Rheumatoid arthritis pathogenesis: From systemic autoimmunity to synovial inflammation

ΠΑΝΕΠΙΣΤΗΜΙΑΚΟ
ΝΟΣΟΚΟΜΕΙΟ ΗΡΑΚΛΕΙΟΥ

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ΙΑΤΡΙΚΗ ΣΧΟΛΗ

References

- Nat Immunol. 2021 Jan;22(1):10-18
- Sci Transl Med. 2016 Mar 23;8(331):331ra38
- Immunity. 2016, 45, 903–916
- Cell Metabolism. 2019, 30, 1–16
- Nat Immunol. 2019 Jul;20(7):928-942
- Nat Rev Immunol. 2017 Jan;17(1):60-75.

Outline

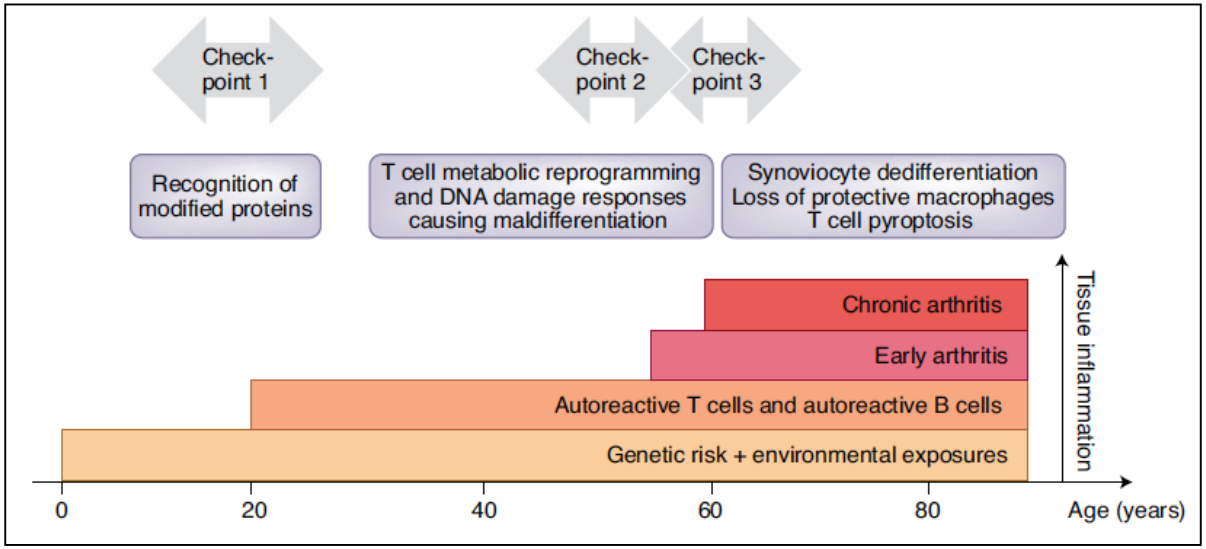
- Autoimmunity development in RA
  - Genes
  - Autoimmunity
  - Citrullination
- Synovial targeting
  - CD4
  - Fibroblasts
  - Macrophages/monocytes

General concept for RA pathogenesis

"RA is an almost lifelong process in with distinct phases:

- ✓ **disease risk:** genetically predisposed individuals lose self-tolerance, produce autoantibodies.
- ✓ **asymptomatic autoimmunity:** characterized by prototypic autoantibodies reactive against post-translationally modified proteins, often citrullinated antigens.
- ✓ **symptomatic synovitis:** Acute joint inflammation transitions

into chronic, destructive synovitis. Tissue responds with a maladaptive wound healing response (pannus), which by itself has destructive features and will lead to irreversible tissue injury"



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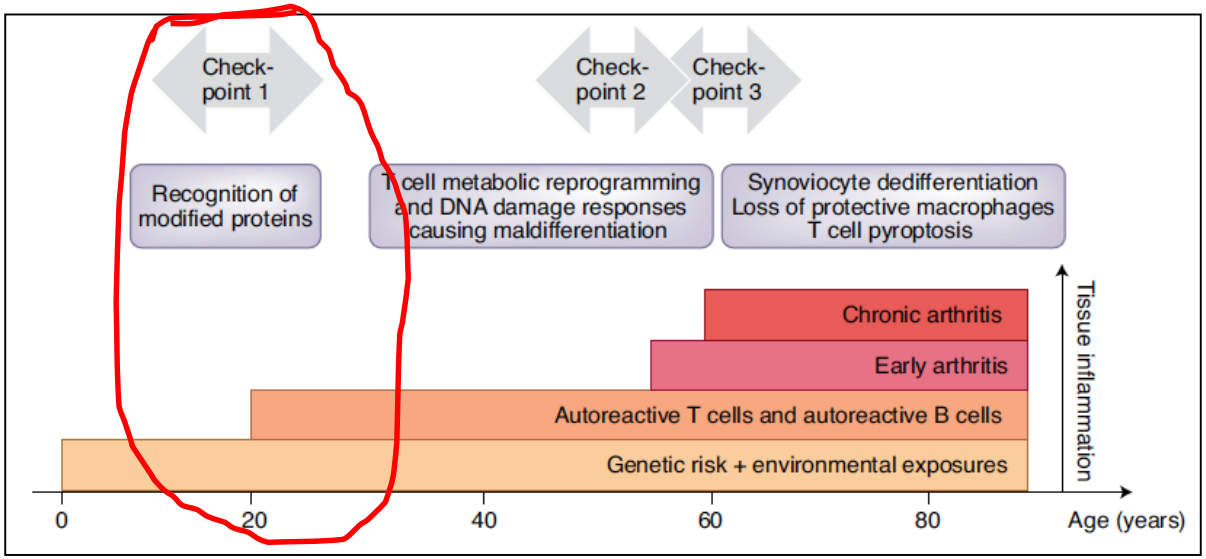
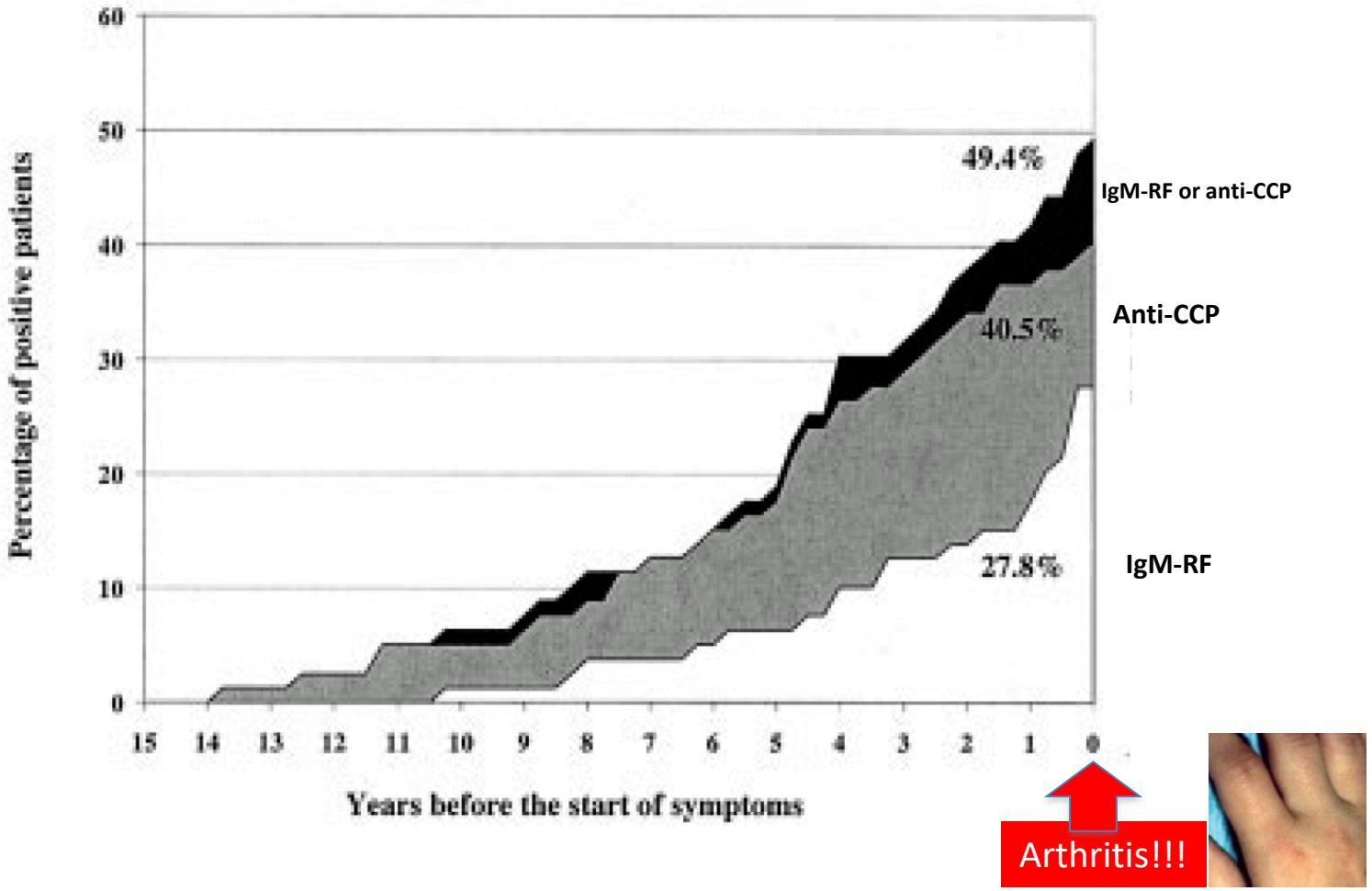


Nat Immunol. 2021 Jan;22(1):10-18

Autoimmunity in RA starts long before symptoms:

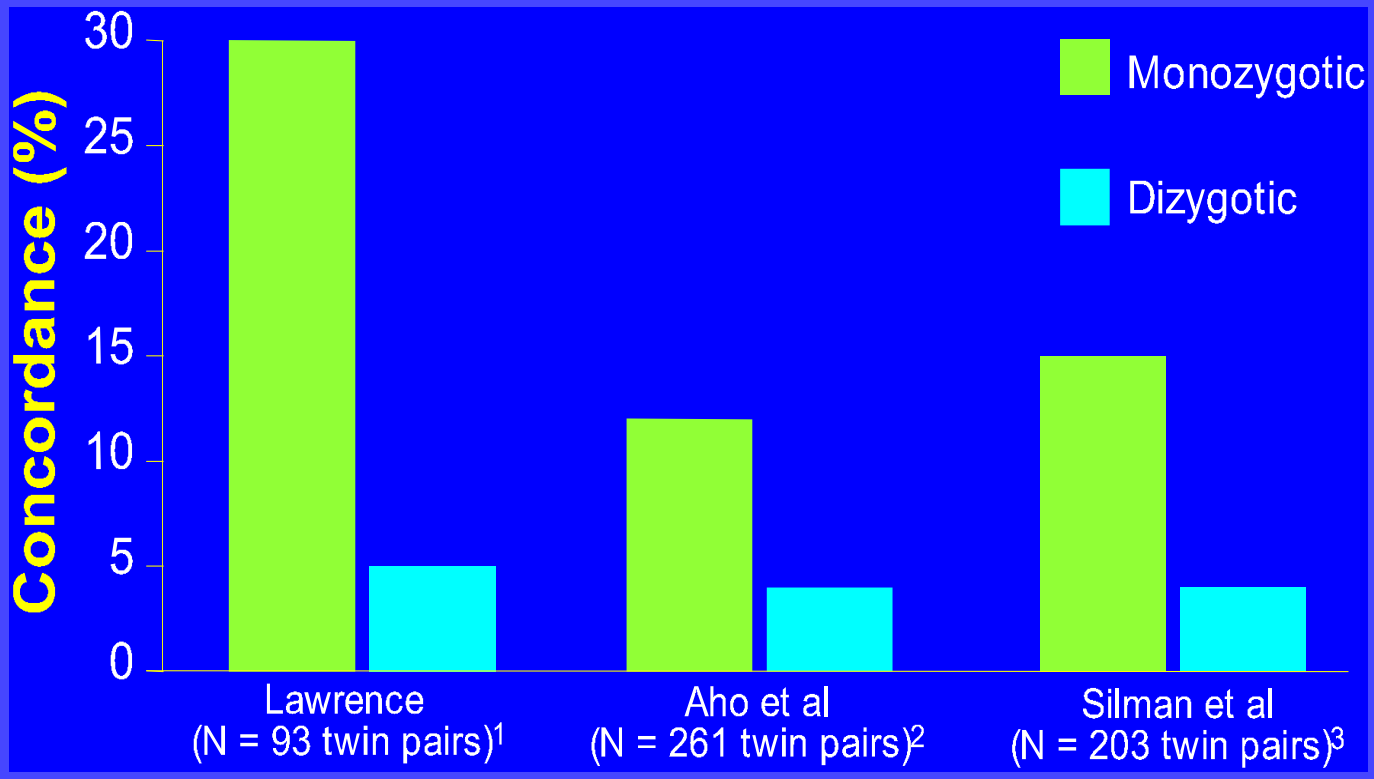
ACPA (anti citrullinated peptide antibodies) and RFs (Rheumatoid factors) decades before symptoms



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

Twin Studies in Rheumatoid Arthritis:

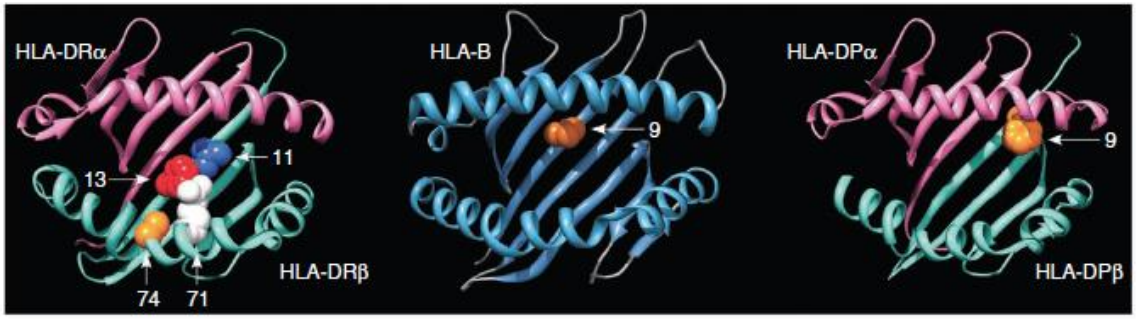
Increased incidence in twins and in families with affected members



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.

