
Other pathways		
BLK	8p23.1	B-lymphoid tyrosine kinase involved in B-cell receptor signaling and B-cell development
CCL21	9q13.3	Chemokine implicated in germinal-center formation
FCGR2A	1q23.2	Low-affinity IgG Fc receptor that regulates macrophage and neutrophil activation and immune-complex clearance
PADI4	1p36.2	Enzyme that converts arginine to citrulline, creating autoantigens in rheumatoid arthritis
PRDM1	6q21	Protein that acts as a repressor of β-interferon gene expression
TNFRSF14	1p36.32	TNF-α–receptor superfamily member with proinflammatory activity

Complex interactions:

Gene–gene interactions that increase disease risk, as described between *HLA-DRB1* and *PTPN22,* exemplify the complexity of the net risk conferred by any given gene

Genetics /Autoimmunity / Clinical correlations:

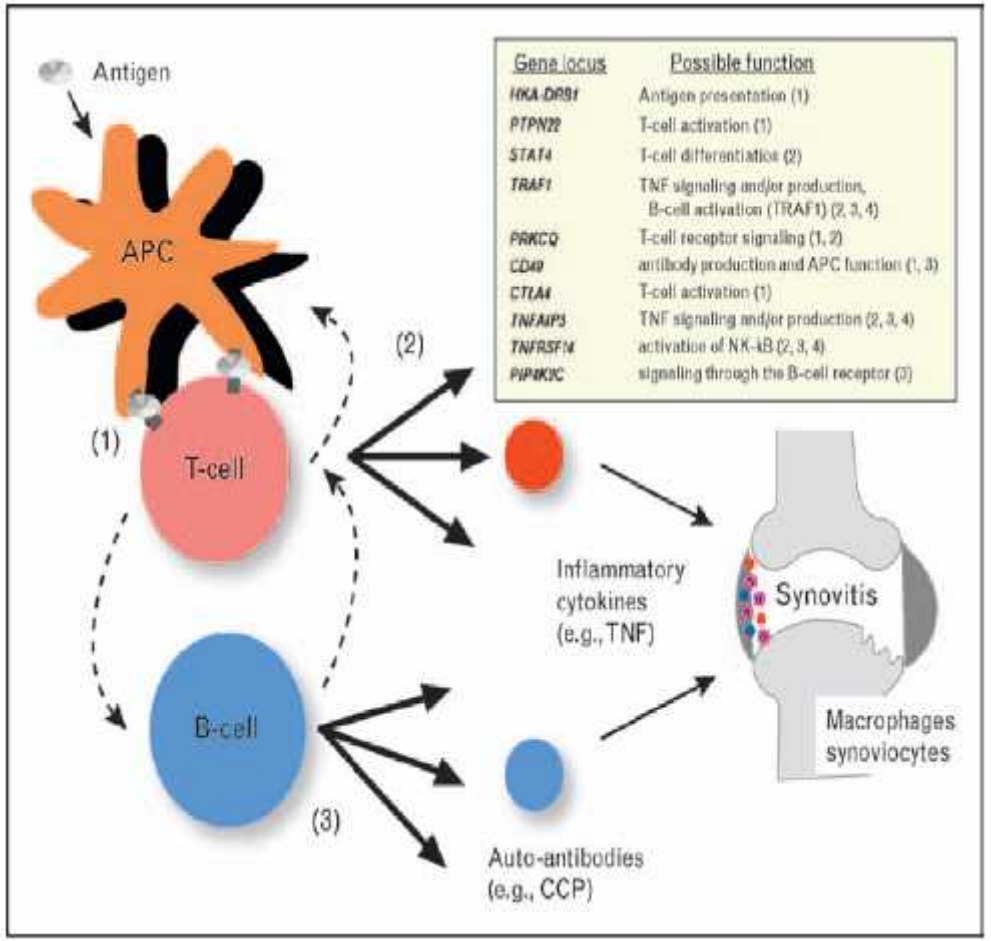
The above genetic background, mainly for ACPA + patieents

N Engl J Med 2011;365:2205

Genes & RA pathogenesis

Thus, the genetic clues to RA pathogenesis
implicate inflammation and adaptive and innate immunity
at the core of pathogenesis.

Function	Genes
Antigen presentation	*DRB1,CD40*
T-cell activation/ differentiation	*PTPN22, STAT4, CTLA4, TNFAIP3*
Ab production	*TRAF1, CD40, TNFAIP3, PIP4K2C*
Inflammation	*TRAF1, TNFAIP3*



Plenge (2009), Cur Op Rheum

Genes, RA pathogenesis & therapeutic perspectives

1. Novel targets:

Although RA genetic studies had little involvement in the development of currently approved therapeutic agents for the disease, **ongoing functional studies to demonstrate the biological roles** of RA-risk variants and genes (such as CD40, LBH, C5, CLEC16A, PTPN22 HLA-DRB1, C5orf30, LYP, IRAK1, IL6R, UBE2L3 and PADI4) at a cellular, immune and whole-organism level will be useful for the design or screening of future therapies.

2. Personalized medicine:

Combination of systematic documentation of **environmental** factors (such as cigarette smoking) and **medical** data (such as anti-CCP antibody status, ACPA subtype, rheumatoid factor status, treatment type, response to therapy and level of bone erosion) related to RA is extremely important in genetic studies.

A study showing an association between a valine at HLA-DRβ1 amino acid position 11 with RA severity, mortality and response to TNF inhibition, as well as with susceptibility to RA exemplifies the value of using a clinically well-defined cohort of patients with RA in genetic studies and precision medicine.

Kim K, Nat Rev Rheumatol. 2017 Jan;13(1):13

Environment and gene interaction in autoimmunity/ disease development

RA is properly considered an immune-mediated disease with a strong genetic influence.

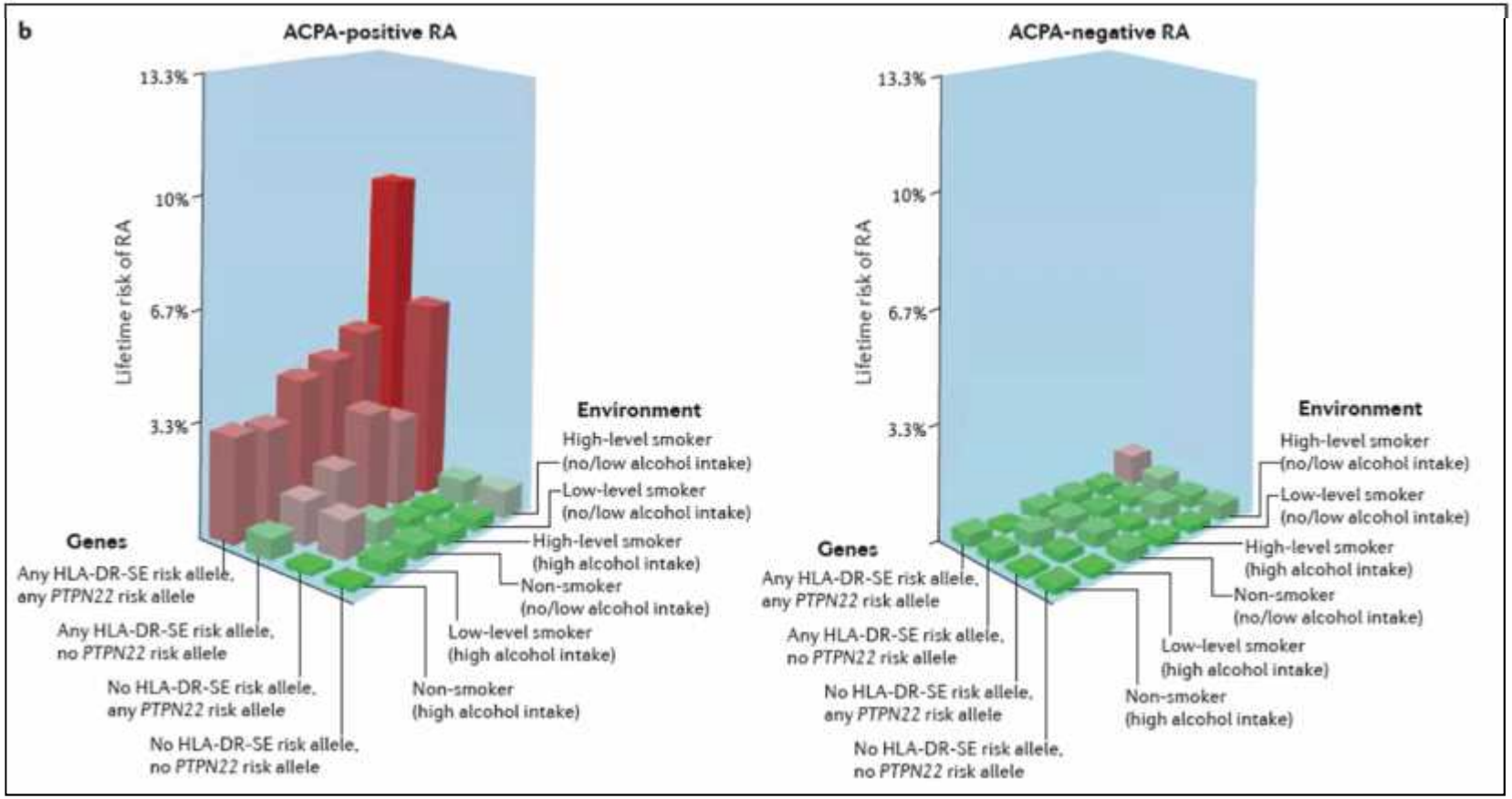
However, **its origins may involve the interface between external influences and the immune system, manifest especially at mucosal surfaces**:

- ✓ *lungs, oral mucosa and gastrointestinal tract*
- ✓ *local tissue stress leads to post-translational modification of peptides with subsequent antibody formation serving as a common mechanism*

Firestein G, McInnes I. Immunity. 2017;46(2):183

Environment and gene interaction in autoimmunity/ disease development

Pronounced gene–gene and gene–environment interactions are present in ACPA-positive RA but absent in ACPA-negative disease



Kallberg. Am. J. Hum. Genet. **80**, 867(2007)
Kallberg. *Ann. Rheum. Dis.* **68**, 222 (2009)

Malmström. *Nature Reviews Immunology* **17**, 60–75 (2017)

Lungs (gums/gut) site of citrullination and autoimmune response

Key points

- Autoantibodies against post-translational modified citrullinated proteins, so-called anti-citrullinated protein antibodies (ACPAs), define a distinct clinical RA phenotype; this phenotype is characterized by an increased frequency of early inflammatory lung changes
- The presence of ACPAs before signs of inflammation in joints suggests that immunity against citrullinated proteins is initiated outside the joint
- Changes in the lung and enrichment of ACPAs in the lungs (bronchoalveolar lavage fluid) occur in both individuals at risk of developing RA as well as patients with early RA
- The lung, therefore, might be a site of initiation of immunity to citrullinated proteins
- Early targeting of the immune reactions in the lung might be a new approach to modulate disease

Katrina. Nat Rev Rheumatol. 2014 Nov;10(11):645

Protein Citrullination – PADs: what, where, why?

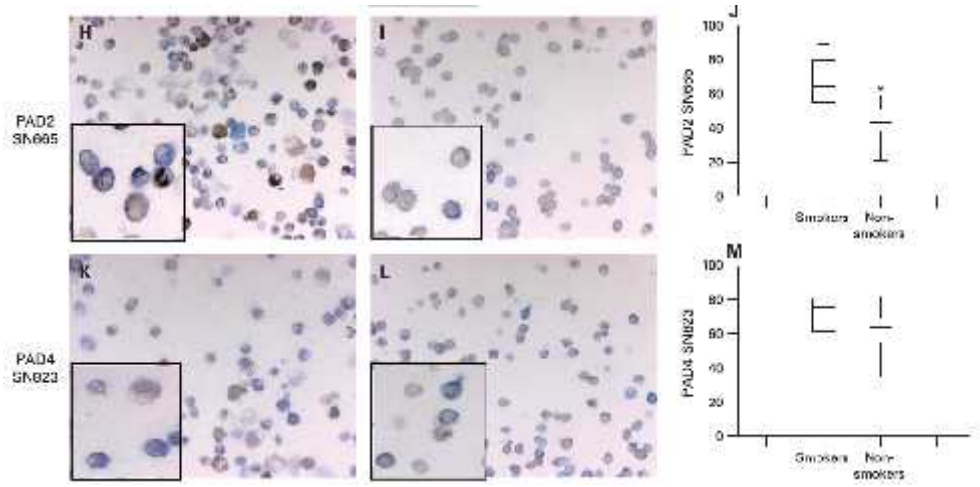
- Citrullination is the posttranslational modification of arginine to citrulline by PAD enzymes (especially PAD2 and PAD4)
- Intracellularly & extracellular milieu
- Shape the epigenome by modifying histones, migrate to the nucleus in stressed cells
- Regulate expression of immunoregulatory tumour suppressor genes such as that encoding p53.

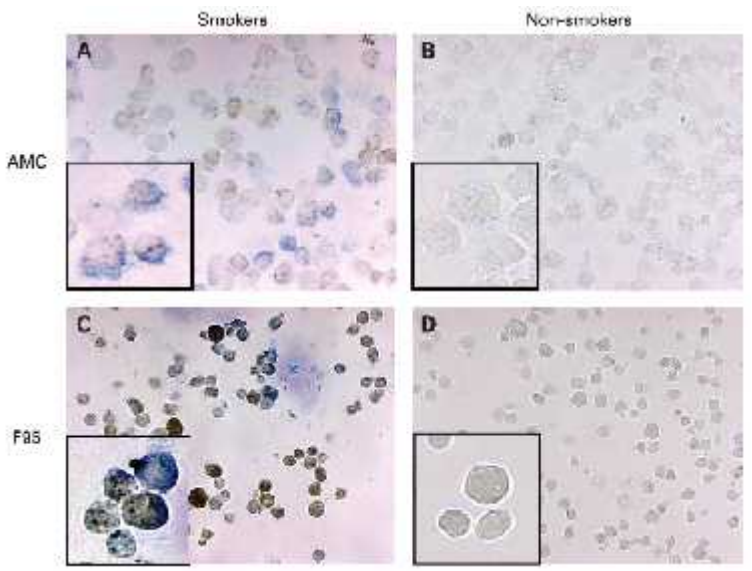
Protein Citrullination – PADs: what, where, why?

- PAD2 and PAD4 are found in synovial tissue and fluid in RA.
- PAD4 Genetic association to RA:
  - Predisposes Japanese male smokers to RA
  - Prolongs its mRNA stability and could increase peptide citrullination (Suzuki et al., 2003).
- Autoimmunity to PADs
  - anti-PAD4 antibodies are seen in people who develop RA up to 4 years prior to the onset of disease and are associated with ACPAs

Lung as the site of citrullination and initial ACPA development

Lungs in Healthy smokers vs non-smokers:
higher expression of PAD2/4 and **citrullinated proteins in BAL**

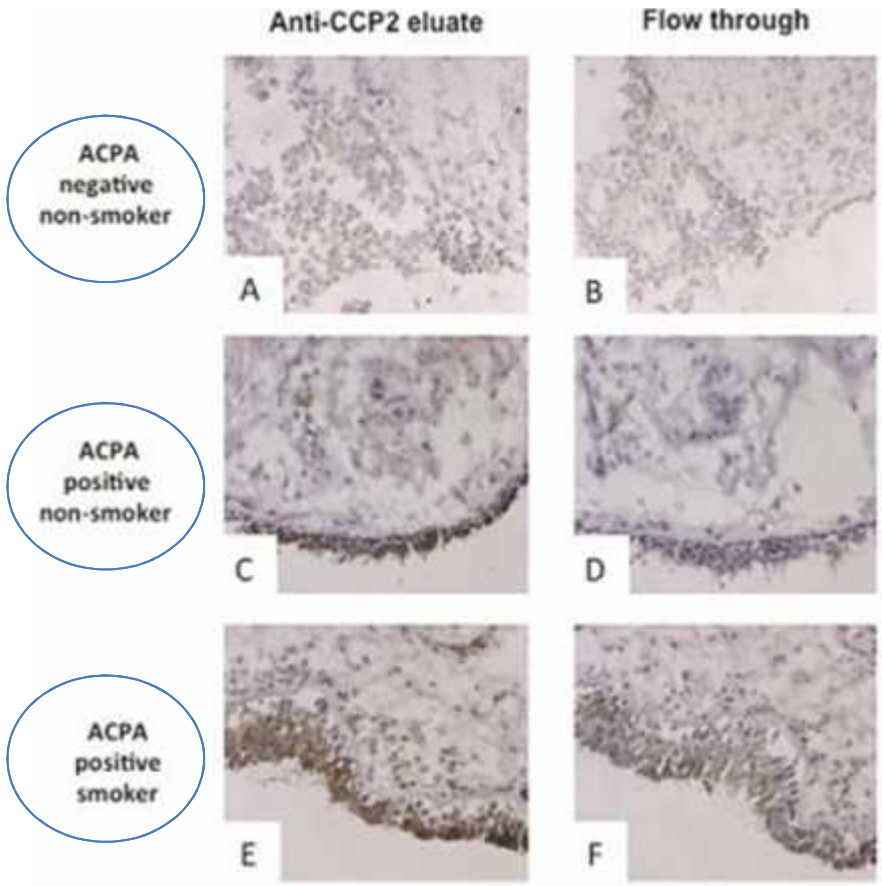


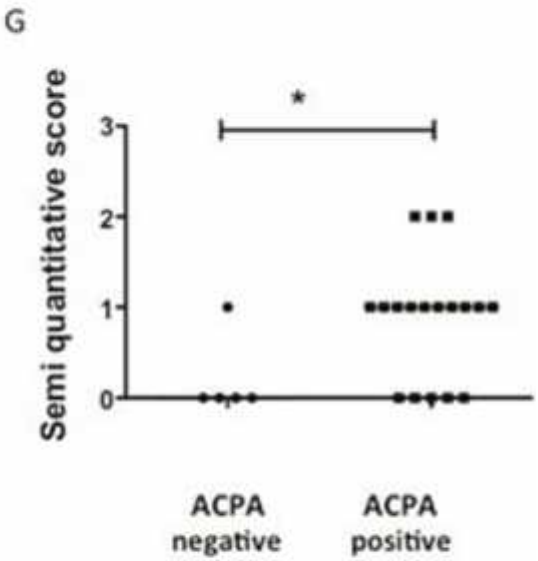


Makrygiannakis D, ARD 2008;67:1488

Lung as the site of citrullination and initial ACPA development

Early, untreated ACPA +ve RA:
higher expression of the **citrullinated protein in large bronchial biopsy tissue**.

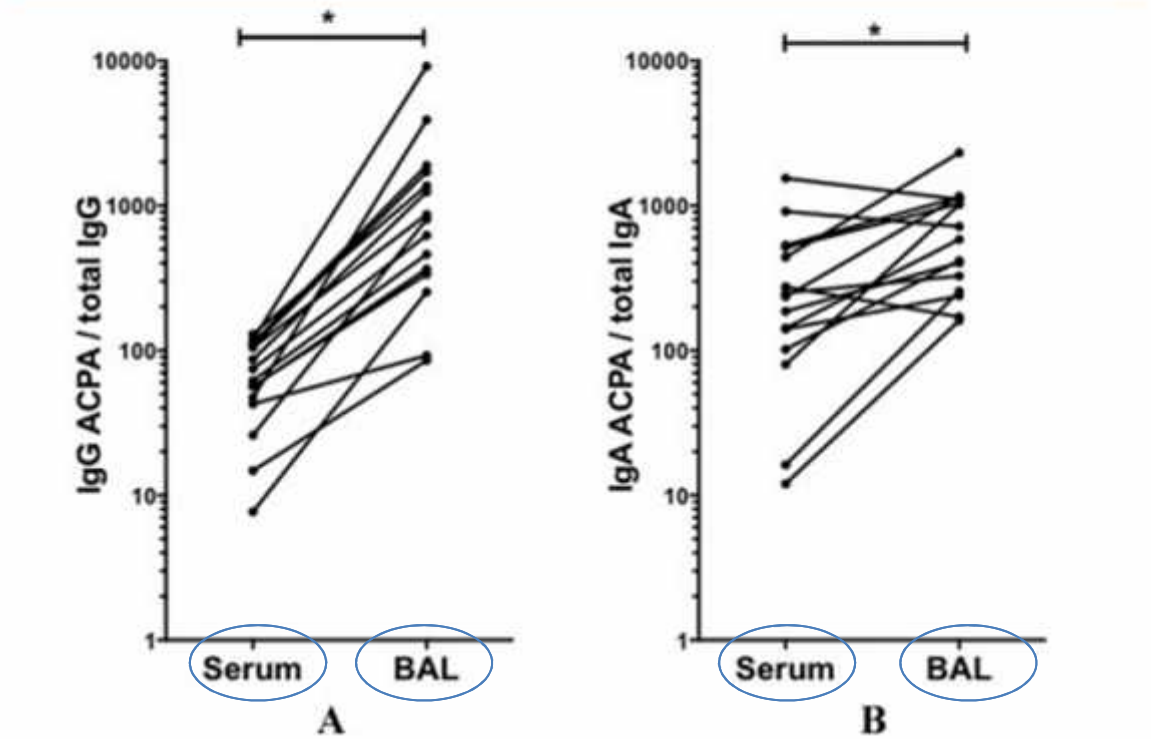




Reynisdottir. A&R 66 January 2014, pp 31

Lung as the site of citrullination and initial ACPA development

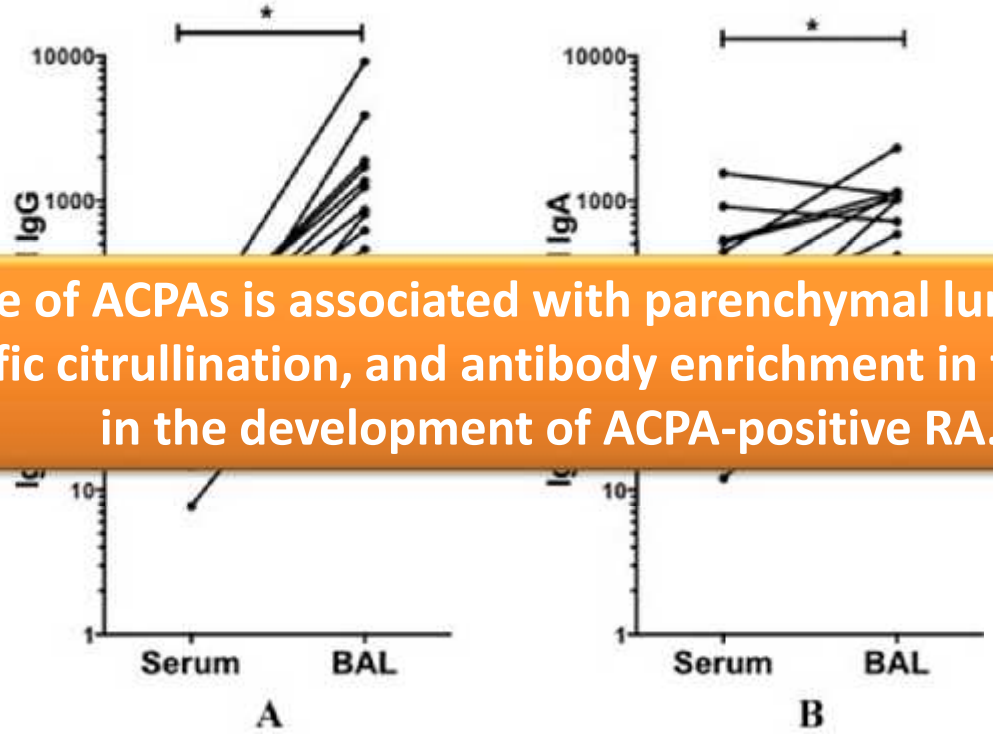
ACPA-positive patients with early, untreated RA:
Enrichment of ACPAs in the lungs vs serum



Reynisdottir. A&R 66 January 2014, pp 31

Lung as the site of citrullination and initial ACPA development

Enrichment of ACPAs in the lungs vs serum of ACPA-positive patients with early, untreated RA

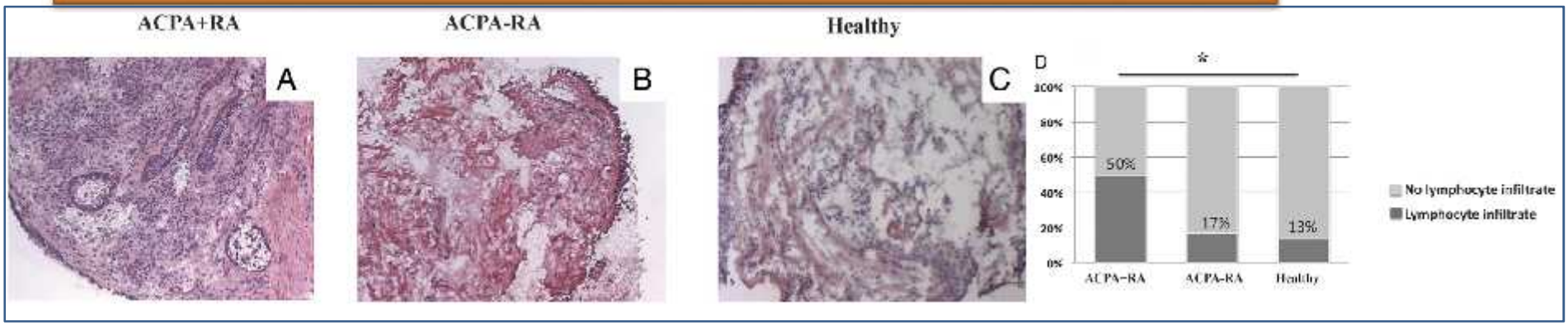


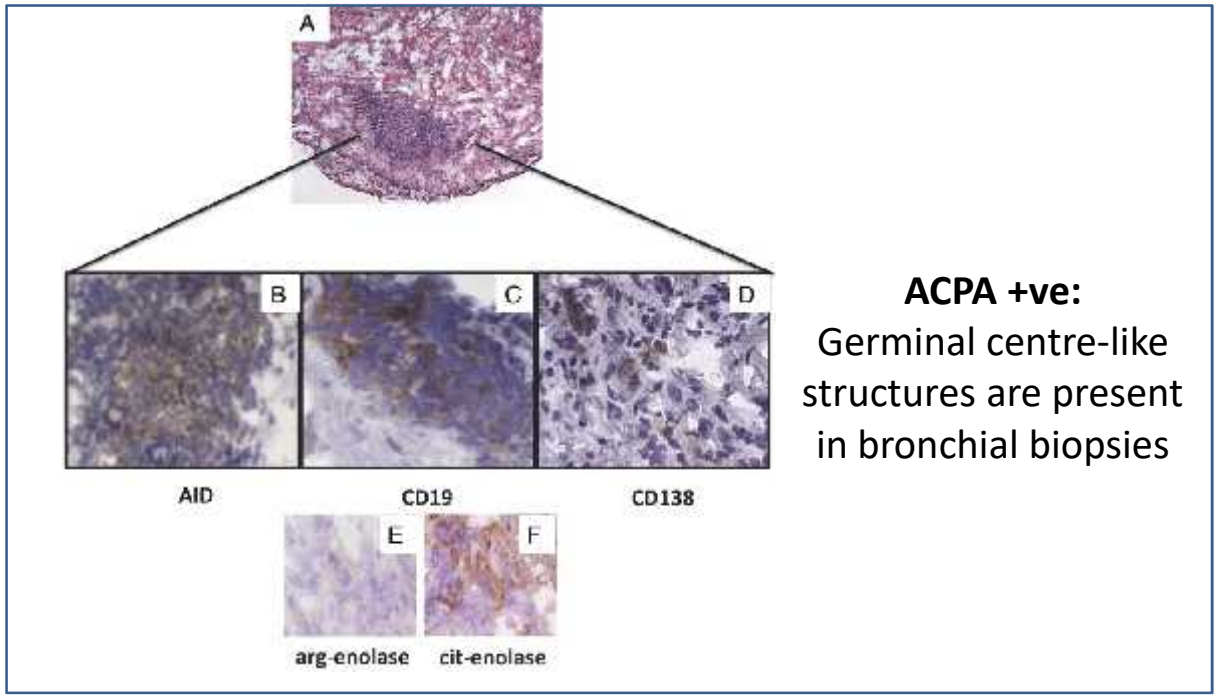
The presence of ACPAs is associated with parenchymal lung abnormalities, site-specific citrullination, and antibody enrichment in the lungs early in the development of ACPA-positive RA.