Genetic risk for RA:
Major contribution of HLA-DR

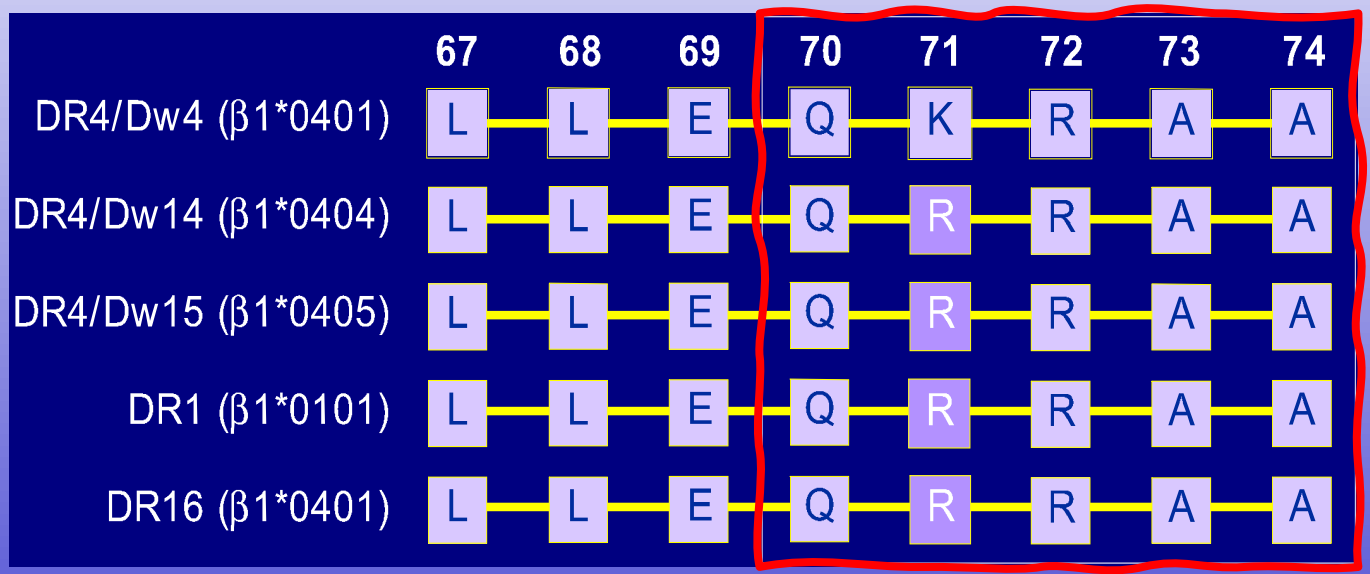
- Studies in twins ➔genetic contribution to RA accounts **for ~60%** of the variation in liability to disease.
- The most important genetic risk : class **MHC class II** region (HLA-DR).
- MHC contribute **18–37%** of the total genetic susceptibility to RA, increasing disease liability **4–6-fold**.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

Shared Epitope Hypothesis

Alleles Associated with Rheumatoid Arthritis



Lipsky PE. In: *Harrison's Principles of Internal Medicine.* 1994:1648-1655.

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.

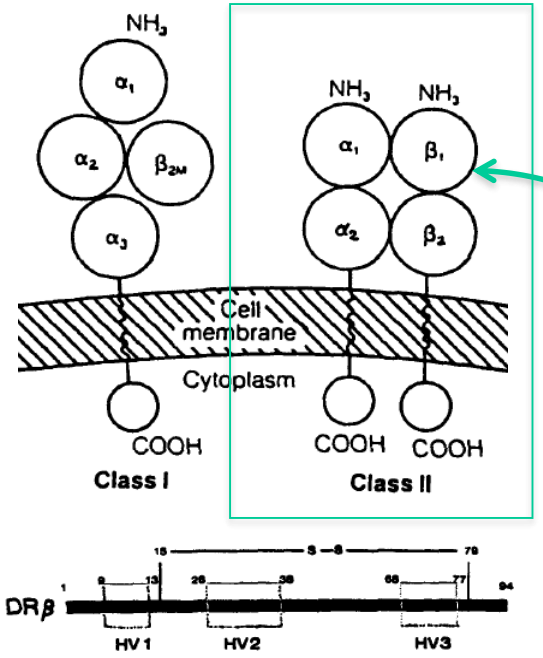


Figure 2. Top, Schematic diagram comparing the structural features of class II molecules at the cell surface with those of class I molecules. Bottom, The first domain, which contains regions of variability that alternate with invariant regions.

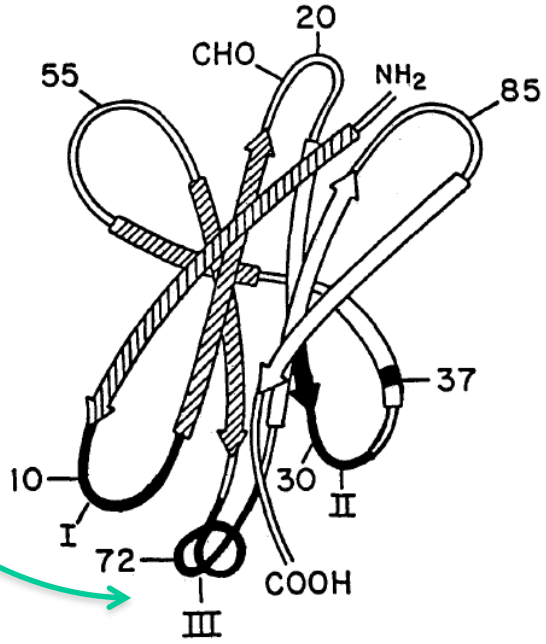


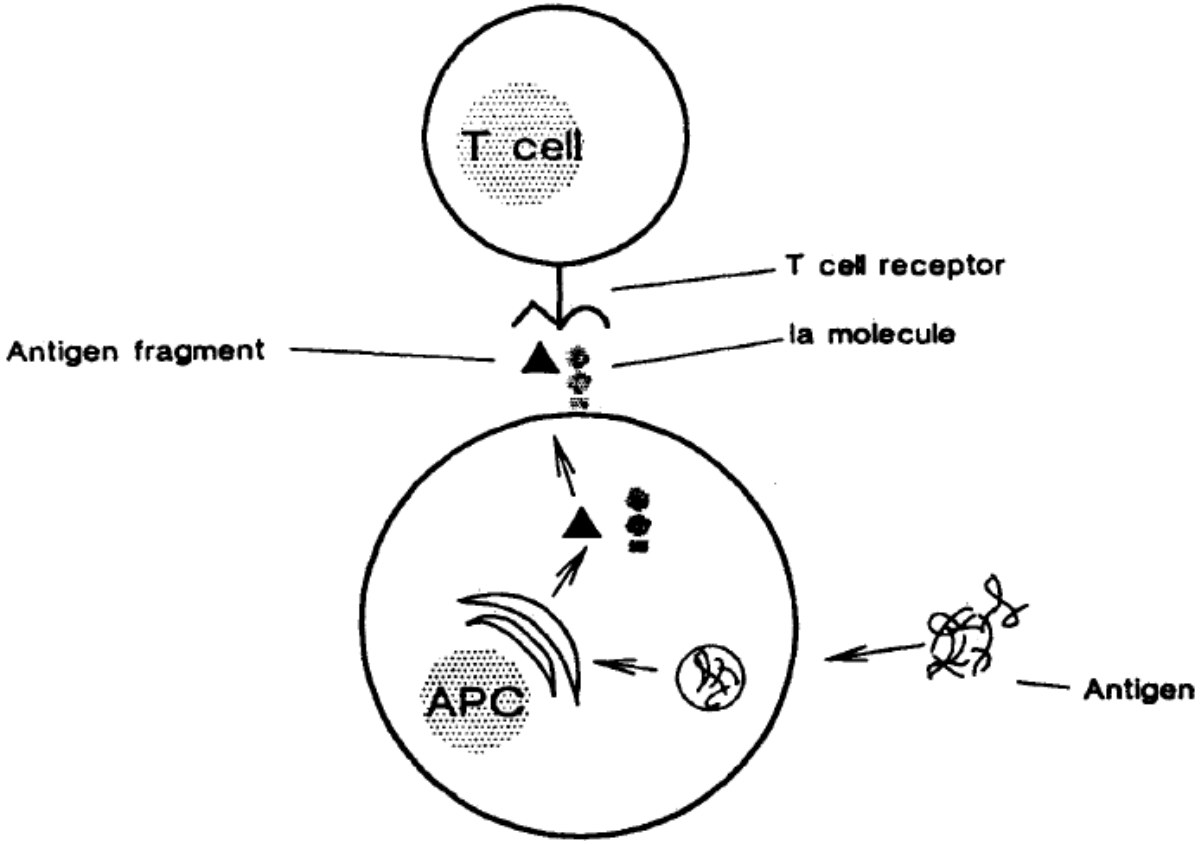
Figure 4. The predicted 3-dimensional structure of a class II β chain. Note that the third hypervariable region around position 70 is predicted to contain a region of α helical structure. It is this region which differs among the DR4 subtypes. Adapted from the *Scandinavian Journal of Immunology* (Norcross 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.



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

RA related Genes ➔ autoimmunity

Τα HLA-DRB1 αλληλόμορφα σχετίζονται ΜΟΝΟ με ACPA θετική νόσο!

Genetic association to autoimmunity

Shared Epitope alleles (HLA-DRB1) are associated with development of anti-CCP (and RFs) antibodies and thus only to seropositive

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

		Dutch EAC RA patients			
		Anti-CCP positive (n = 195)		Anti-CCP negative (n = 213)	
SE	Dutch controls (n = 423), no. (%)	No. (%)	OR (95% CI)	No. (%)	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

Mechanism of the shared epitope / ACPA-association:

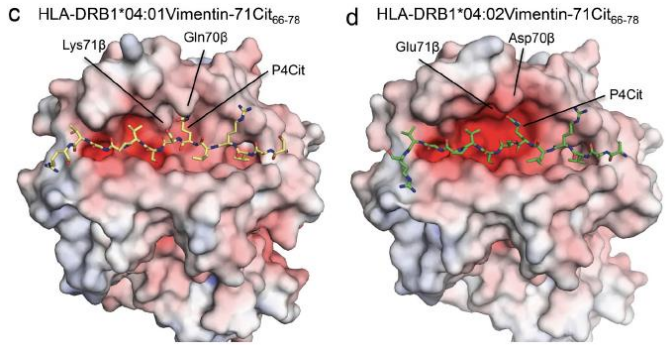
DR-B1 alleles bind more avidly citrulline-containing peptides

- It is partly attributable to the finding **that citrulline-containing peptides bind more avidly than unmodified molecules to the binding pocket of DRB1** molecules that contain the epitope, with subsequent activation of CD4+ T cells.

J. Immunol. 171, 538–541 (2003).

- The shared epitope also seems to function as an **immunostimulatory** ligand that polarizes T-cell differentiation towards type 17 T helper (TH17) cells, which are associated with autoimmunity.

J. Immunol. 185, 1927–1934 (2010).



J. Exp. Med. 2013 Vol. 210 No. 12 2569

Genes ➔ T cell function: HLA-DR4+ & T cell dysfunction

Telomere damage and abnormal T cell differentiation
in healthy HLA-DR4+ individuals *and*
asymptomatic first-degree relatives of patients with RA

Genes → T cell function: HLA-DR4+ & T cell dysfunction

HLA-DRB1 alleles are associated to T-cell senescence

Premature telomeric loss in rheumatoid arthritis is genetically determined and involves both myeloid and lymphoid cell lineages

Stefan O. Schönland[†], Consuelo Lopez[†‡], Thomas Widmann[†‡], Julia Zimmer[†], Ewa Bryl[†], Jörg J. Goronzy[†§], and Cornelia M. Weyand[†§¶]

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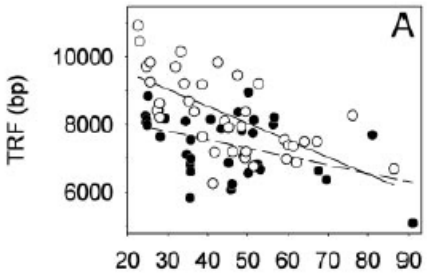
HLA-DR4 Affects Telomeric Length in CD4 T Cells

HLA-DR4^{+} (●) HLA-DR4^{-} (○)



PNAS 2003 Nov 11;100(23):13471-6.

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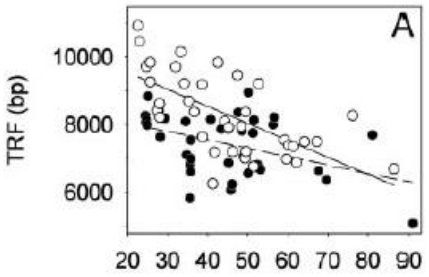
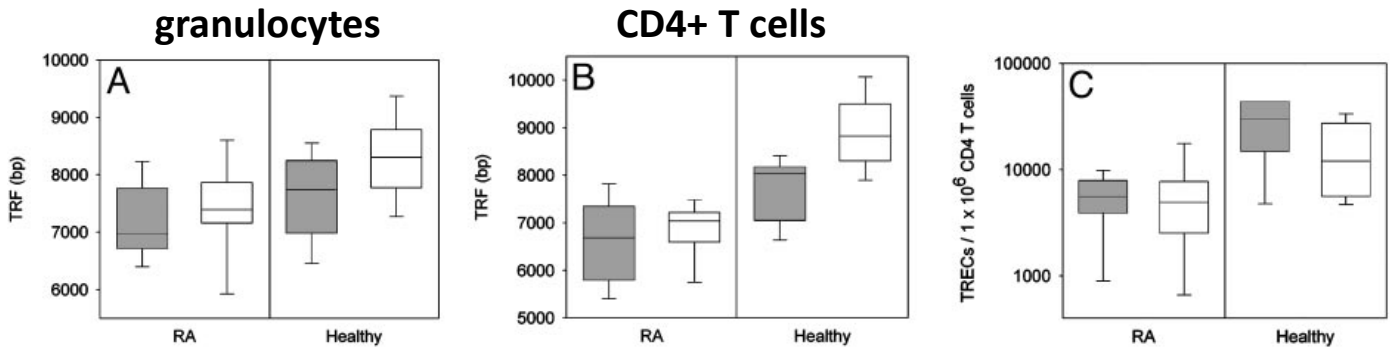


Premature telomeric erosion in lymphocytes and granulocytes and decreased frequencies of TREC T cells in patients with RA.