Reynisdottir. A&R 66 January 2014, pp 31

Immune activation in BAL and bronchial biopsies of patients with **early untreated ACPA-positive RA**

Patients with **ACPA-positive <u>untreated early rheumatoid arthritis</u>**
Lymphocytic infiltration in the bronchial biopsies



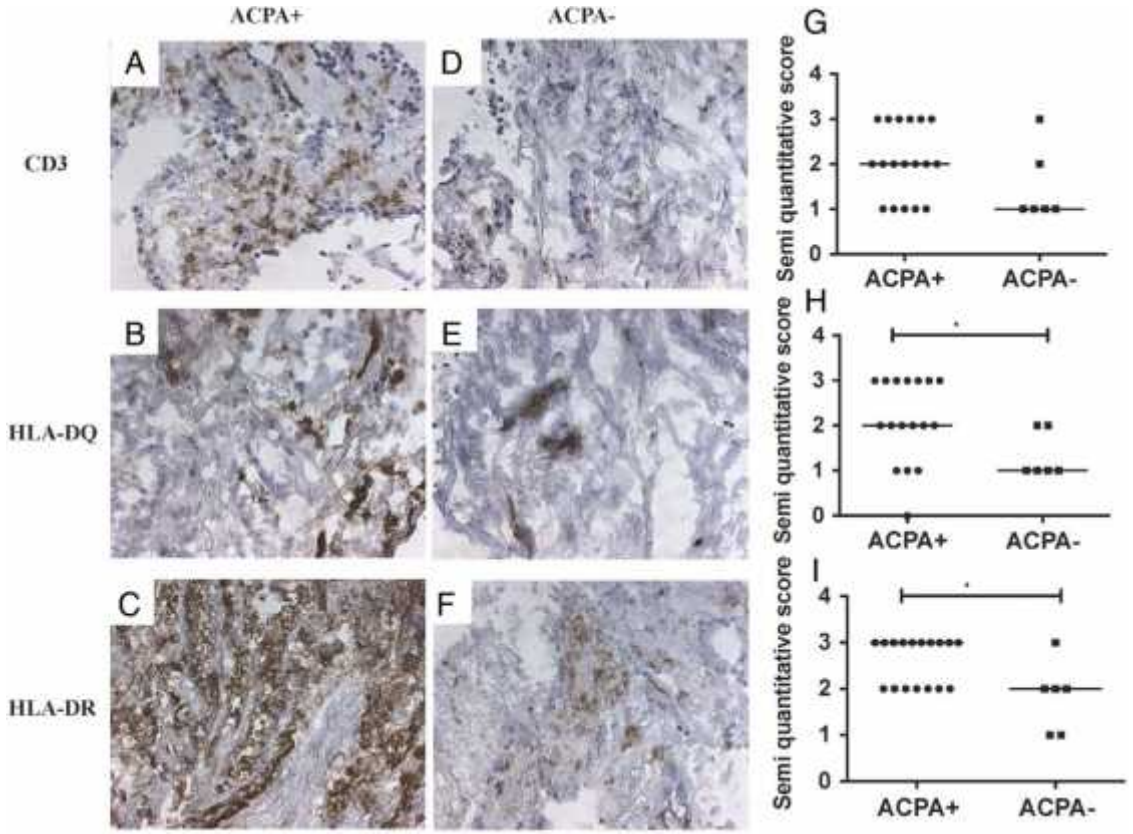


ACPA +ve:
Germinal centre-like structures are present in bronchial biopsies

Reynisdottir. Ann Rheum Dis 2016;75:1722

Immune activation in BAL and bronchial biopsies of patients with **early untreated ACPA-positive RA**

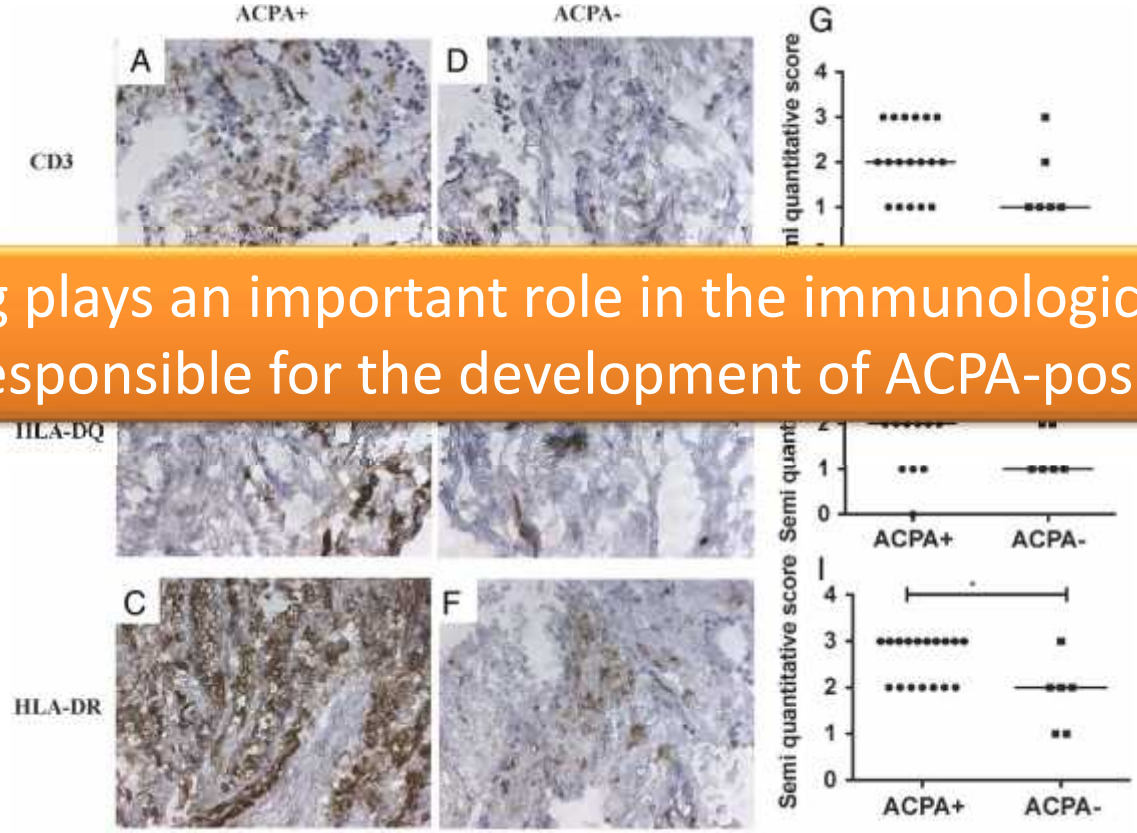
ACPA-positive untreated patients with early rheumatoid arthritis (RA)
Immune activation in bronchial biopsies



Reynisdottir. Ann Rheum Dis 2016;75:1722

Immune activation in BAL and bronchial biopsies of patients with **early untreated ACPA-positive RA**

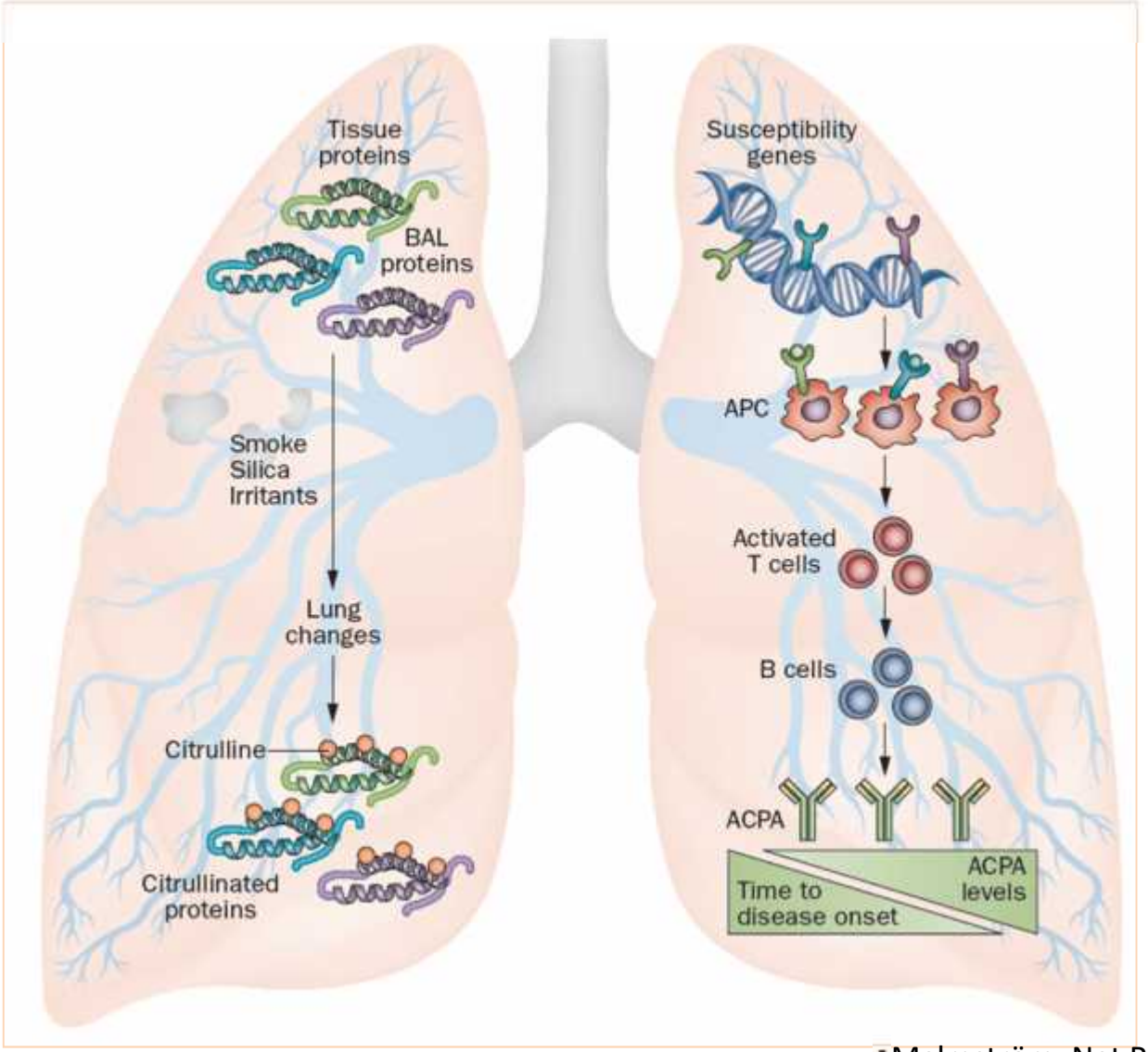
Immune activation are present in bronchial biopsies of patients with ACPA-positive untreated early rheumatoid arthritis (RA)



"lung plays an important role in the immunological reactions responsible for the development of ACPA-positive RA"

Reynisdottir. Ann Rheum Dis 2016;75:1722

LUNG: the location of citrullination and the stochastic combination of altered peptides, innate leukocyte subsets, and mucosal inflammation conspire a permissive microenvironment for the creation of ACPAs.



- Malmström, Nat Rev Rheumatol. 2014:645

Citrullination to autoimmunity & RA

Citrullinated proteins are not known to exist in the joint prior to the onset of RA (though they have not been assayed in that setting) BUT the hypothesis is:

- Citrullination of proteins in the cartilage and synovium could occur during an early phase by:
  - Nonspecific injury or inflammation in the joints ➔ citrullinated epitopes ➔ a genetically predisposed host resulting in high titre of ACPAs ➔ precipitate the clinical development of RA

(analogous to antibody transfer models of arthritis such as passive K/BxN and CAIA)

Citrullination – Periodonditis – RA

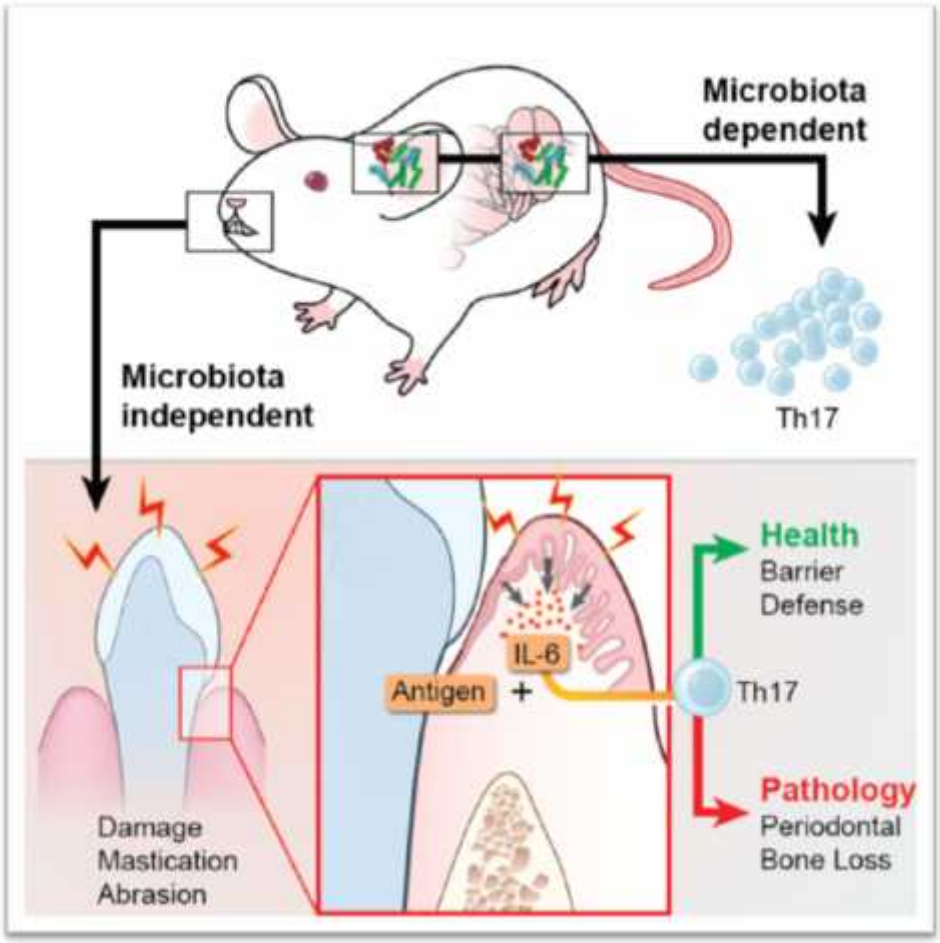
- **Tissue of citrullintion**: lung, synovium, gut, chronic periodontal disease
- **Chronic periodontitis** is known to be associated with RA and *Porphyromonos gingivalis (PG)* is frequently implicated.
- *P. gingivalis* expresses PAD ➔ citrullinate proteins particularly of the cytoskeleton, such as cytokeratin, vimentin, and filaggrin.
- PAD from *P. gingivalis* citrullinates peptides at carboxy-terminal arginine residues, whereas human PADs citrullinate internal arginines ➔ creating **epitopes recognized by ACPAs.**
- Chronic periodontitis is associated with some of the *HLADRB1* alleles linked to RA, as well as with the presence of RF

It remains unclear whether *P. gingivalis* is the only bacterium in the microbiome of the oral cavity that is associated with new-onset RA

AR 2010, 62, 2662–2672.

Immunity

On-going Mechanical Damage from Mastication Drives Homeostatic Th17 Cell Responses at the Oral Barrier



- Physiologic damage through mastication promotes the generation of **oral Th17** cells
- Th17 cells develop **independently of commensal microbe** colonization
- This process is dependent on **epithelial cells -derived IL6**
- oral Th17-cell-mediated protective immunity and inflammation (bone loss – periodontitis)
- **TH17 do not migrate to LN**

Dutzan et al., 2017, Immunity 46, 133

From the lungs to systemic immunity (I)

ACPAs & cytokines

From the lungs to systemic immunity (I)

ACPAs & cytokines

Evolution from pre-RA to clinical arthritis is characterized by:

- increased titre of ACPAs
  - citrullinated enolase, vimentin and fillagrin
- reactivity against higher number of citrullinated peptides
- epitope spreading
- increased levels of cytokines/chemokines

- ✓ *Sokolove, J.* Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS ONE* 7, e35296 (2012).
- ✓ *Brink, M.* Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis. Arthritis Rheum. 65, 899–910 (2013).
- ✓ *Kokkonen, H*. Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis Rheum. 62, 383–391 (2010).

ACPA and synovial localization of pathology:

"However, it is becoming increasingly clear from studies of both B cell and T cell responses in RA that **this disease is not the result of an aberrant immune response to a single autoantigen but to many autoantigens**, of which we still do not understand the relative importance and/or hierarchy."

Moreover, there has been a conceptual problem:
linking immunity to citrullinated proteins, which are ubiquitously present during inflammation, to disease in a specific organ system such as the bones and joints.

What drives the synovial localization of ACPAs, activated T/B cells?

Malmström. *Nature Reviews Immunology* **17**, 60–75 (2017)

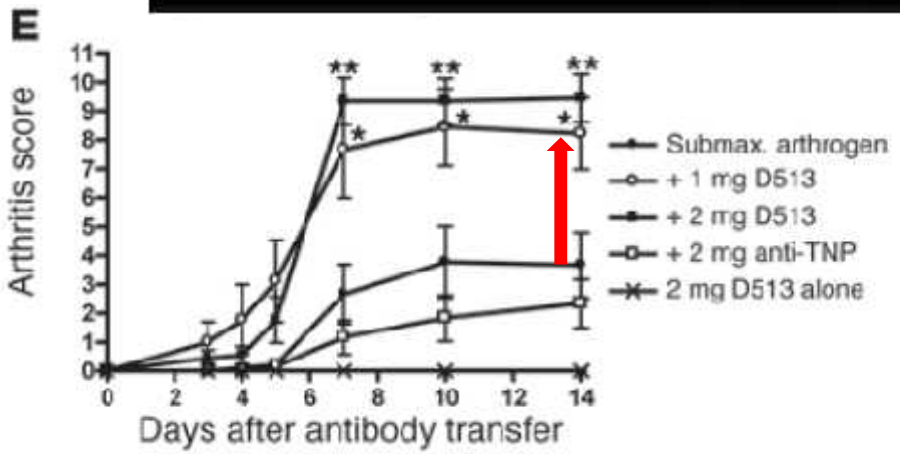
ACPA and synovial localization of pathology:

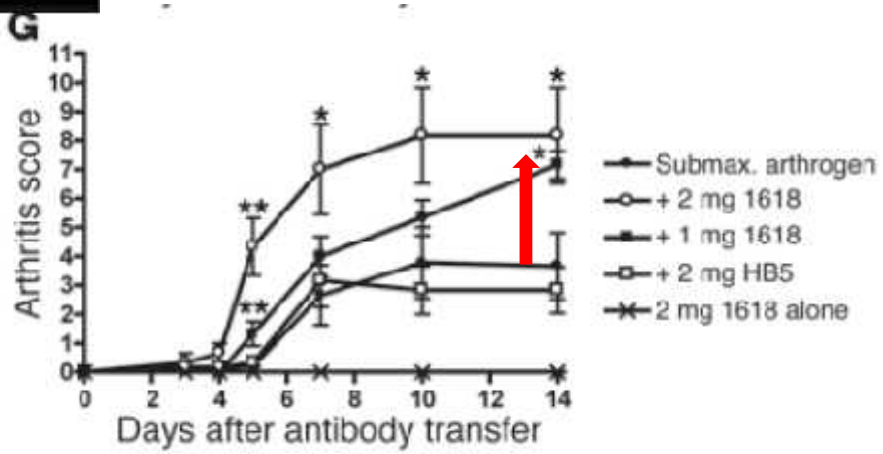
Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis

Kristine A. Kuhn,[1] Liudmila Kulik,[2] Beren Tornooka,[2,3] Kristin J. Braschler,[2] William P. Arend,[2] William H. Robinson,[3,4] and V. Michael Holers[1,2]

DBA1 mice: Antibodies specific to citrullinated proteins substantially enhance submaximal arthritis

D513: bounds citrullinated fibrinogen, vimentin, clusterin and keratin
1618: bounds citrullinated fibrinogen, filaggrin and vimentin



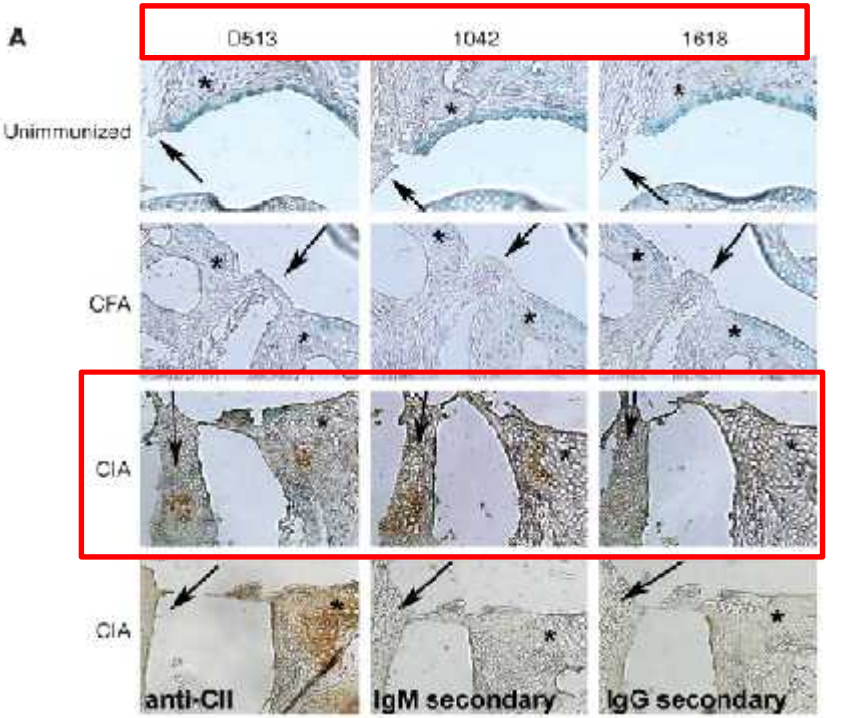


J Clin Invest 2006. 116:961–973

ACPA and synovial localization of pathology:

DBA1 mice: Antibodies specific to citrullinated proteins react **to citrullinated synovial proteins**

D513: bounds citrullinated fibrinogen, vimentin, clusterin and keratin
1618: bounds citrullinated fibrinogen, filaggrin and vimentin



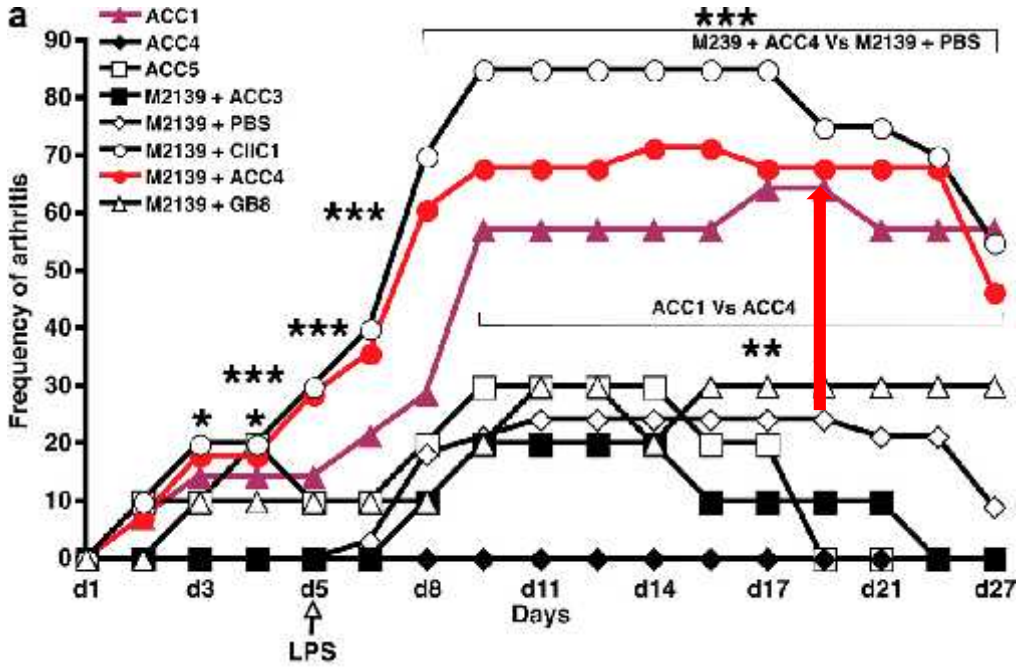
J Clin Invest 2006. 116:961–973

ACPA and synovial localization of pathology:

Structure and pathogenicity of antibodies specific for citrullinated collagen type II in experimental arthritis

Antibodies specific to citrullinated Collagen II react **to synovial proteins in HUMANS (RA-OA) and potentiate arthritogenic potential of anti-collagen ab**

"Antibodies to citrullinated CII can induce arthritis in naive mice by themselves, and the arthritis severity is enhanced by combining them with antibodies to non-citrullinated CII.



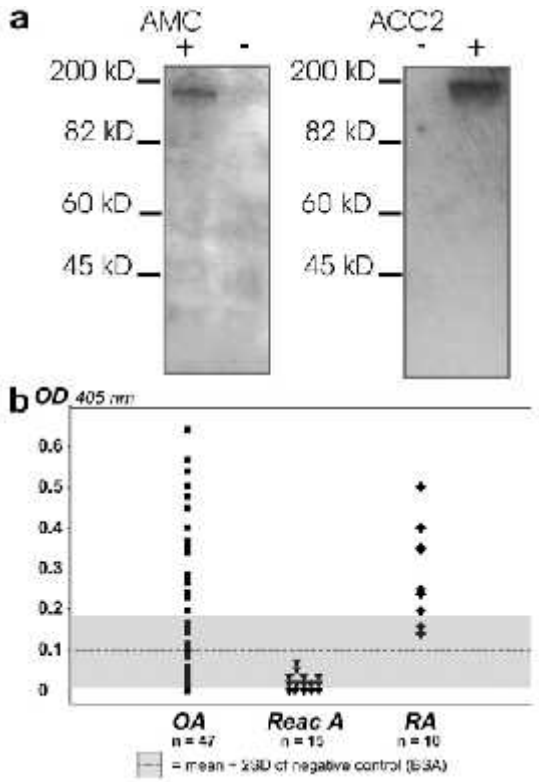
Oysal. J.Exp.Med. 2012, (206). 449

ACPA and synovial localization of pathology:

Structure and pathogenicity of antibodies specific for citrullinated collagen type II in experimental arthritis

Antibodies specific to citrullinated Collagen II:
- **potentiate arthritogenic potential of anti-collagen ab**
- react **to synovial proteins in HUMANS (RA-OA)**

Detection of citrullinated CII in human synovial fluid.