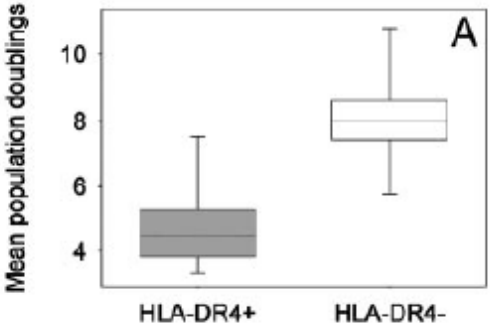


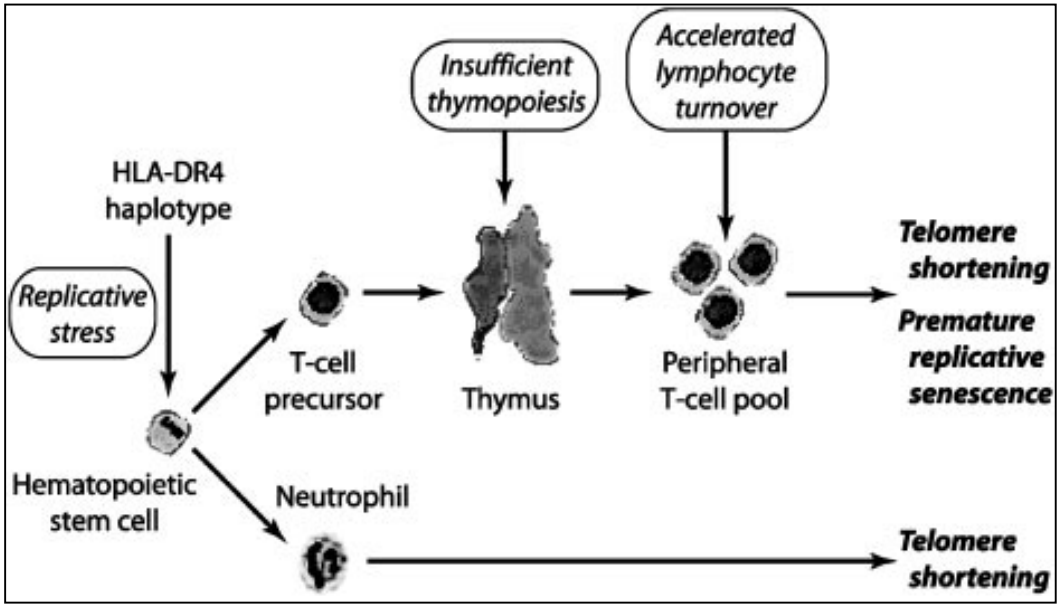
HLA-DR4$^-$ (white) and HLA-DR4$^+$ (gray)

PNAS 2003 Nov 11;100(23):13471-6.

HLA-DRB1*04 alleles or genes in linkage disequilibrium regulate stem cell replication and contribute to the **accumulation of senescent and autoreactive T cells in rheumatoid arthritis.**

Telomere Shortening in HLA-DR4 T Cells Correlates with Functional Markers of Cellular Senescence.





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Context dependent mechanisms

If not in the joints where autoimmunity starts?

Context dependent mechanisms

If not in the joints where autoimmunity starts?

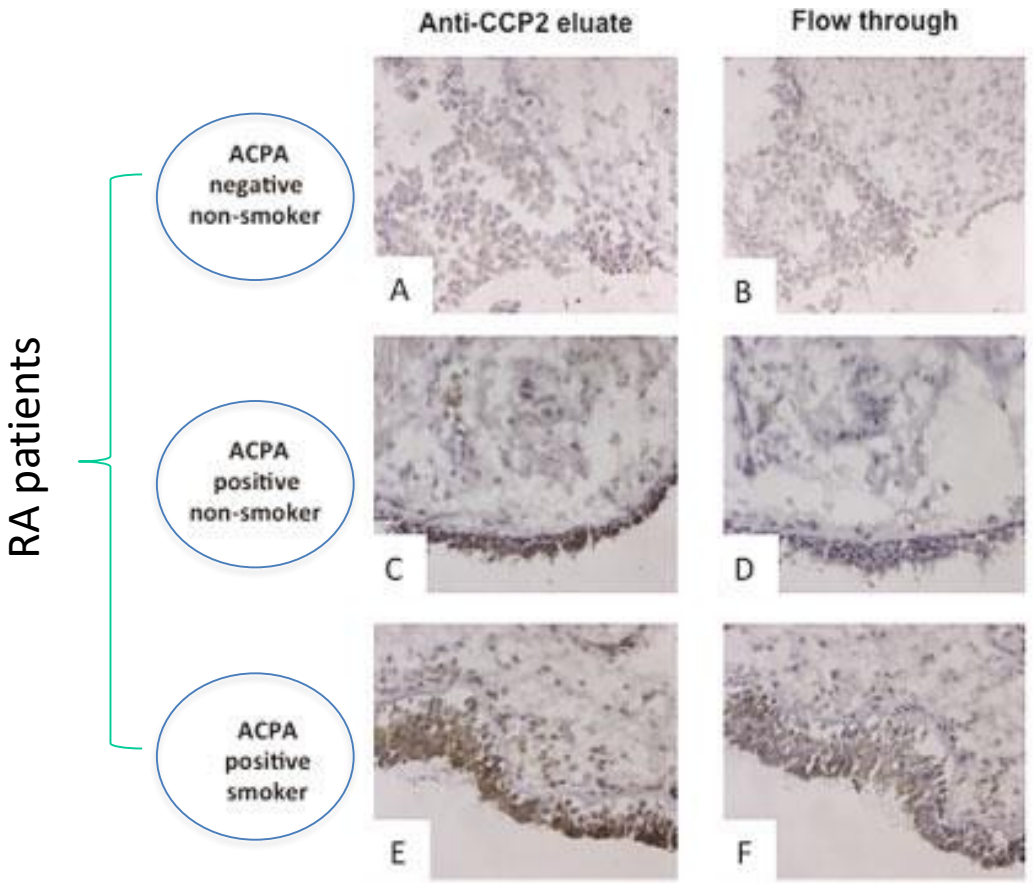
- **Mucosal tissue sites** and **neutrophil extracellular traps** may provide relevant contextual signals through which **citrullinated antigens** gain immunogenicity, supporting that early steps of tolerance loss may occur in the lung and the gut.

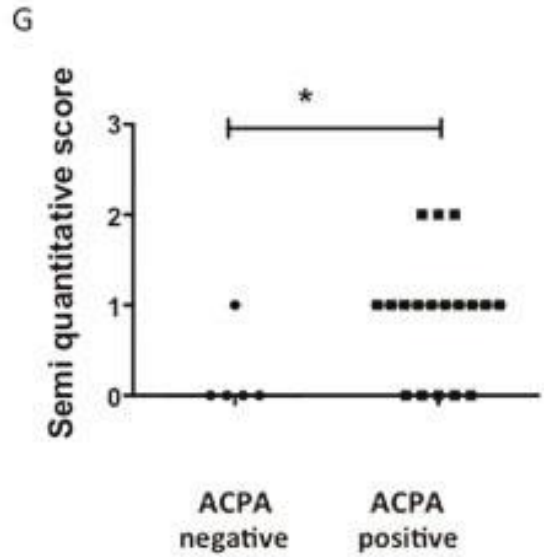
- Nevertheless, how tolerance to the modified peptides is broken remains unclear, and a common denominator is not clear

Context dependent mechanisms

Lung as the site of citrullination and initial ACPA development

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



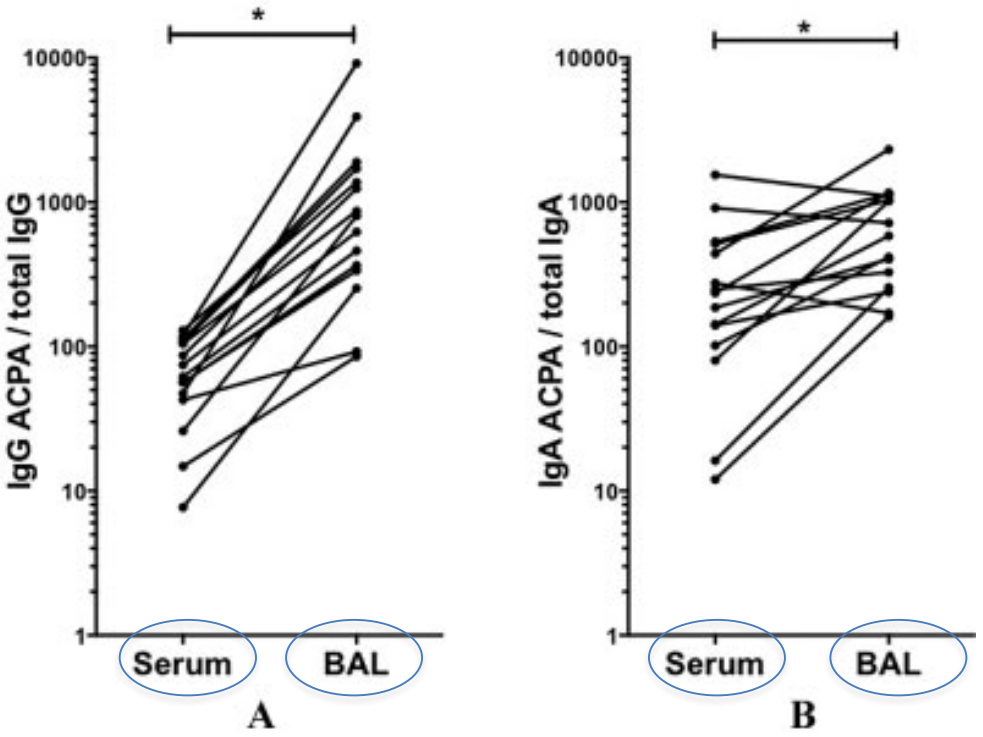


Reynisdottir. A&R 66 January 2014, pp 31

Context dependent mechanisms

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



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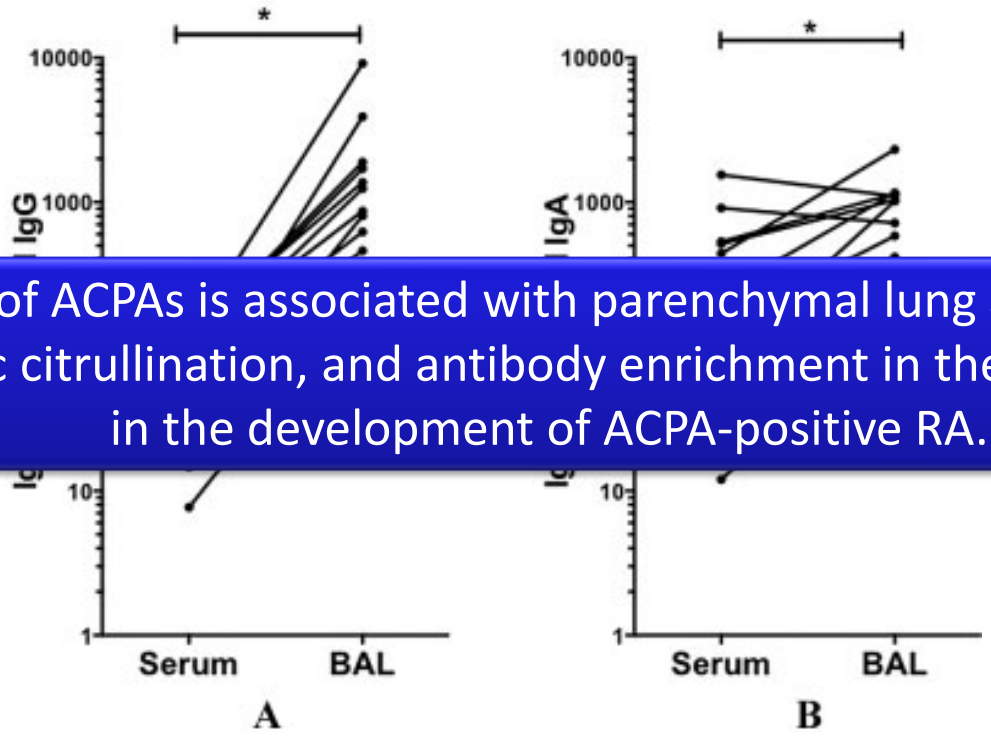


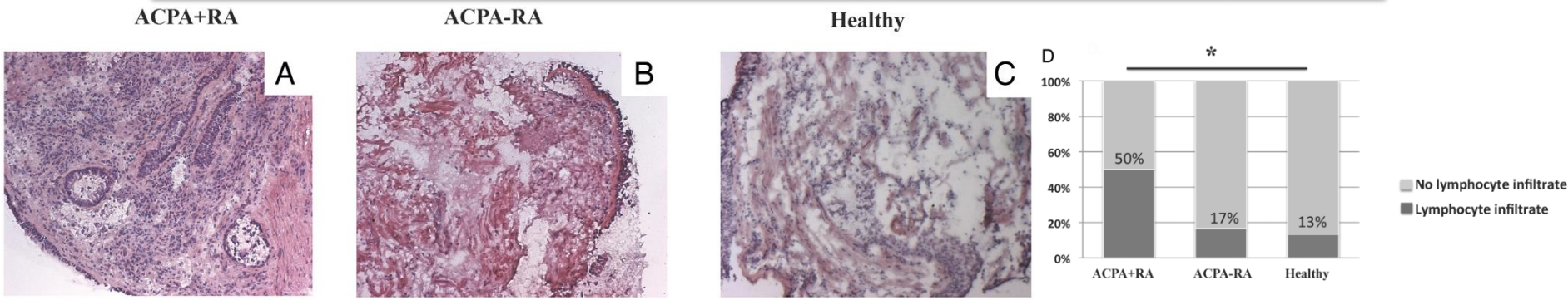
The presence of ACPAs is associated with parenchymal lung abnormalities, site-specific citrullination, and antibody enrichment in the lungs early in the development of ACPA-positive RA.

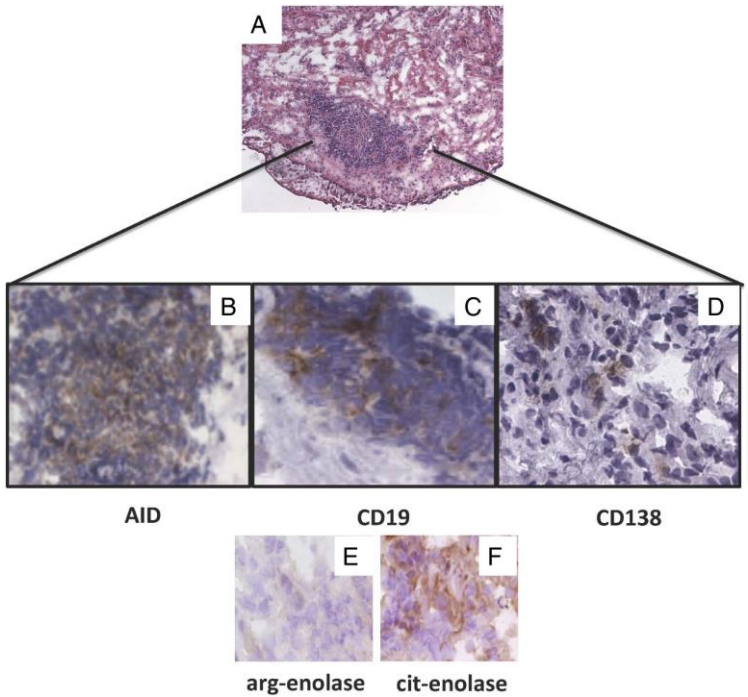
Reynisdottir. A&R 66 January 2014, pp 31

Context dependent mechanisms

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

Lymphocytic infiltration in the bronchial biopsies of patients with ACPA-positive untreated early rheumatoid arthritis





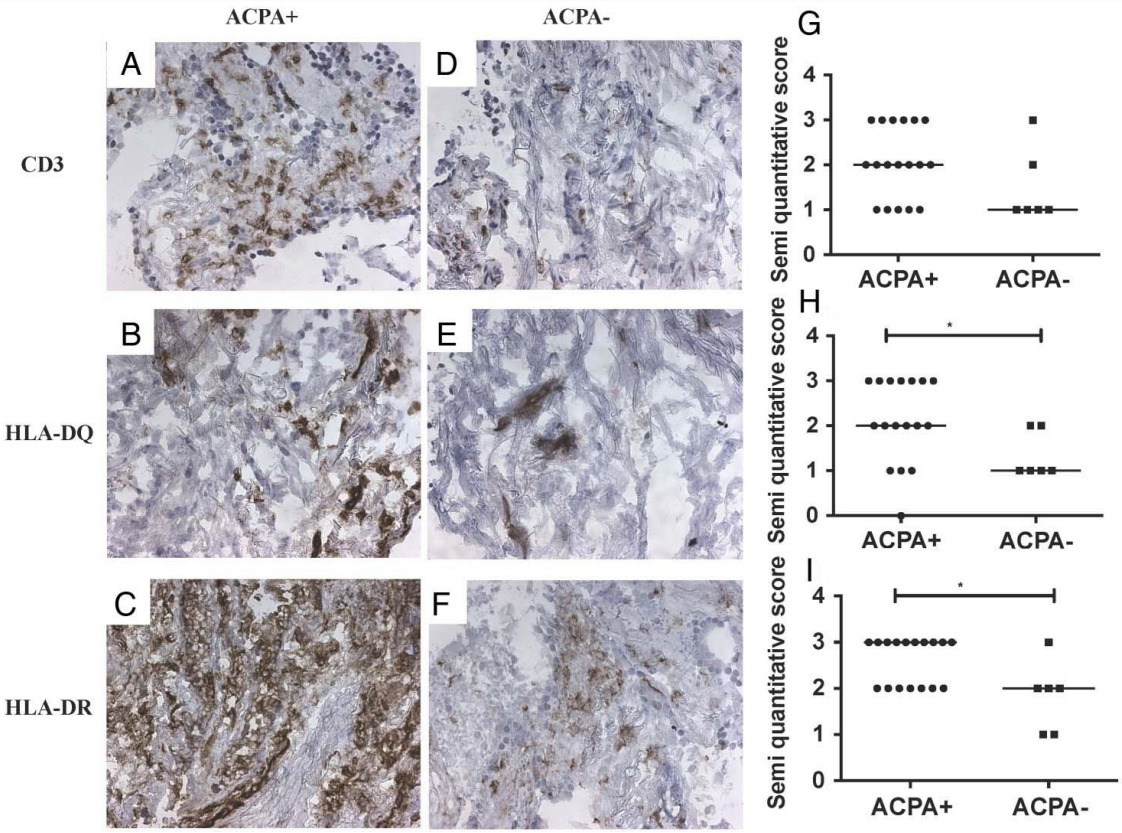
Germinal centre-like structures are present in bronchial biopsies

Reynisdottir. Ann Rheum Dis 2016;75:1722

Context dependent mechanisms

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 RA



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Context dependent mechanisms

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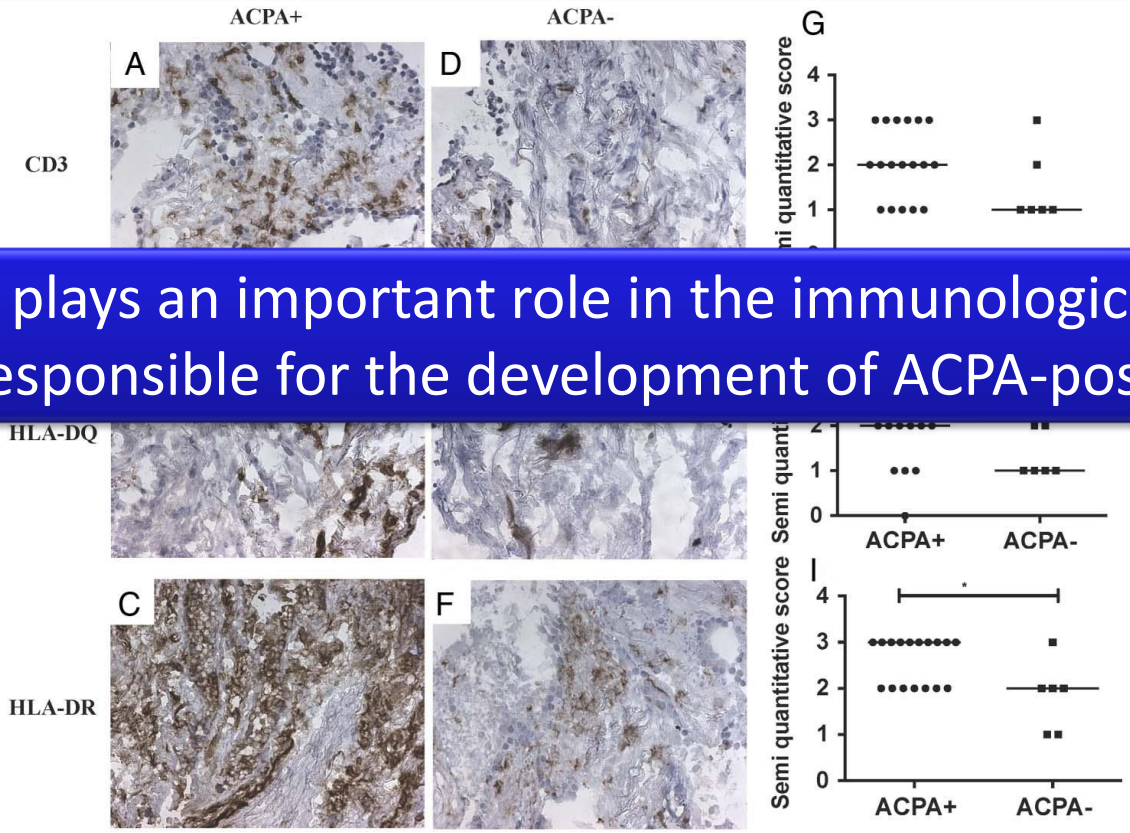


Lung plays an important role in the immunological reactions responsible for the development of ACPA-positive RA

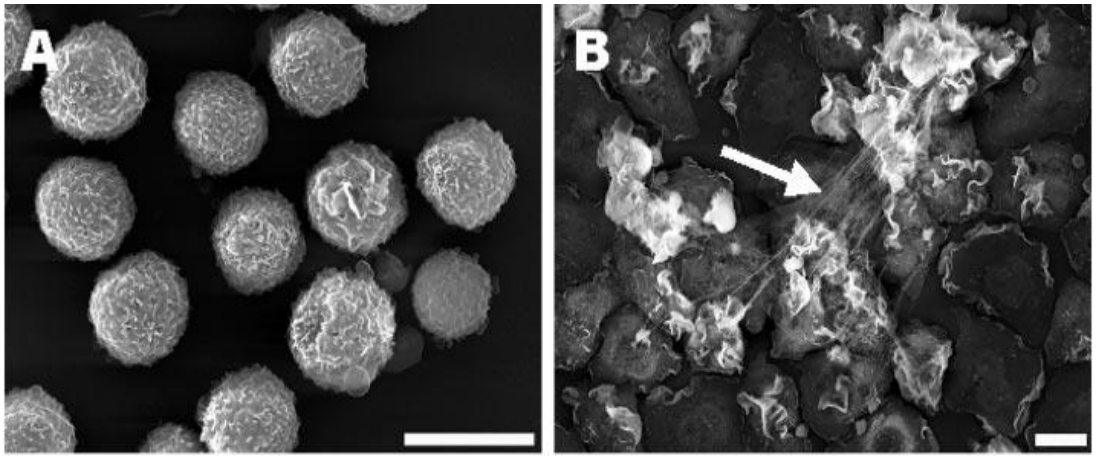
Reynisdottir. Ann Rheum Dis 2016;75:1722

Context dependent mechanisms

Neutrophil Extracellular Traps

- ✓ web-like structures, composed of decondensed chromatin complexed with >30 different neutrophil proteins that can capture, neutralize, and kill a variety of microbes.

Resting neutrophils PMA-stimulated

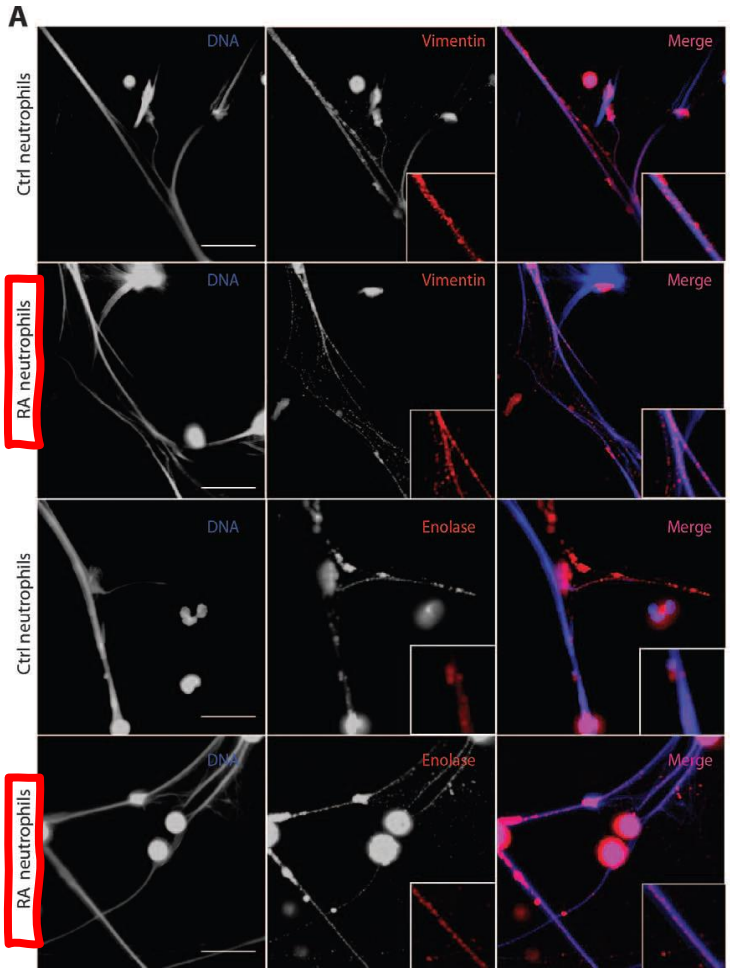


Brinkmann et al., Science 2004

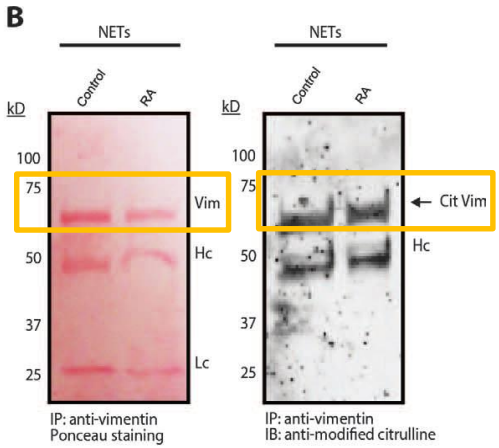
Context dependent mechanisms

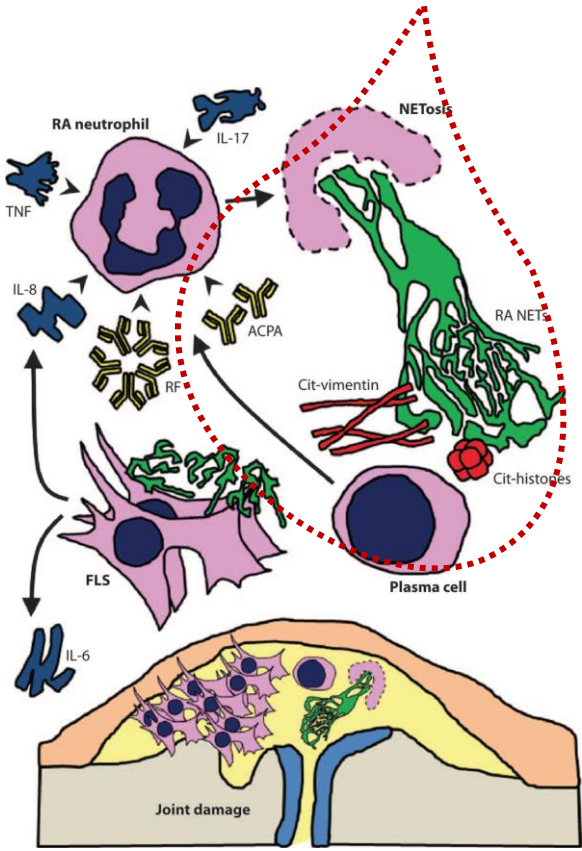
NETs externalize citrullinated autoantigens involved in RA pathogenesis: **Citrullinated vimentin**

- Vimentin and a-enolase decorate control and RA NETs.



- Vimentin externalized in control and RA NETs is citrullinated.