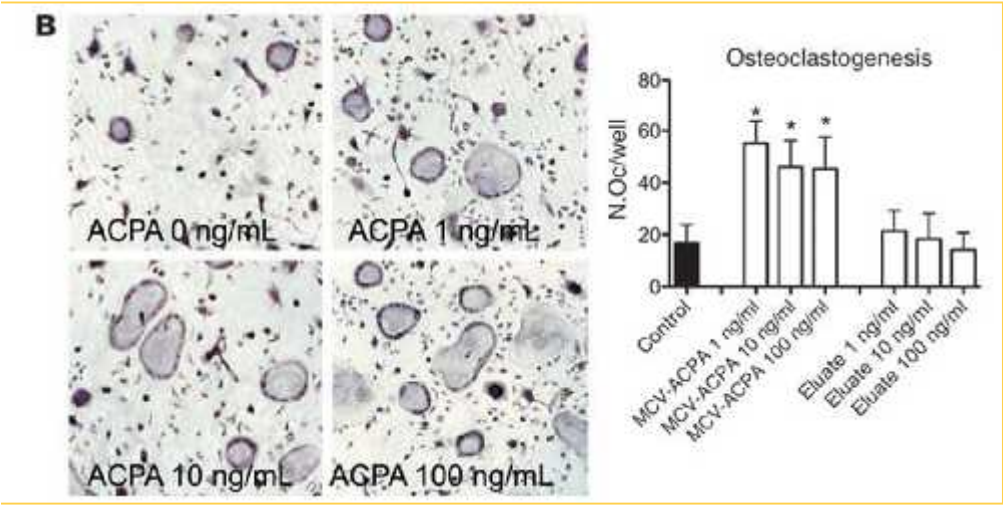
ELISA for detection of citrullinated CII in synovial fluid from patients with OA, Reactive arthritis and RA.

Oysal. J.Exp.Med. 2012, (206). 449

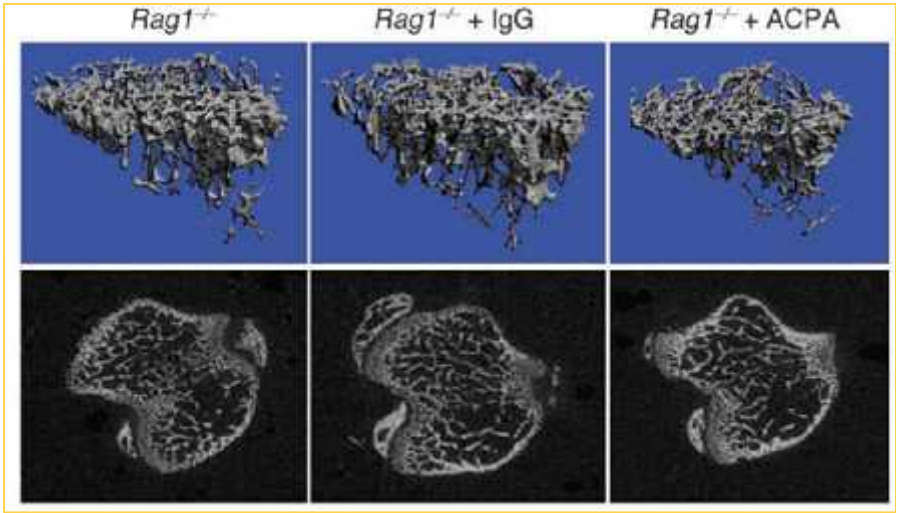
ACPA and synovial localization of pathology: OSTEOCLASTS

ACPA can promote osteoclasts and induce bone loss

Human autoantibodies against mutated citrullinated vimentin (MCV) bound to osteoclast surfaces, but also led to robust induction of osteoclastogenesis and bone-resorptive activity.



Adoptive transfer of purified human MCV autoantibodies into mice induced osteopenia and increased osteoclastogenesis



J Clin Invest. 2012;122(5):1791

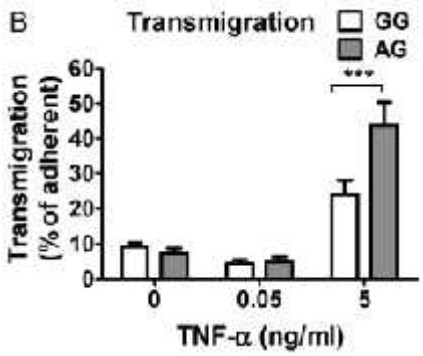
ACPA and synovial localization of pathology: OSTEOCLASTS

Genetic risk and synovial localization of pathology

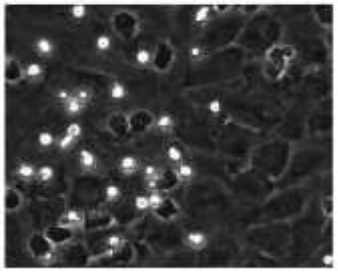
Heterozygosity for PTPN22 R620W increased neutrophil migration across inflamed endothelium

The autoimmune-associated genetic variant PTPN22 R620W enhances neutrophil activation and function in patients with rheumatoid arthritis and healthy individuals

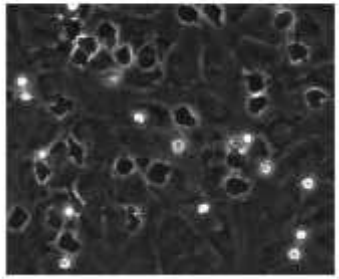
Rachel Bayley,[1] Kerry A Kite,[1] Helen M McGettrick,[1] Jacqueline P Smith,[2] George D Kitas,[2] Christopher D Buckley,[1] Stephen P Young[1]



C. Transmigration of GG neutrophils



D. Transmigration of AG neutrophils



Bayley R, et al. Ann Rheum Dis 2014;0:1

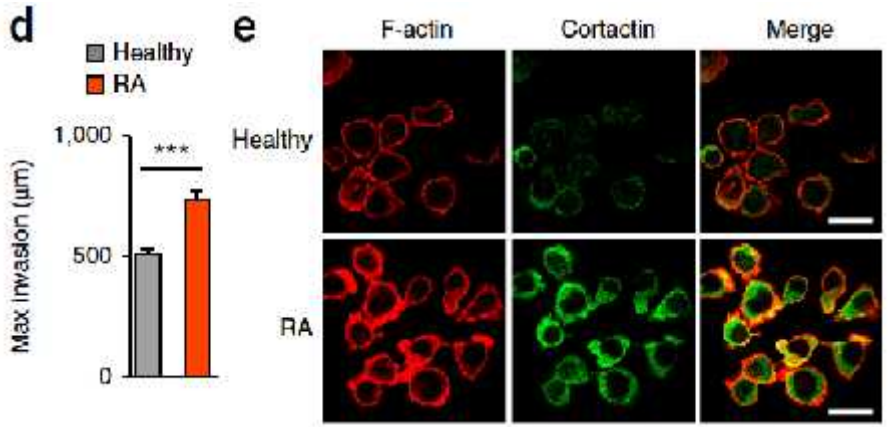
T cell migratory and inflammatory properties: metabolomics

T cell migratory and inflammatory properties: metabolic reprogramming

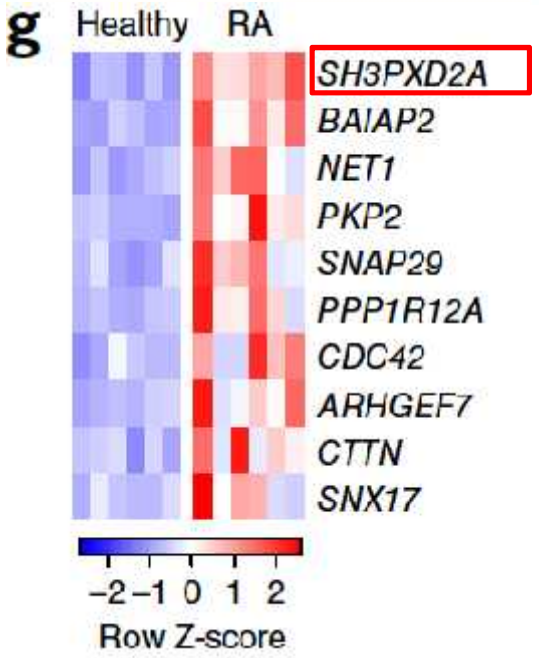
Metabolic control of the scaffold protein TKS5 in tissue-invasive, proinflammatory T cells

Yi Shen[1], Zhenke Wen[1], Yinyin Li[1], Eric L. Matteson[2], Jison Hong[1], Jörg J Goronzy[1] & Cornelia M Weyand[1]

RA T cells are "tissue-invasive"



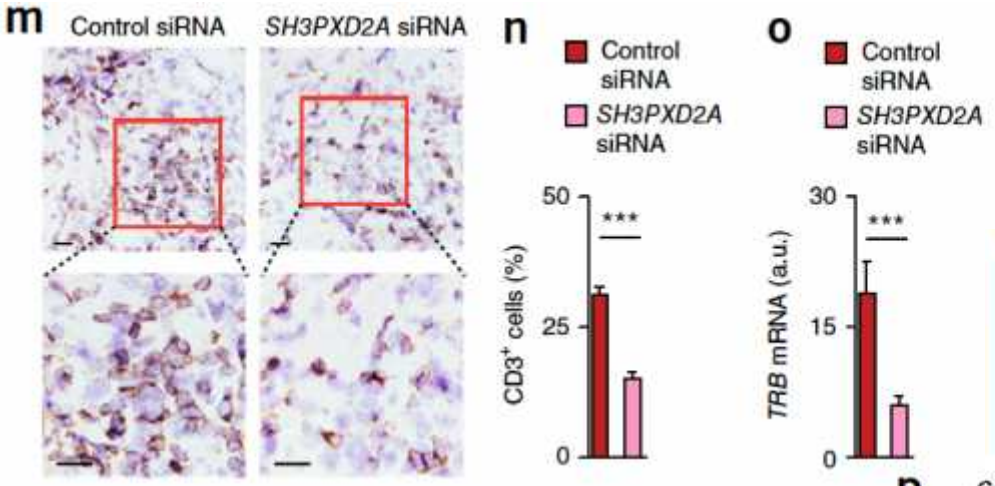
RA T cells expressed higher levels of ***SH3PXD2A***, which encodes the **TKS5** that **facilitates the formation of cellular projections**



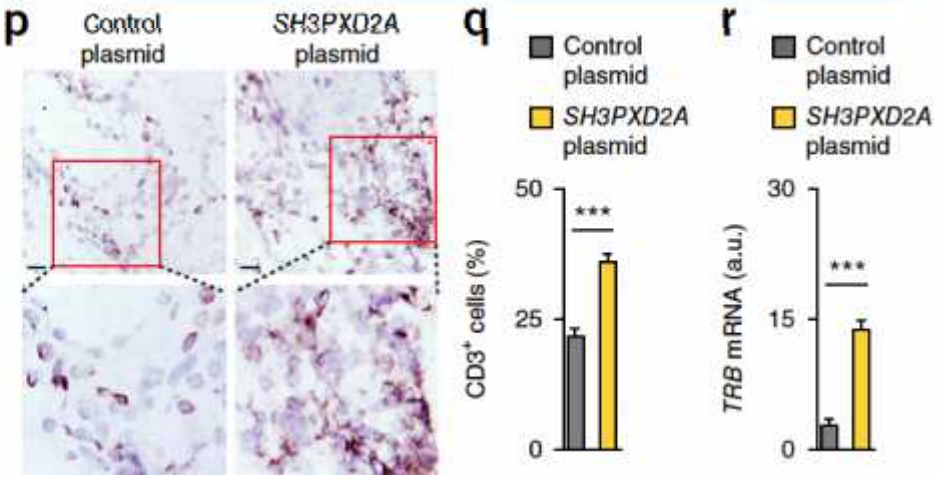
Chen et al. Nat Immunol. 2017;18(9):1025

T cell migratory and inflammatory properties: metabolic reprogramming

Knockdown of *SH3PXD2A* disrupted the invasive capabilities of RA T cells



Conversely, TKS5 overexpression in healthy T cells resulted in enhanced T cell invasion



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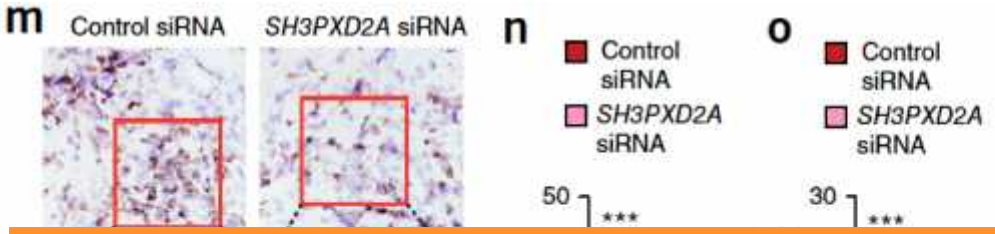
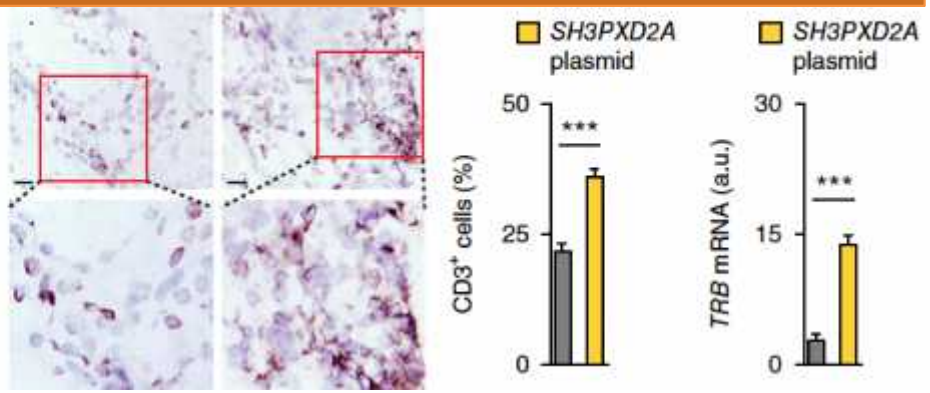


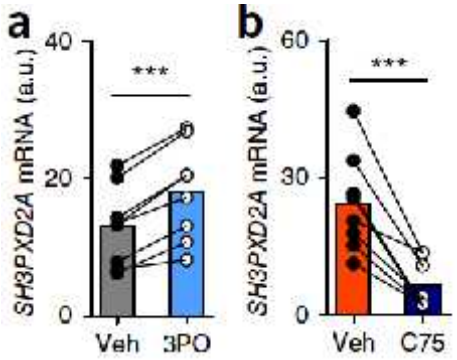
- ✓ Thus, RA T cells were equipped to dynamically form membrane ruffles and invaded non-lymphoid tissue sites.
- ✓ The scaffolding protein TKS5 seemed to be nonredundant for this invasive behavior.