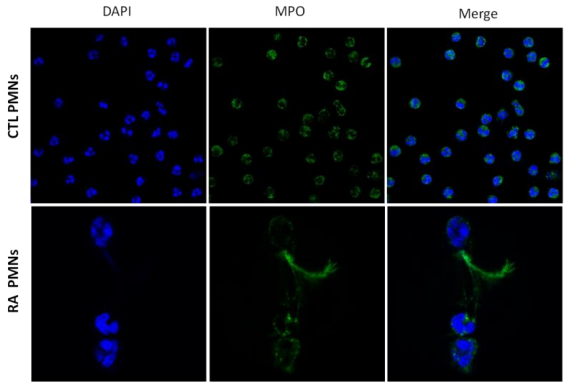
Sci Transl Med 5, 178ra40 (2013)

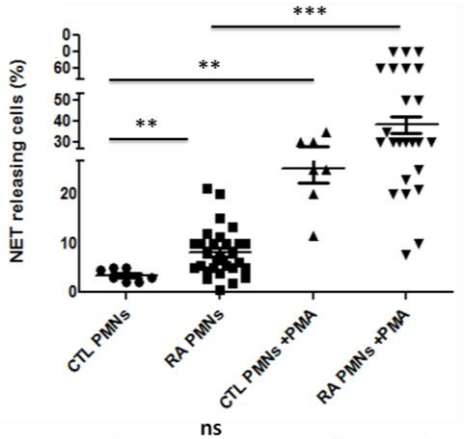
Increased spontaneous NET release of RA PB-neutrophils

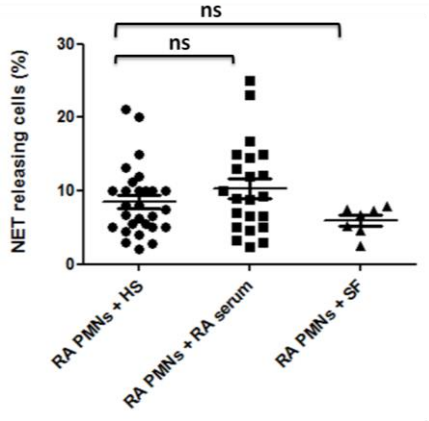
Research Article

Neutrophil extracellular traps exacerbate Th1-mediated autoimmune responses in rheumatoid arthritis by promoting DC maturation

*Garyfalia Papadaki[1,2], Konstantinos Kambas[3], Christiana Choulaki[1,2], Katerina Vlachou[1,2,4], Elias Drakos[5], George Bertsias[1,2], Konstantinos Ritis[3], Dimitrios T. Boumpas[1,4,6,7], Paul R. Thompson[8], Panayotis Verginis[*4] and Prodromos Sidiropoulos[2]*

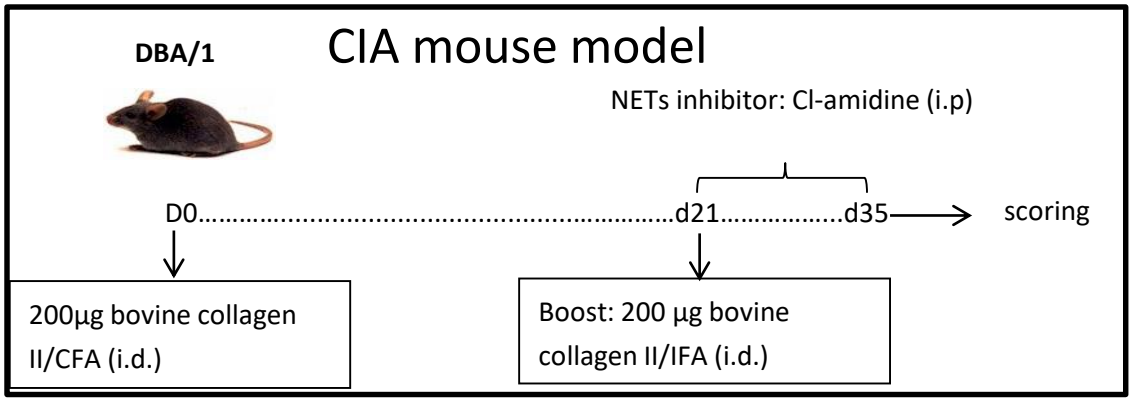


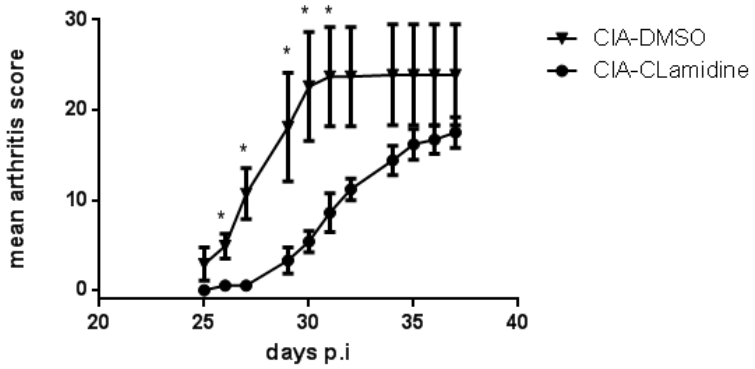


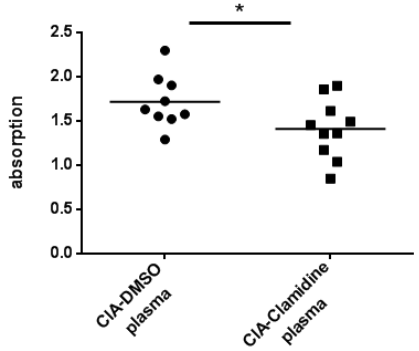


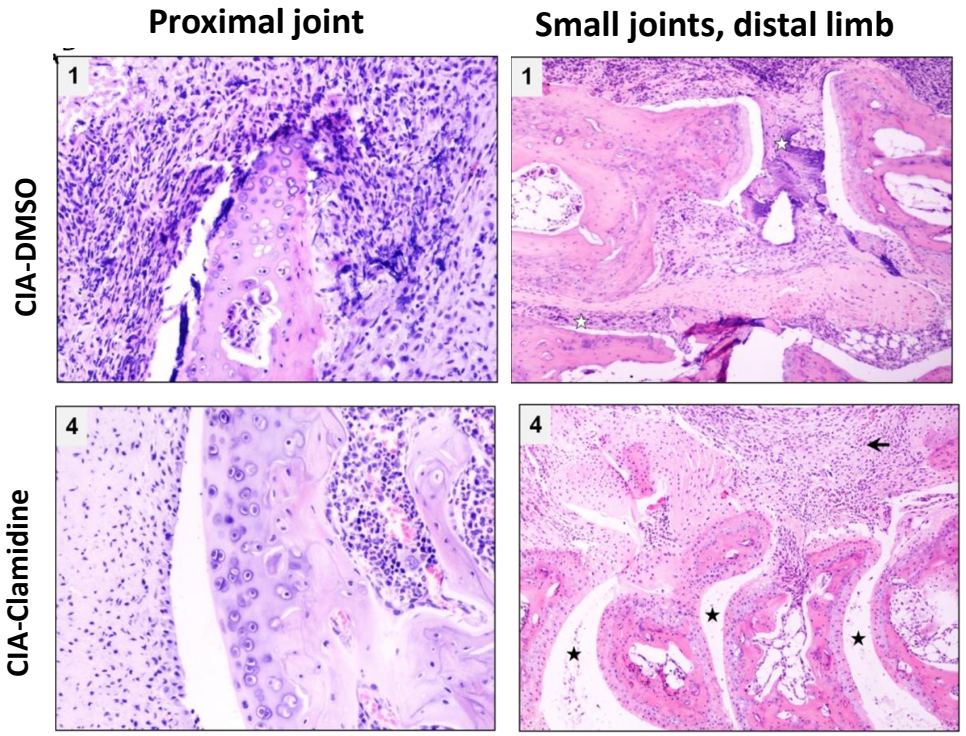
Papadaki. Eur J Immunol. 2016:2542

NETs inhibition attenuates CIA severity and anti-collagen antibodies





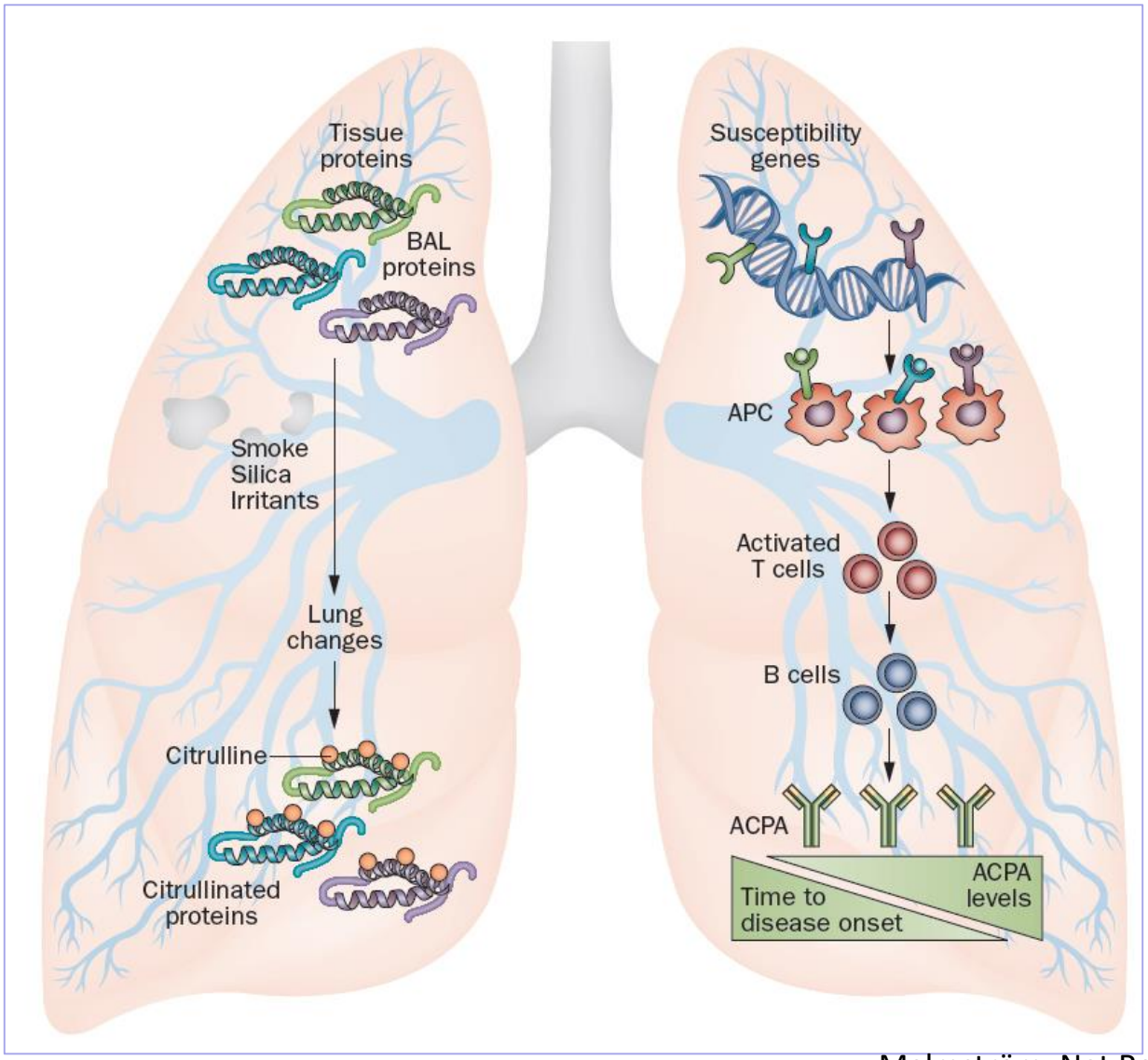




Papadaki. Eur J Immunol. 2016:2542

Context dependent mechanisms

Initiation of RA-associated immunity against citrullinated proteins in the lungs.



Malmström, Nat Rev Rheumatol. 2014:645

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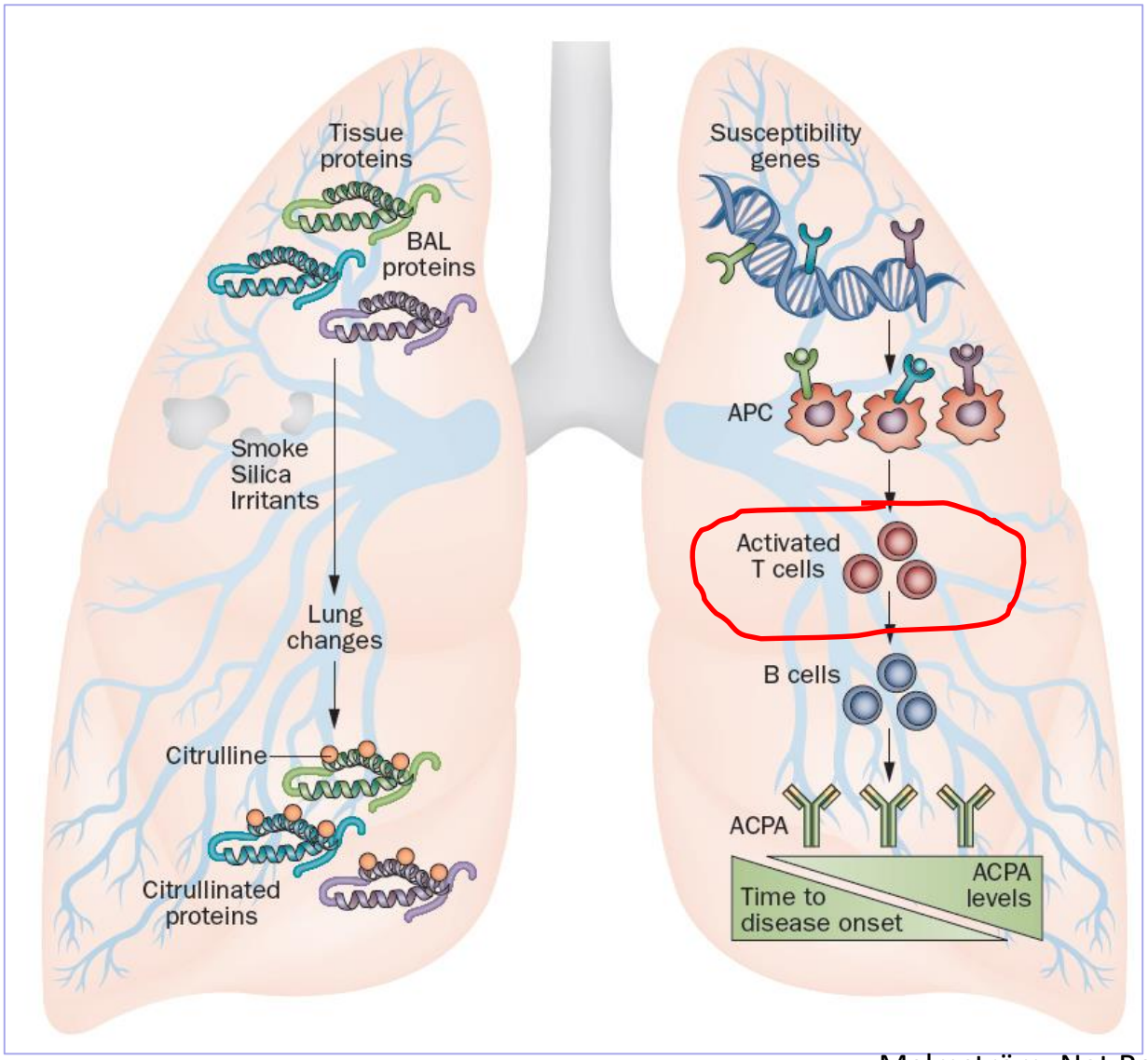
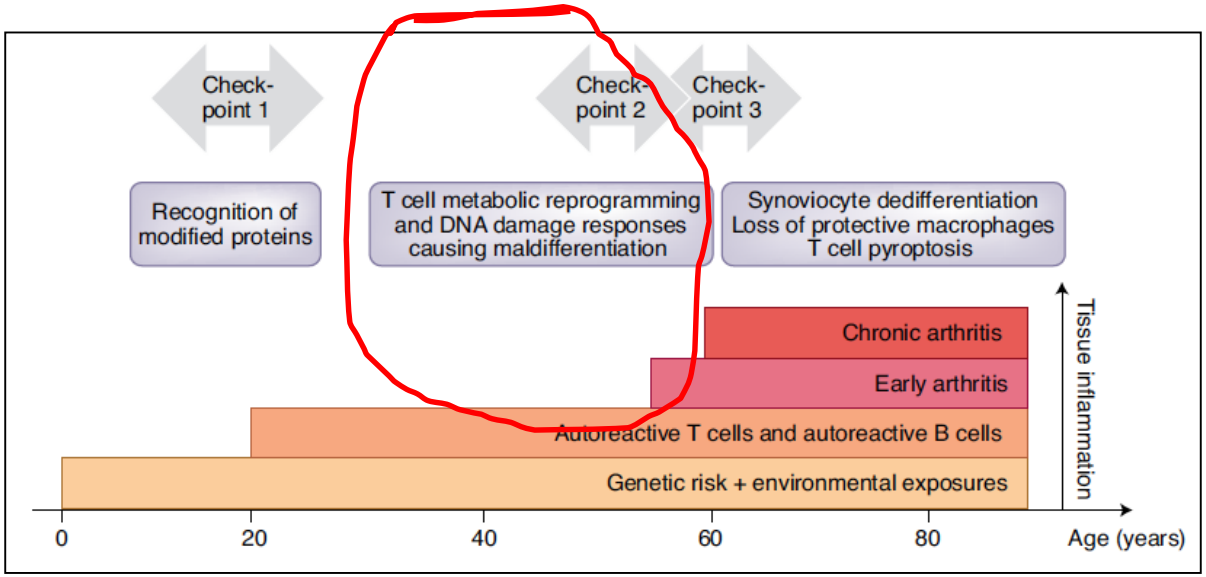


Malmström, Nat Rev Rheumatol. 2014:645

General concept for RA pathogenesis

"RA is an almost lifelong process in with distinct phases:

- ✓ **disease risk:** genetically predisposed individuals lose self-tolerance, produce autoantibodies.
- ✓ **asymptomatic autoimmunity:** characterized by prototypic autoantibodies reactive against post-translationally modified proteins, often citrullinated antigens.
- ✓ **symptomatic synovitis:** Acute joint inflammation transitions into chronic, destructive synovitis. Tissue responds with a maladaptive wound healing response (pannus), which by itself has destructive features and will lead to irreversible tissue injury"



Nat Immunol. 2021 Jan;22(1):10-18

Immune factors contributing to synovial localization of inflammation

"The onset of synovial inflammation, is closely linked to cell-intrinsic defects in CD4+ T cells and is functionally caused by a mis-differentiation step during the conversion of naive resting CD4+ T cells into memory and effector T cells"

- ✓ **CD3+ T** cells are present in most **early synovitis** cases and the **histologic phenotype** of synovial biopsy samples **predicts** disease persistence and severity.

  Ann. Rheum. Dis. 78, 761–772 (2019). Cell Rep. 28,2455–2470.e5 (2019)

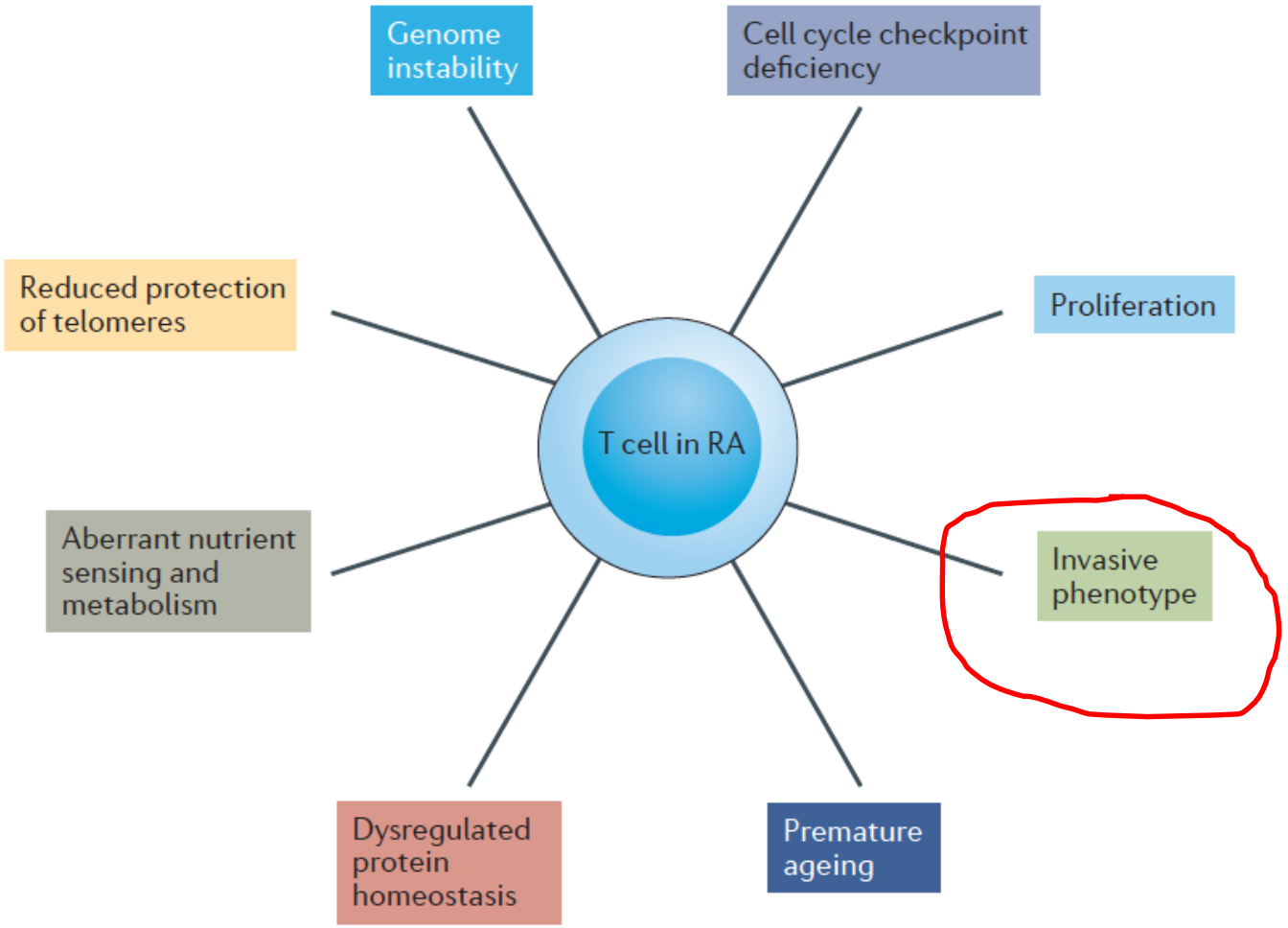
- ✓ **Decreased frequency of naïve CD4+ T** cells is the strongest predictor for the progression from **ACPA positivity to synovitis**.

  Sci. Rep. 10, 3669 (2020)

- ✓ **Differential methylation** patterns of naïve CD4+ T cells characterize the **earliest stages** of joint inflammation in patients who were drug naïve

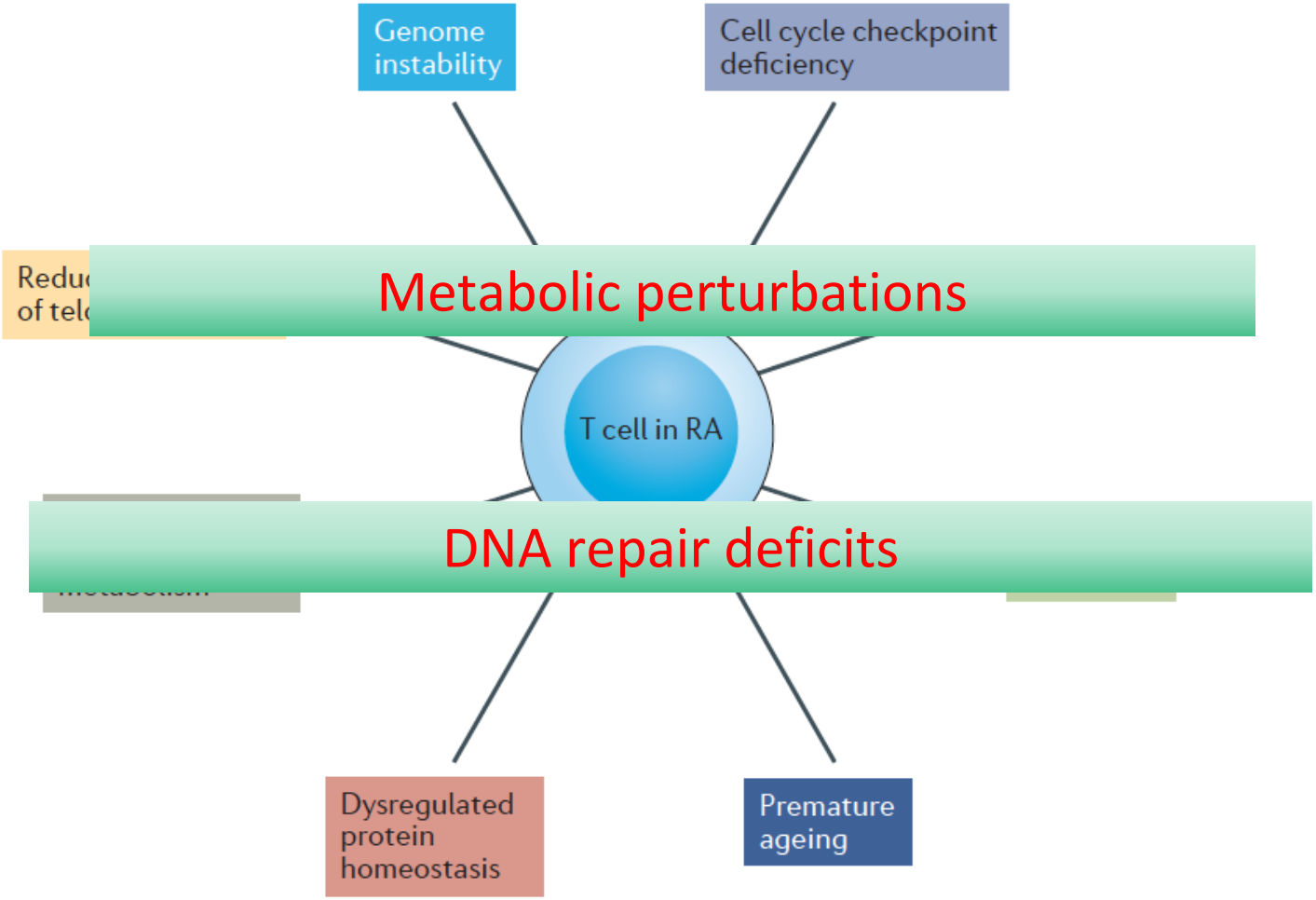
  Clin. Epigenetics 12,54 (2020)

Hallmarks of T cells in rheumatoid arthritis



Nat Rev Rheumatol. 2017 May;13(5):291-301

Hallmarks of T cells in rheumatoid arthritis



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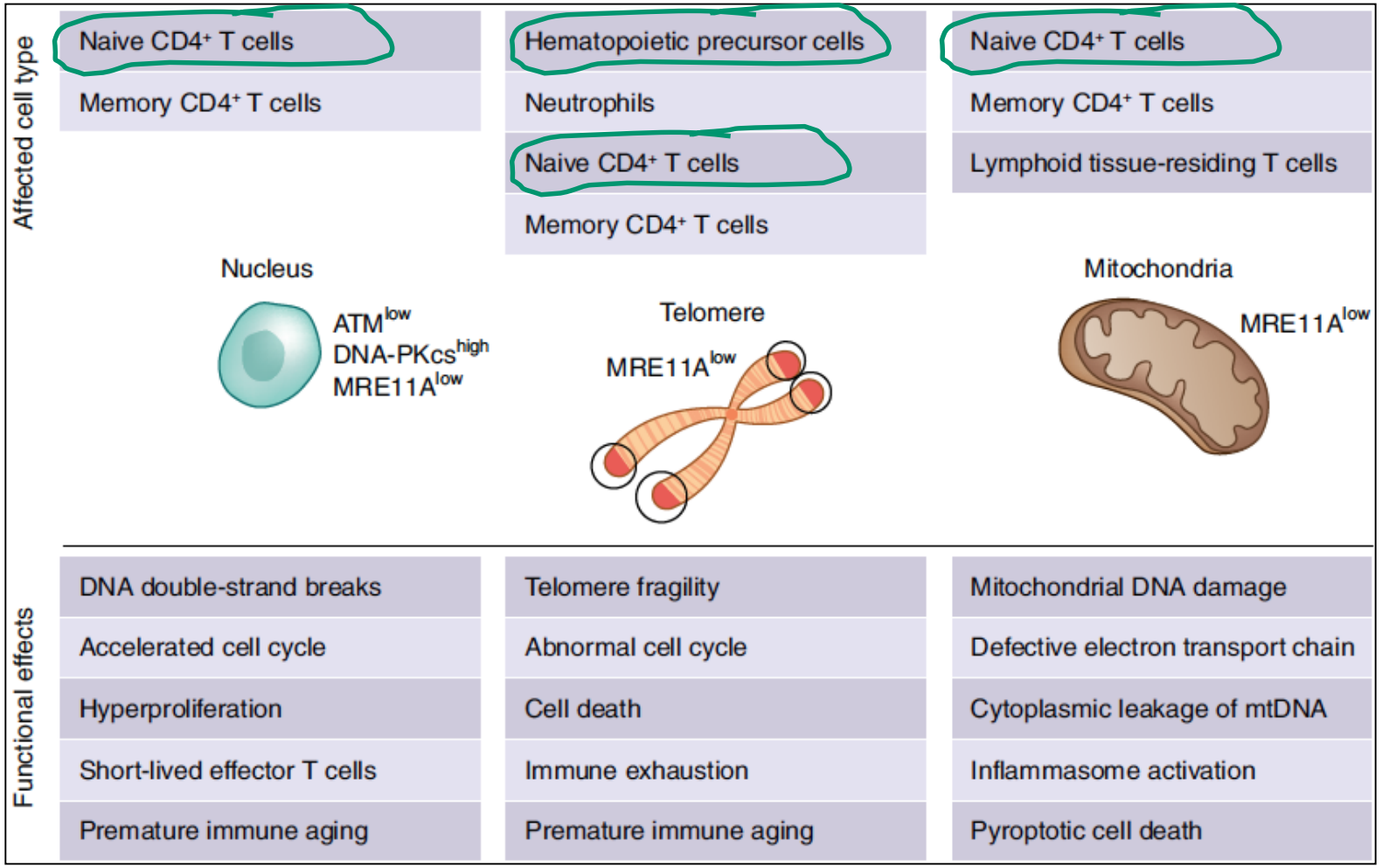
DNA repair deficits in rheumatoid arthritis