Chen et al. Nat Immunol. 2017;18(9):1025

T cell migratory and inflammatory properties: metabolic reprogramming

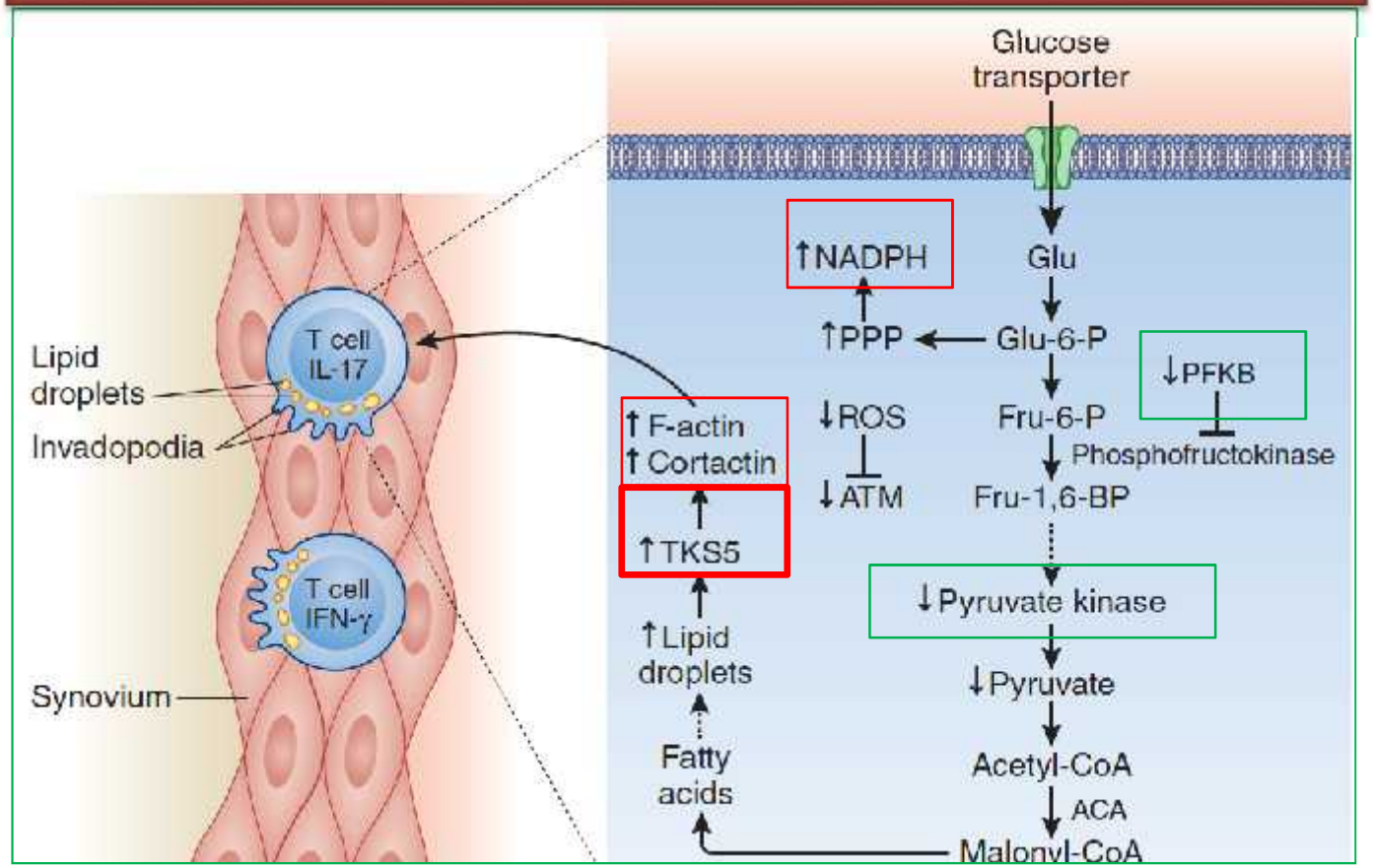
TKS5 expression is metabolically regulated



- ✓ Healthy CD4+ T cells treated PFKFB3 inhibitor 3PO (mimic RA glycolysis arrest) (a)
- ✓ Patient-derived T cells treated with FA synthase (FAS) inhibitor C75 (inhibit increased NADPH-dependent FA synthesis) (b)

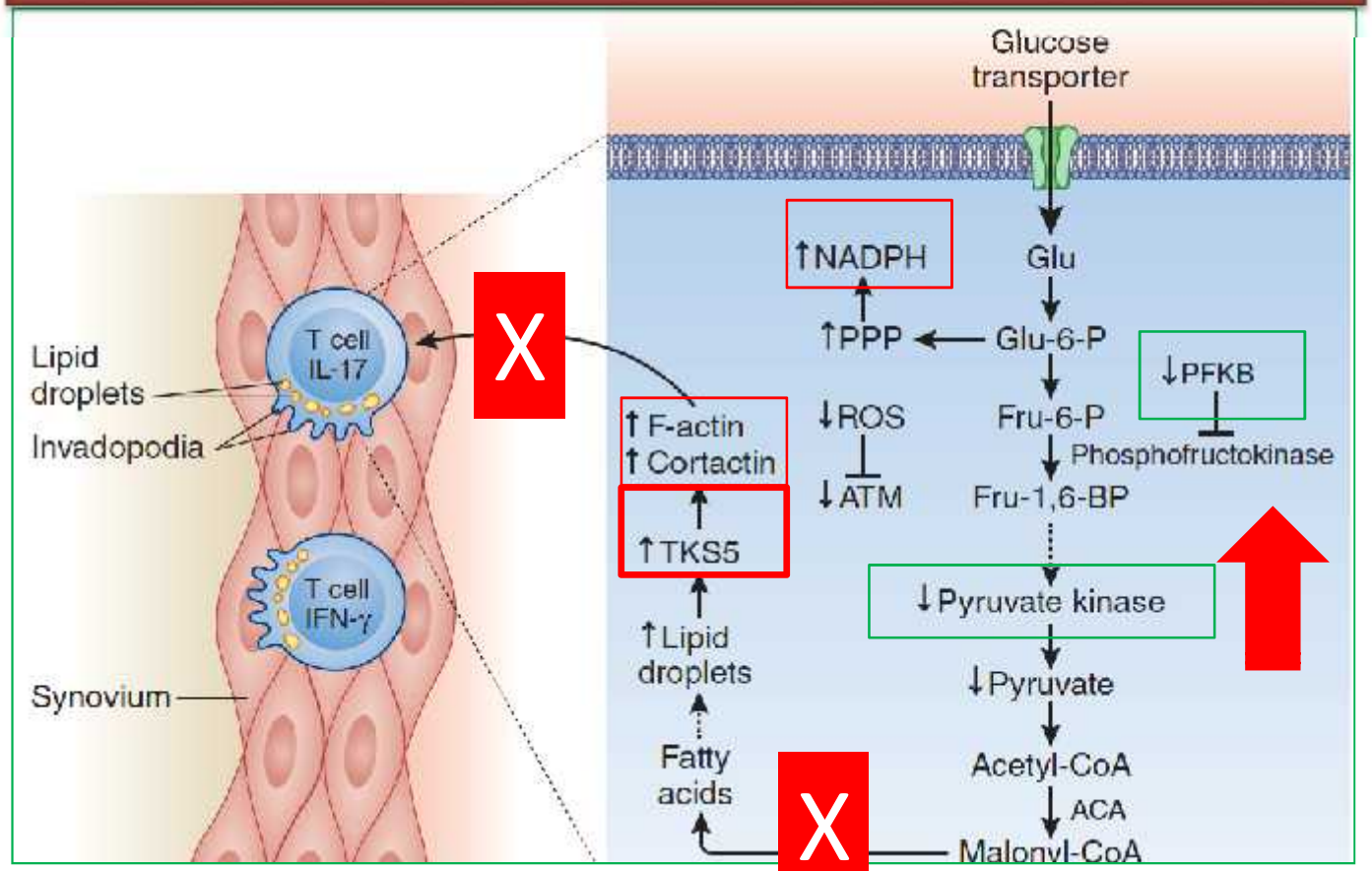
Chen et al. Nat Immunol. 2017;18(9):1025

T cell migratory and inflammatory properties: metabolic reprogramming



Chen et al. Nat Immunol. 2017;18(9):1025
Tsokos. Nat Immunol. 2017; 18(9):955

T cell migratory and inflammatory properties: metabolic reprogramming

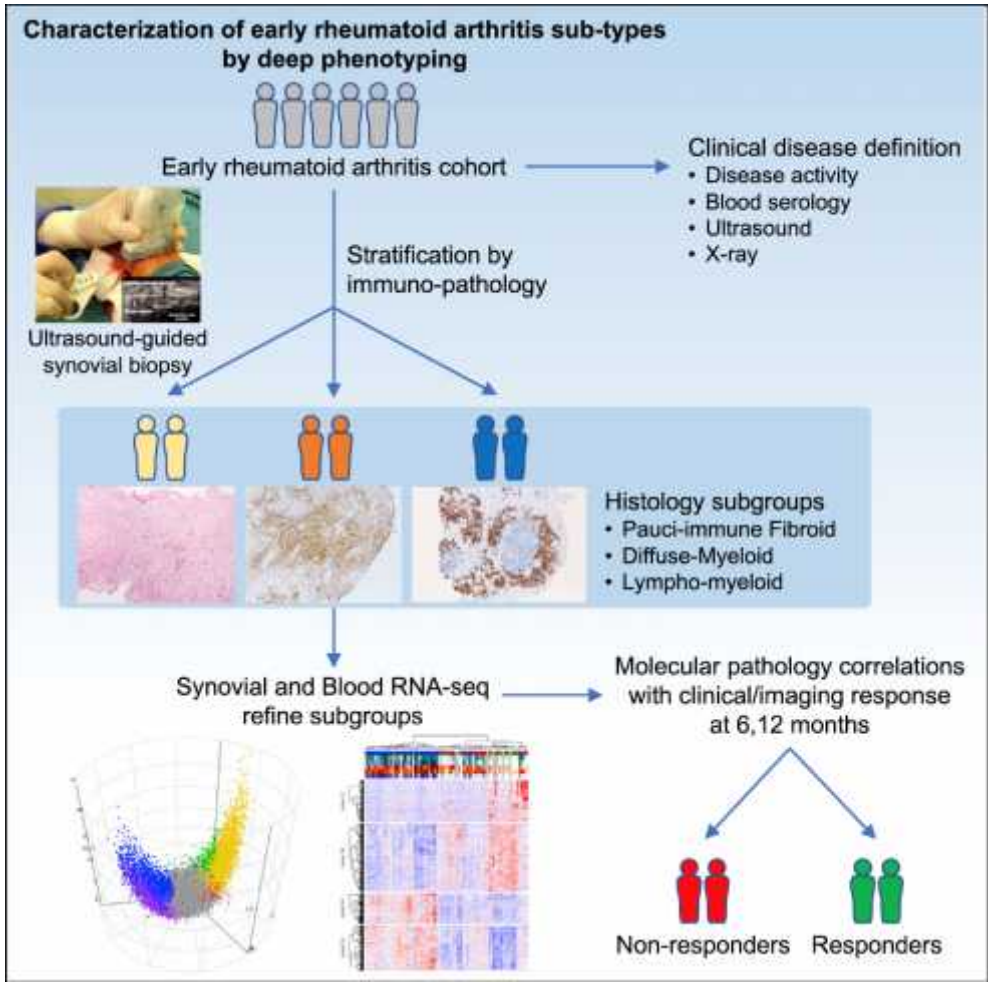


- ✓ T cells from the peripheral blood of people with RA **reduced invasiveness and inflammatory** properties when treated with:
  - the FAS inhibitor C75 or
  - the pyruvate-kinase activator ML265

Chen et al. Nat Immunol. 2017;18(9):1025
Tsokos. Nat Immunol. 2017; 18(9):955

Cell Reports

Molecular Portraits of Early Rheumatoid Arthritis Identify Clinical and Treatment Response Phenotypes