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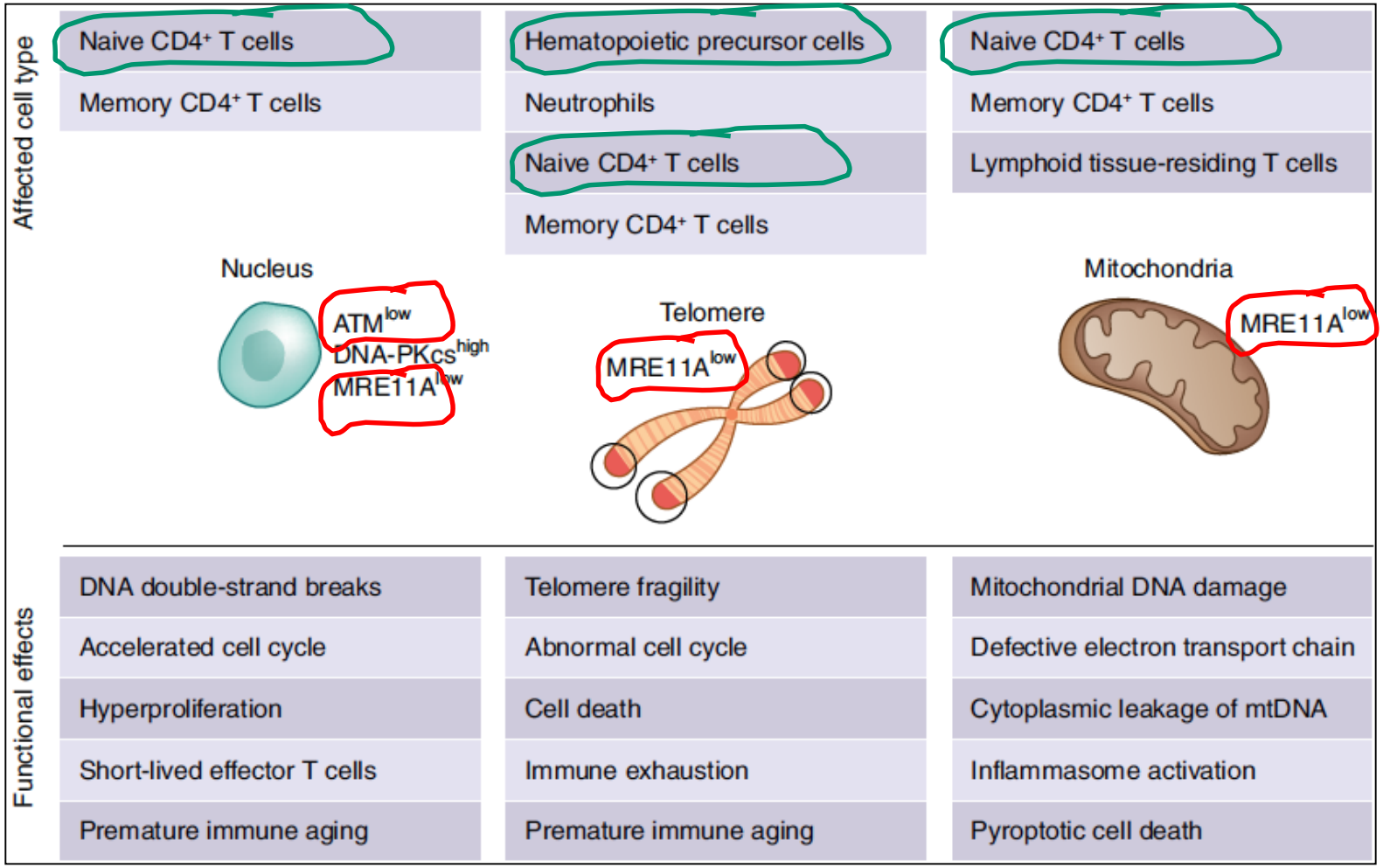

Nat Immunol. 2021 Jan;22(1):10-18

RA CD4 have increased DNA damage and fail to mobilize DNA repair mechanism.

ATM is in the apex of the pathway

J Exp Med. 2009 Jun 8;206(6):1435-49

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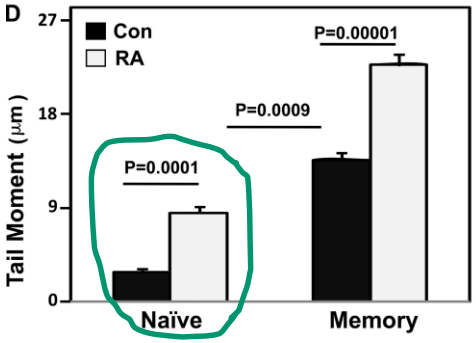
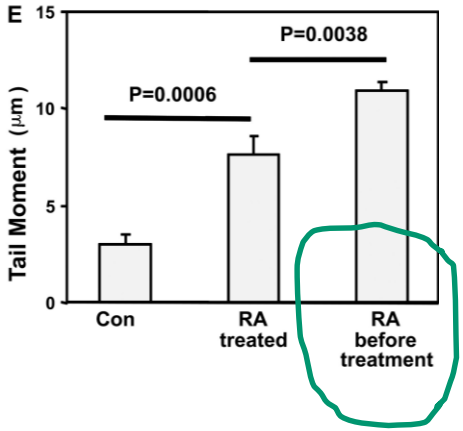
Accumulated DNA damage in CD4 T cells in RA.

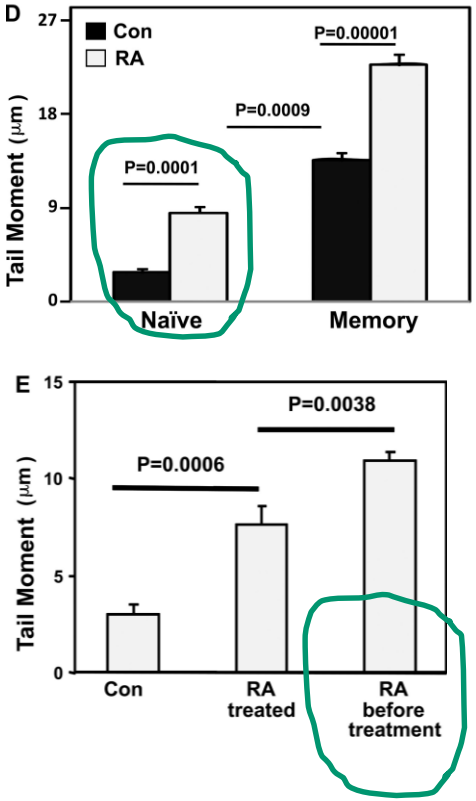




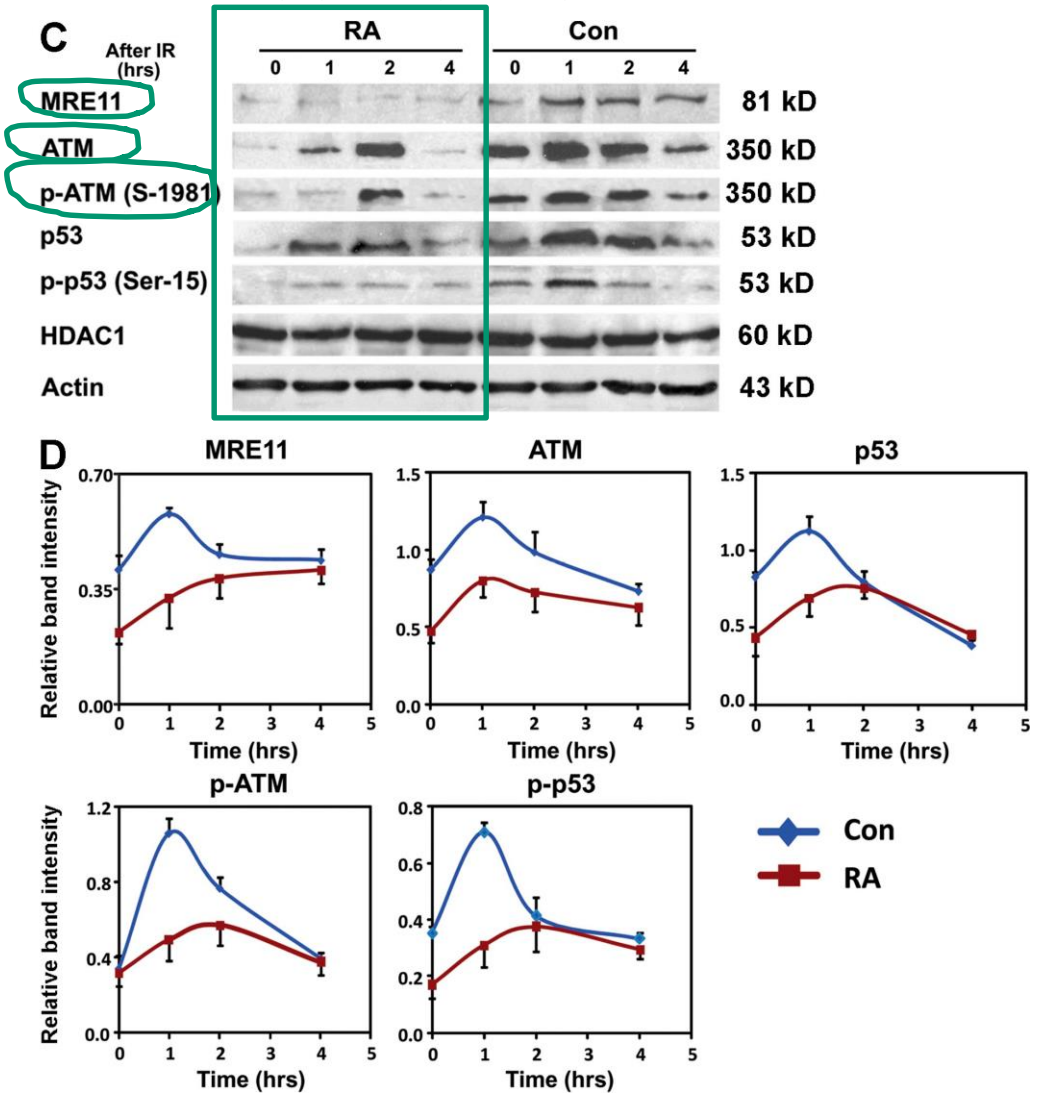
J Exp Med. 2009 Jun 8;206(6):1435-49

ATM is in the apex of the pathway of impaired DNA damage in RA

Accumulated DNA damage in CD4 T cells in RA.



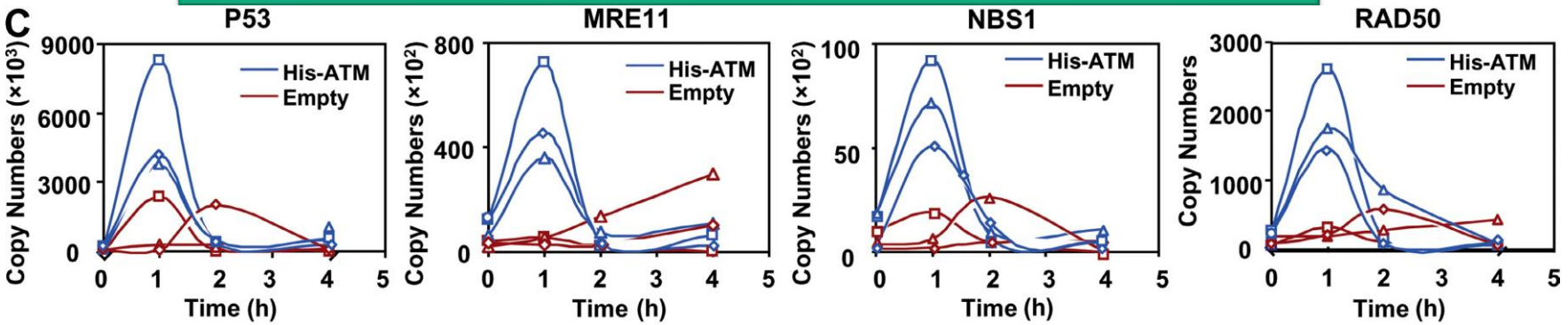
Irradiation-induced up-regulation of the DNA damage pathway is delayed in RA T cells.

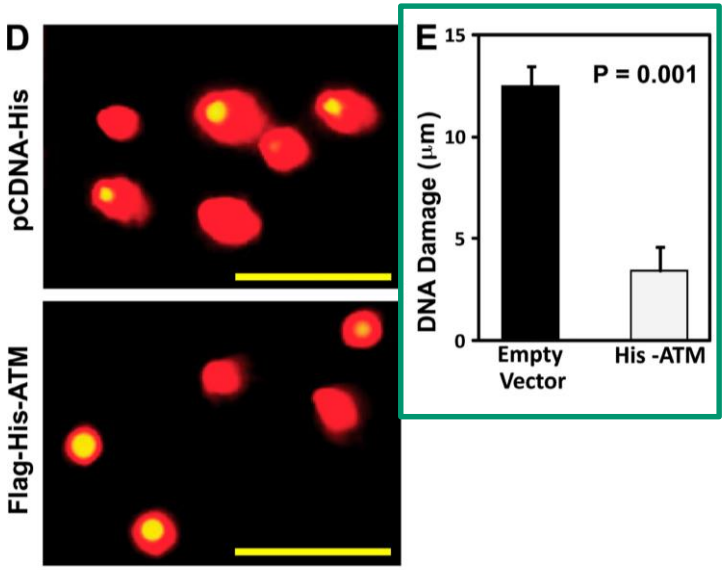


J Exp Med. 2009 Jun 8;206(6):1435-49

ATM is in the apex of the pathway of impaired DNA damage in RA

Overexpression of ATM restores the defect in DNA damage repair and in apoptosis sensitivity in RA CD4 T cells





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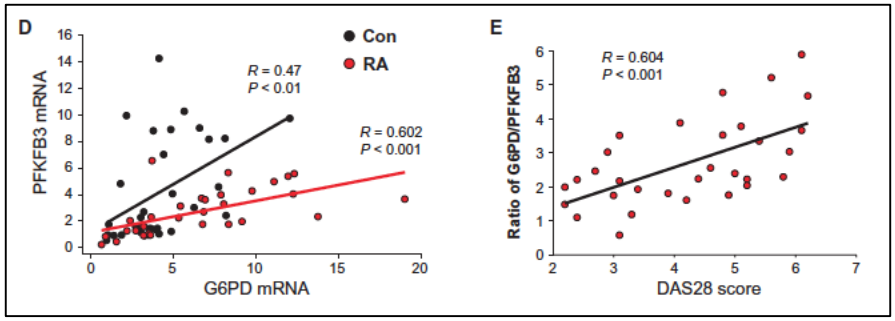
In naïve RA CD4 **Glucose shunting toward the PPP** results in ↑ of NADPH and reduced glutathione and **loss of ROS**