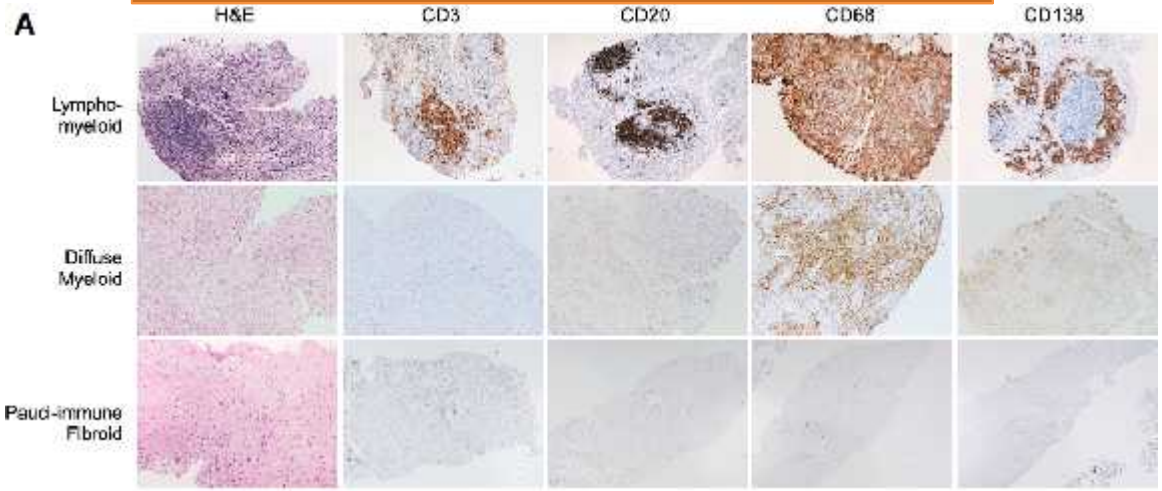


- ✓ Deep phenotyping and RNA-seq of early RA individuals pre-treatment
- ✓ Synovial plasma cell gene expression predicts future progressive joint damage on X-ray
- ✓ Blood interferon gene signature associates with synovial B and plasma cell infiltration

Lewis et al., 2019, Cell Reports 28, 2455

Cell specific RNA-seq signatures correlates to histology

Immunohistochemistry of synovial biopsies



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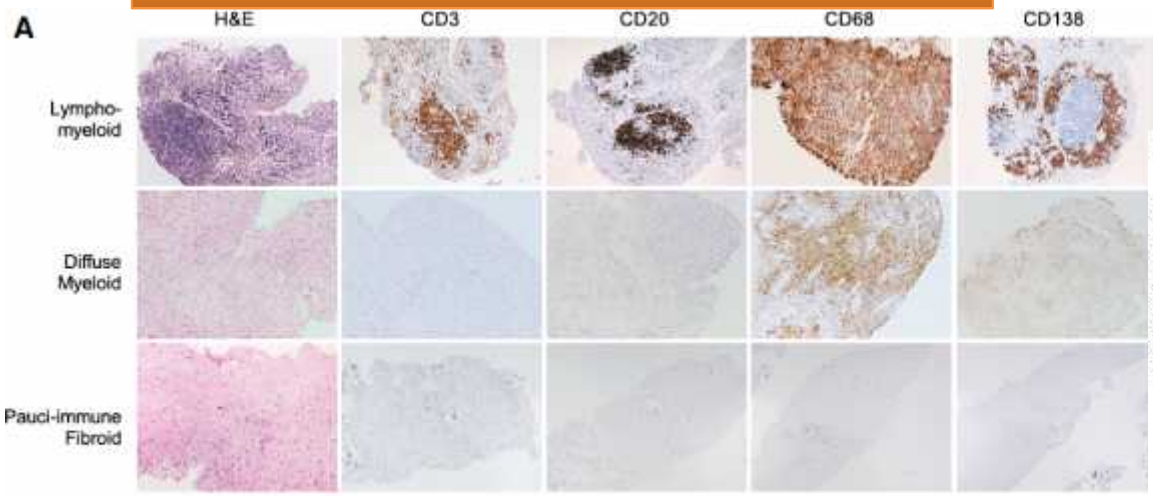
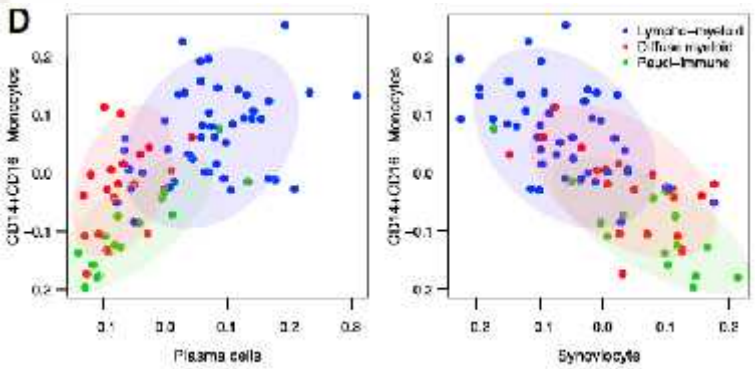
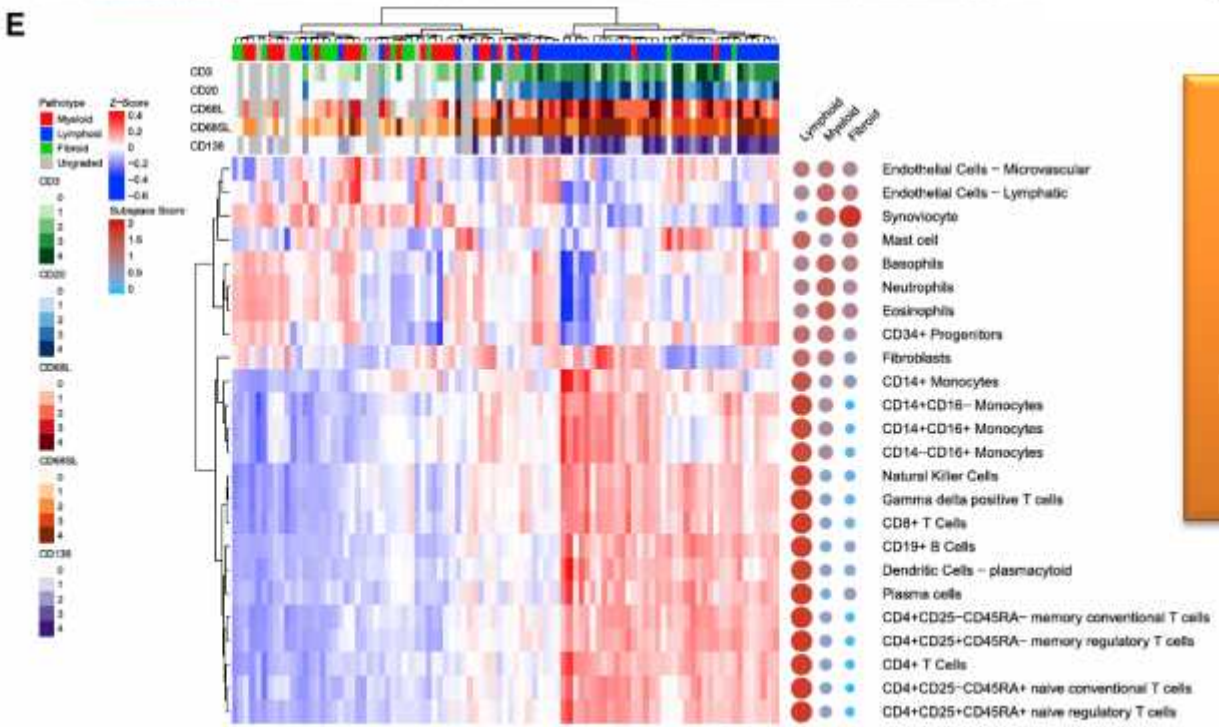


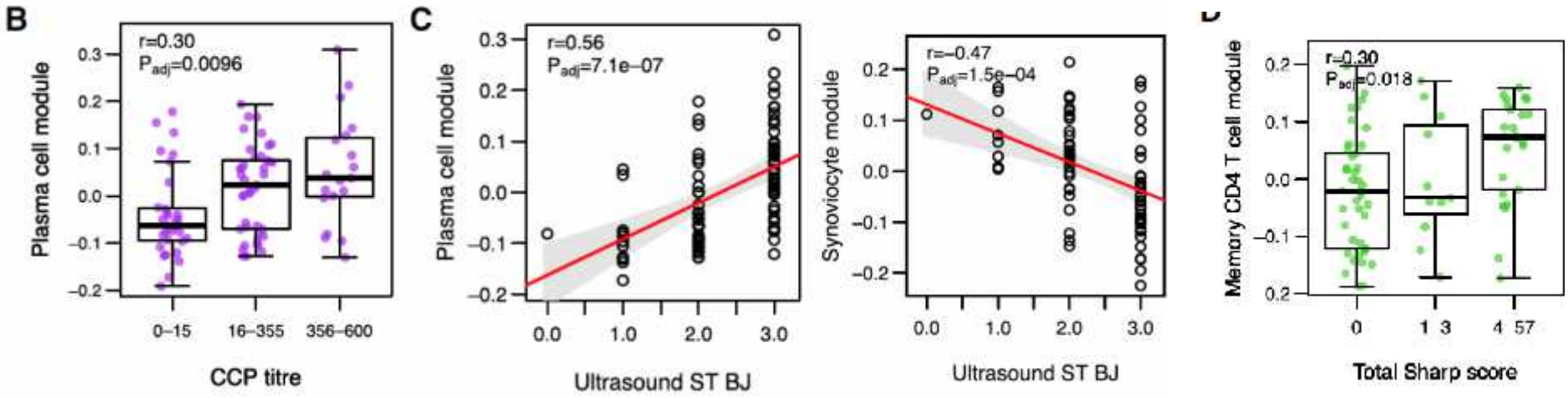




Cell-specific RNA-seq gene module scores

Heatmap showing hierarchical clustering of cell-specific gene module scores and collapsed module space (right) highlighting cellular composition of synovial biopsies from each pathotype.

Cell-Specific Synovial Gene Modules Correlate to Clinico-radiographic Phenotype

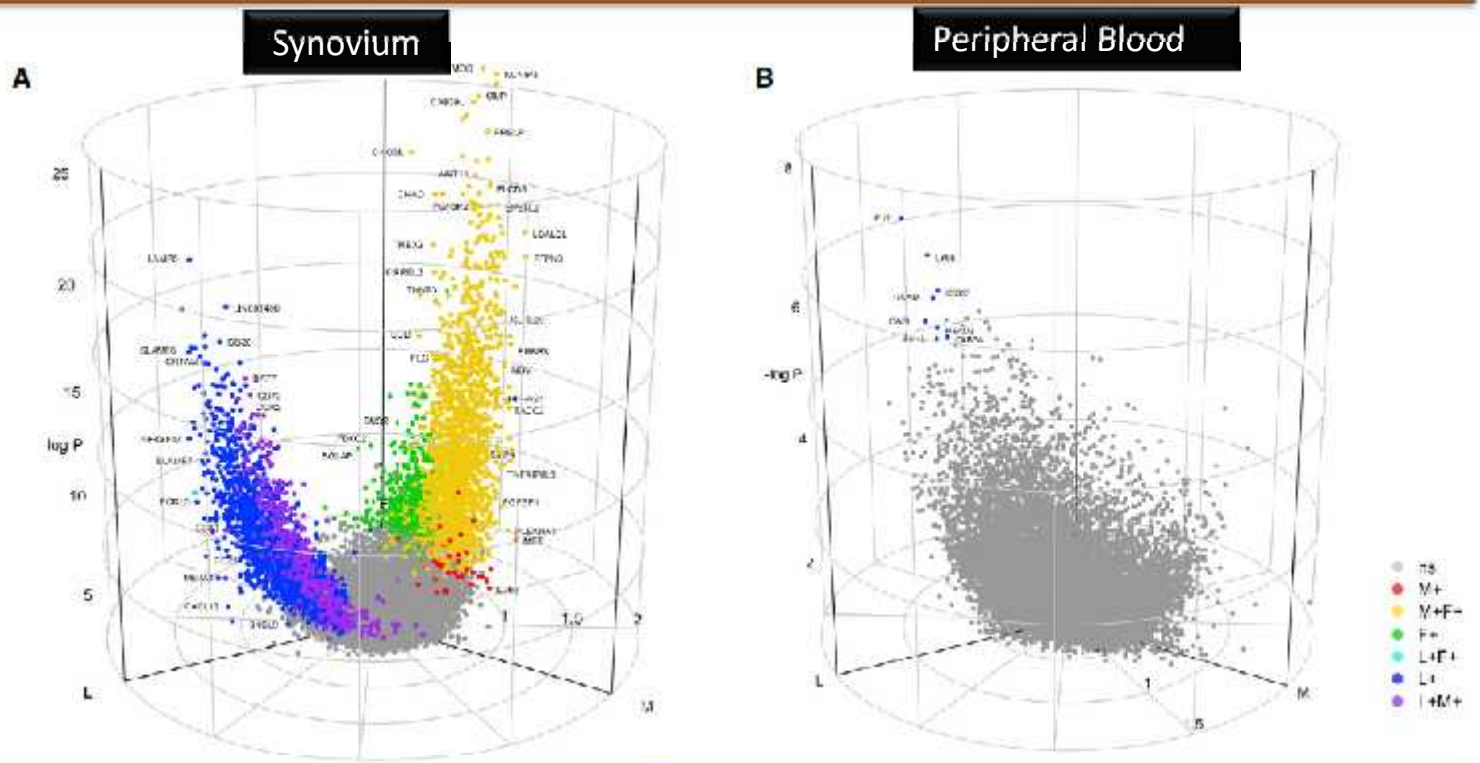


"These data suggest that infiltration of multiple immune cell types associated with **ectopic lymphoid responses** in the synovial tissue may be **linked to more destructive disease** from **early on in the course of RA**."

Lewis et al., 2019, Cell Reports 28, 2455

Synovium is much more informative compared to peripheral

Differential gene expression:
3,000 transcripts in synovium compared to only 8 in PB



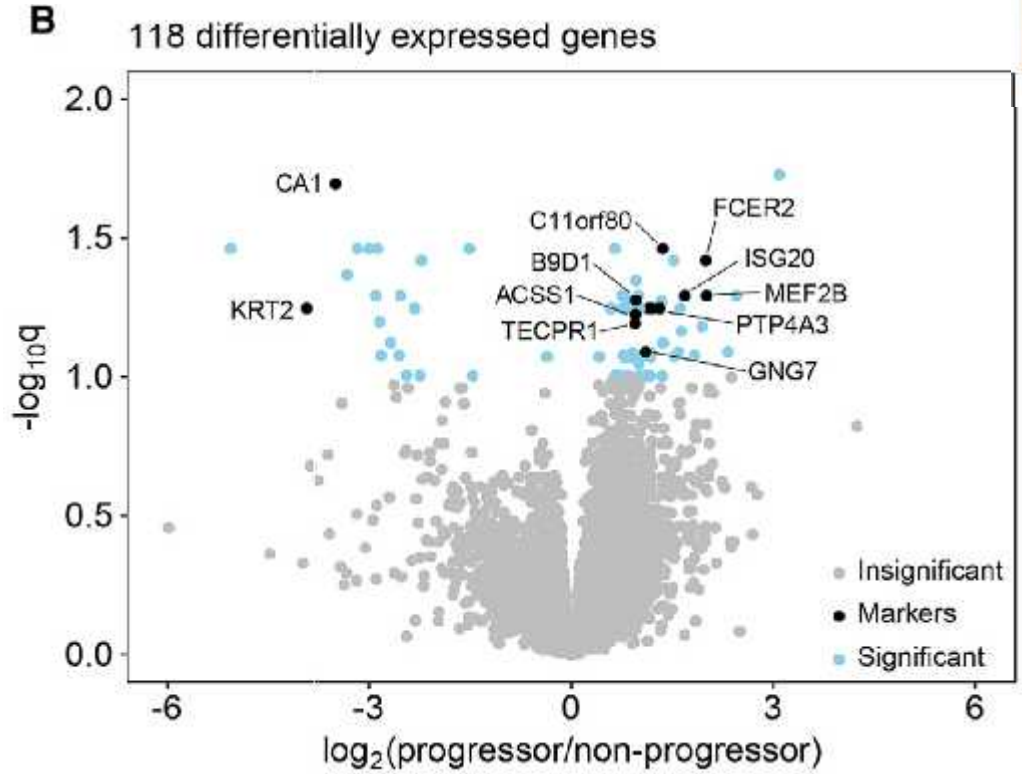
The synovium gives clean delineation of the lympho-myeloid group, particularly in those individuals with synovial plasma cells the blood transcriptome shows significantly less differentiation between pathotypes

Lewis et al., 2019, Cell Reports 28, 2455

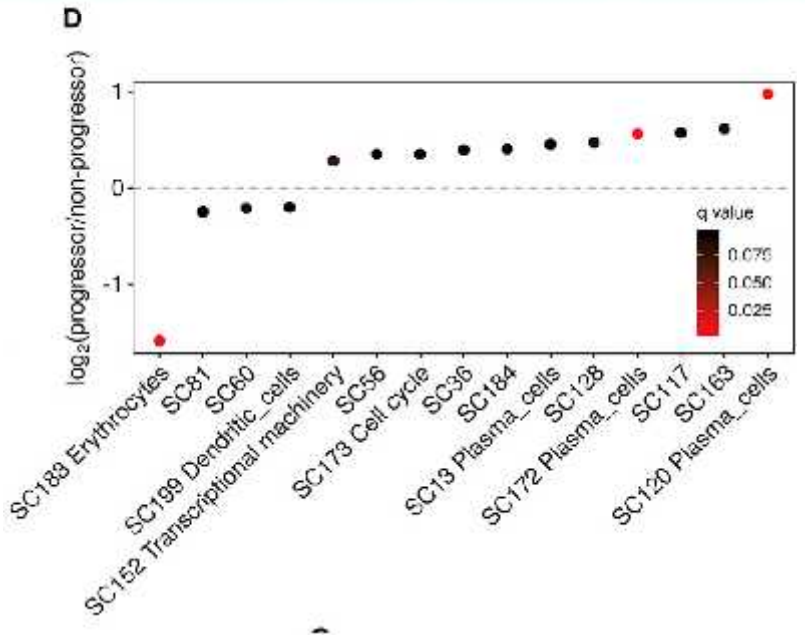
Synovium Gene expression gives clinically relevant information

Synovium Plasma Cell Gene Expression at Baseline **Predicts Worse Prognosis at 12 Months**

Total Synovium **Gene Expression Baseline** Synovium Plasma Cell signature **predicts damage**



Single-cell RNA-seq-annotated WGCNA modular analysis shows that increased **plasma cell module expression predicts bone erosion**



Lewis et al., 2019, Cell Reports 28, 2455

MOU1
MOU2

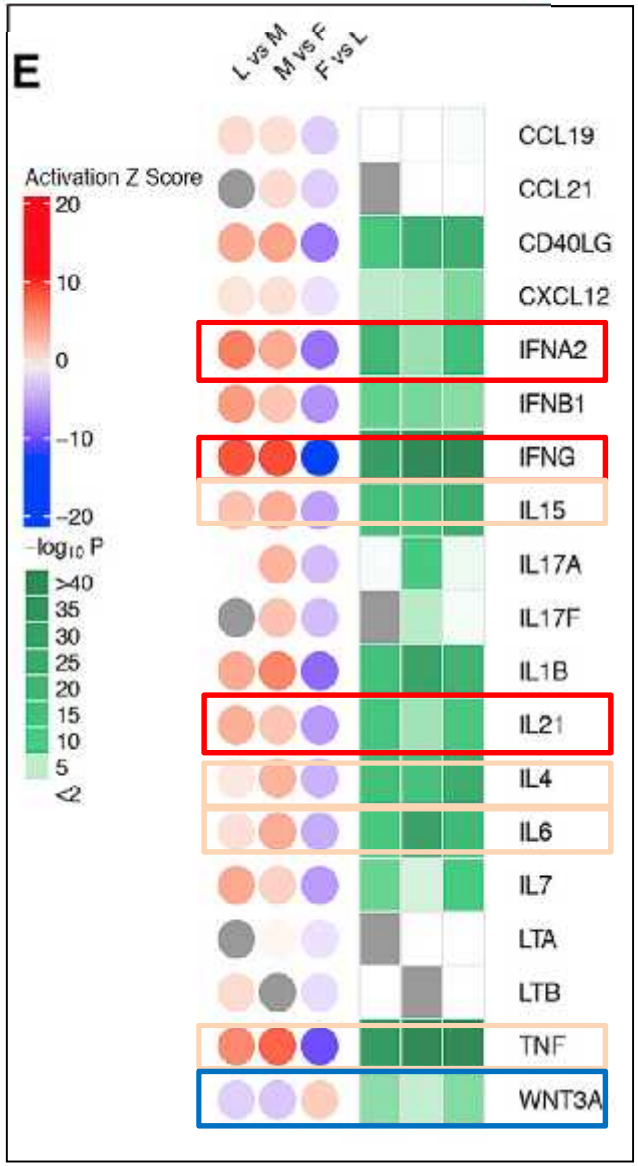
Different regulators support ectopic lymphoid, myeloid or fibroid structures within synovium

Upstream regulator analysis showed key regulators for each pathotype:

✓lympho-myeloid pathotype
- IFN-g, IFN-a2, IFN-b1, IL-7, IL-21, CD40L

✓diffuse myeloid pathotype,
- TNF, IFN-g, IL-1b, IL-4, IL-6, IL-15

✓pauci-immune fibroid pathotype
- WNT3A.



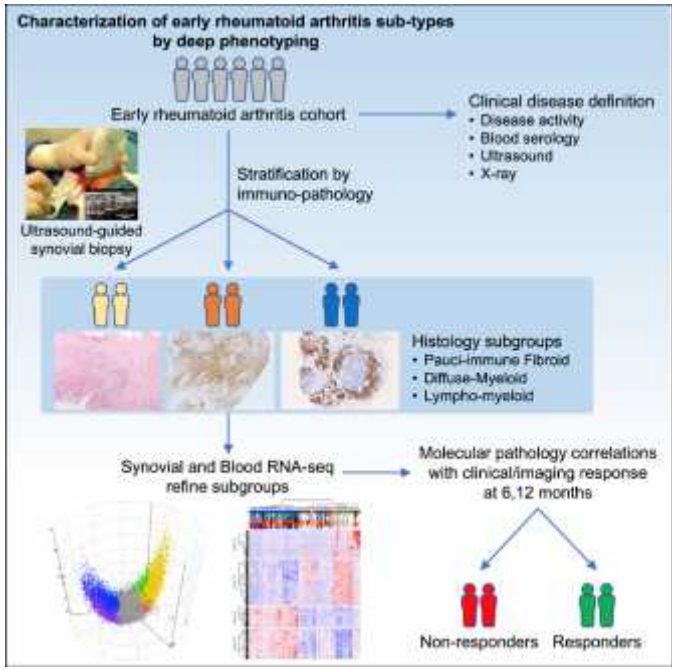
Lewis et al., 2019, Cell Reports 28, 2455

MOU1 Microsoft Office User, 31-Oct-19

MOU2 Upstream regulator analysis showed key regulators for each pathotype:
lympho-myeloid pathotype
IFN-g, IFN-a2, IFN-b1, IL-7, IL-21, and CD40L, dominant theme of B cell proliferation, differentiation, and plasma cell development and the previously reported association of IL-7 pathway with synovial B cell
The follicular helper T cell cytokine IL-21 is important for ELS maturation
We also confirmed the association of the chemokine CXCL12 with ELS formation which is consistent with its role in maintaining long-lived plasma cells
diffuse myeloid pathotype,
TNF, IFN-g, IL-1b, IL-4, IL-6 ,and IL-15
pauci-immune fibroid pathotype
WNT3A.
Microsoft Office User, 31-Oct-19

Cell Reports

Molecular Portraits of Early Rheumatoid Arthritis Identify Clinical and Treatment Response Phenotypes



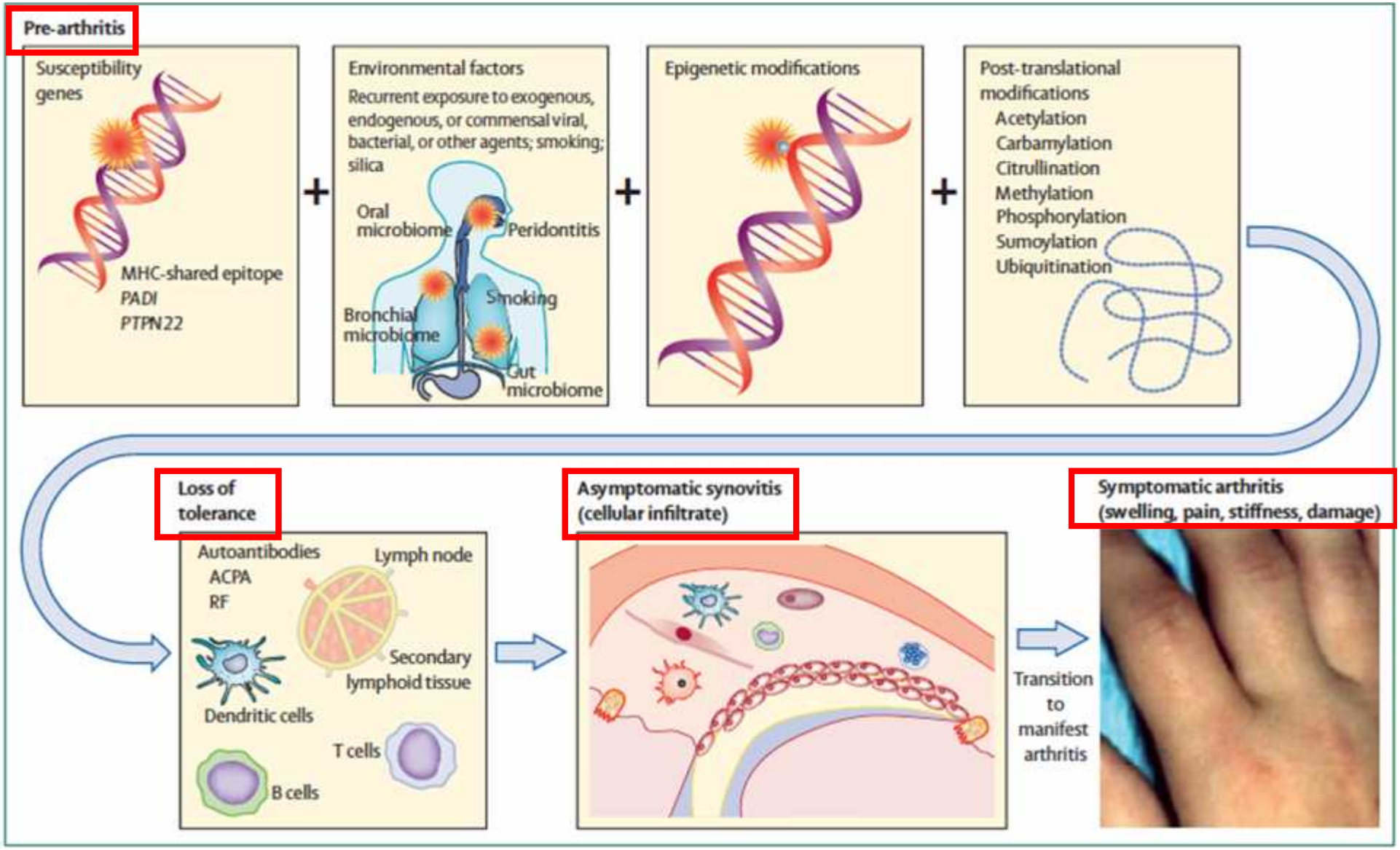
In-depth RNA-seq analysis of synovial tissue and peripheral blood:

- Showed differential synovial histology
- Revealed pathways supporting different cellular infiltration
- Identified clinical correlates: increased risk of rapid disease progression

"Support that idea that optimal stratification of RA therapies would be enhanced by sampling of both synovium and blood biomarkers."

Lewis et al., 2019, Cell Reports 28, 2455

Putting everything together genes, enviroment and immune inflammatory response



Smolen. Lancet 2016; 388: 2023–38