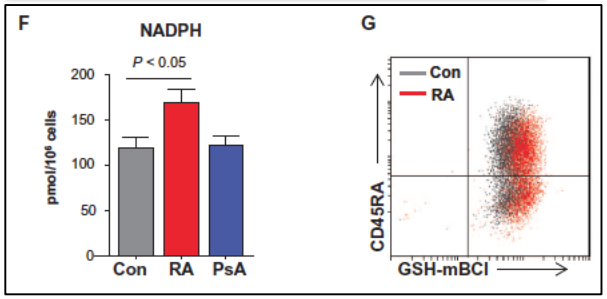
Sci Transl Med. 2016 Mar 23;8(331):331ra38

In naïve RA CD4 **Glucose shunting toward the PPP** results in ↑ of NADPH and ↑ reduced glutathione and **loss of ROS**

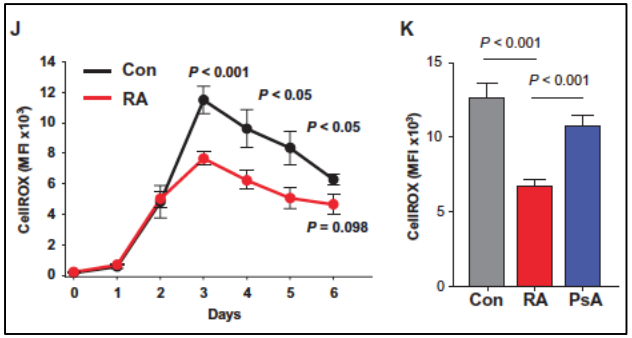
RA naïve CD4 Glucose shunting to PPP: ratio of G6PD/PFKFB3 is shifted toward G6PD



Accumulation of NADPH and reduced glutathione



ROS levels in RA T cells were significantly reduced Kinetics of ROS generation mirrored glycolytic activity

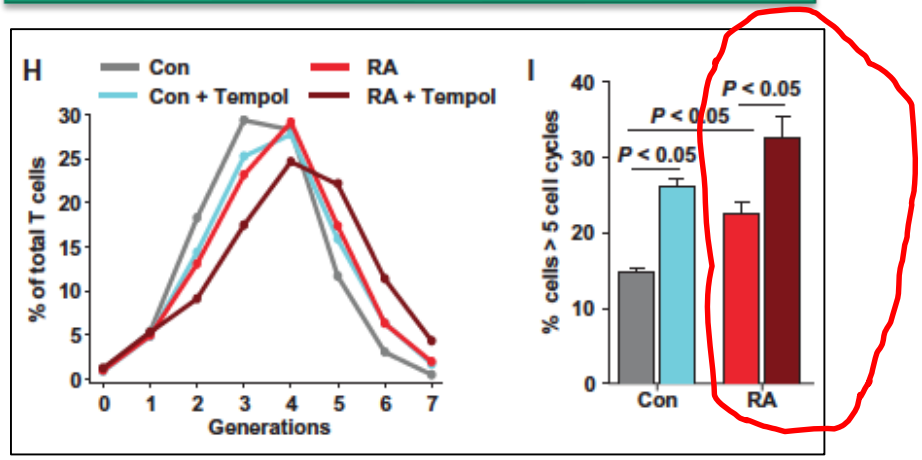


Sci Transl Med. 2016 Mar 23;8(331):331ra38

Intracellular **ROS regulates cell cycle progression**, proliferative efficiency, and **naïve-to-memory conversion.**

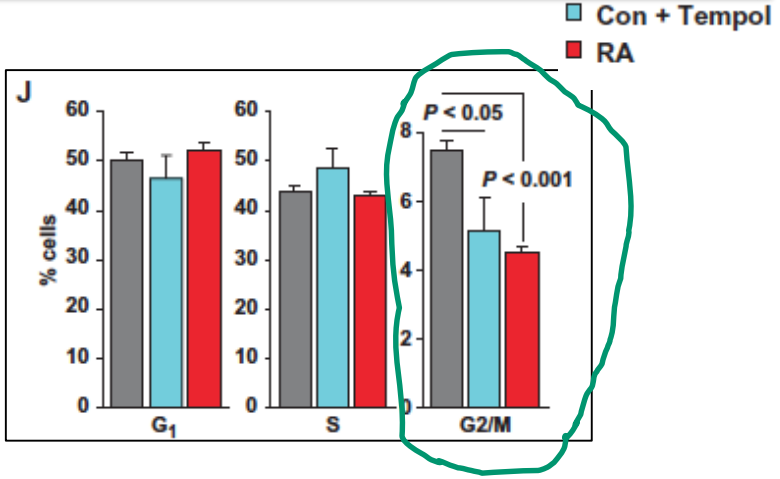
ROS-deficient RA T cells hyperproliferate, fail to maintain the naïve phenotype, and bypass the G2/M cell cycle checkpoint.

- ✓ In RA T cells, **proliferation is spontaneously higher** and further increased by **ROS scavenging**.
- ✓ Proliferative rates in Tempol-treated wild-type T cells is similar to untreated RA T cells.



ROS regulate G2/M transition:

- ✓ RA T cells effectively bypassed the G2/M checkpoint

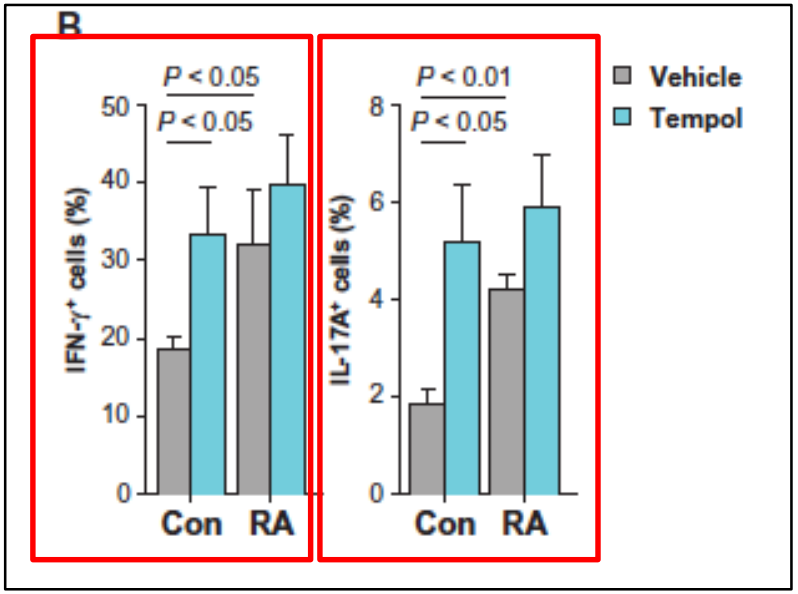


Sci Transl Med. 2016 Mar 23;8(331):331ra38

RA naive CD4 cells upon stimulation express **lower ATM** and have a differentiation skewing towards TH1 & TH17.

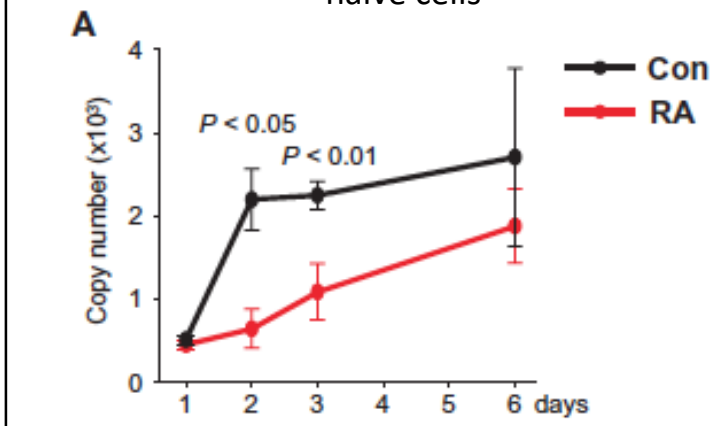
Low ROS mimics RA

- ✓ RA naïve CD4 are prone to differentiate to TH1 & TH17
- ✓ ROS scavenging mimics RA differentiation skewing



In RA T cells, ATM transcripts responded slower vs controls upon TCR stimulation

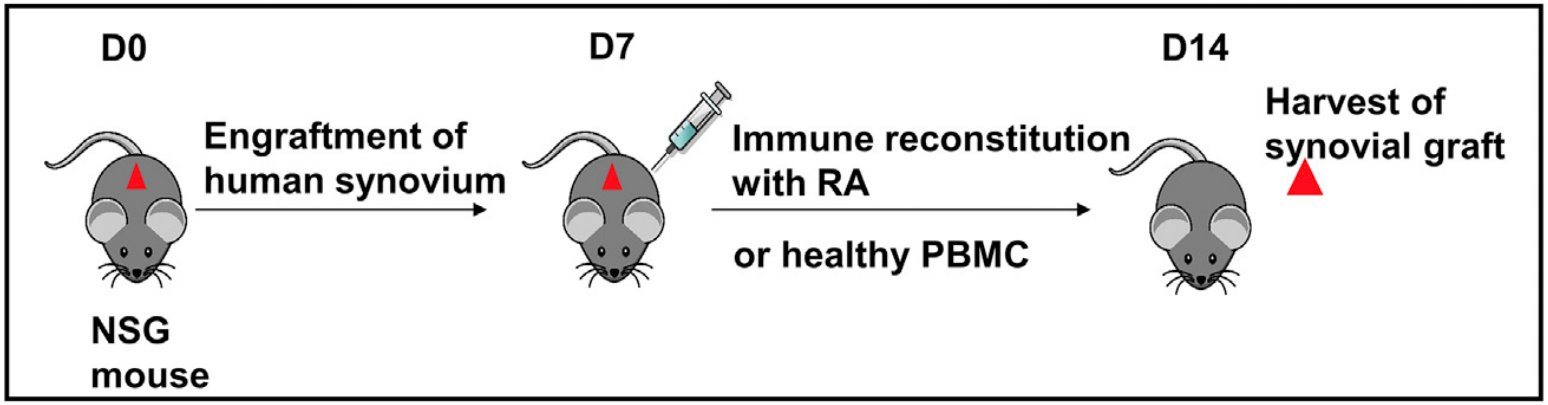
ATM transcripts upon TCR stimulation of CD4 naïve cells



Sci Transl Med. 2016 Mar 23;8(331):331ra38

Human synovial tissue–NSG chimeras

Synovial tissue engrafted into NSG mice which were reconstituted with peripheral blood mononuclear cells (PMBCs). Synovial grafts were explanted 7 to 14 days later



NSG severely immunodeficient mice

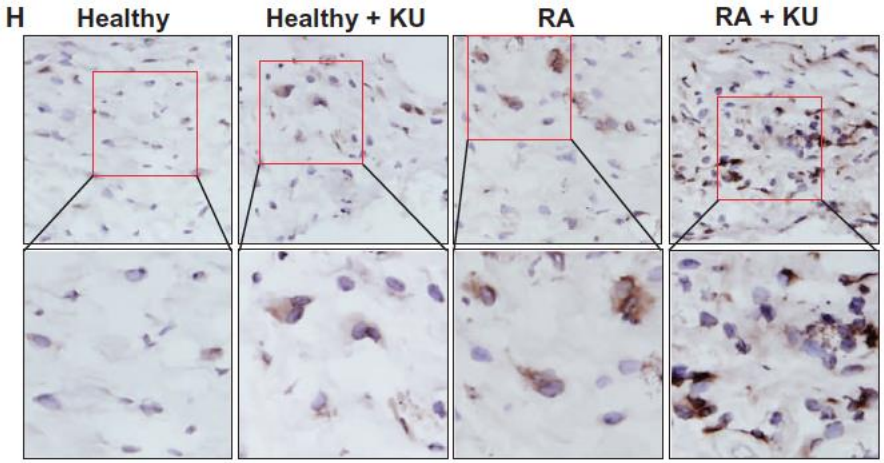
The mice carry two mutations on the NOD/ShiLtJ genetic background

- ✓ Severe combined immune deficiency (*scid*) and
- ✓ Complete null allele of the IL2 receptor common gamma chain (*IL2rg*^{null})

ATM regulates the lineage commitment and the arthritogenic potential of T cells

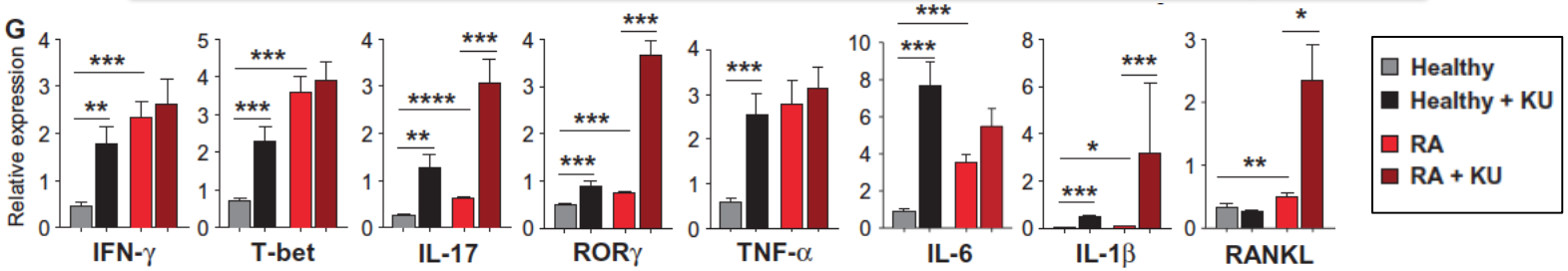
Setting:

- ✓ CD45RO$^+$ depleted PBMCs from healthy individuals or RA patients were adoptively transferred into synovium-engrafted NSG mice.
  - ✓ **Mice were treated with the ATM inhibitor KU-55933**



ATM inhibition increased expression of the **osteoclastogenic ligand RANKL** (brown)

✓ ATM inhibition (KU-55933) strongly up-regulated IL-17, RORg, TNF-a, IL-1b, and IL-6, and expression of the osteoclastogenic ligand RANKL

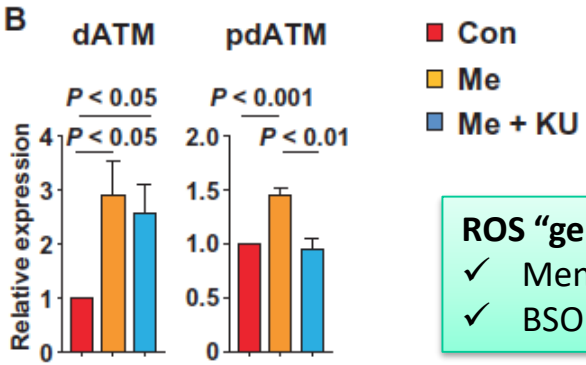


Sci Transl Med. 2016 Mar 23;8(331):331ra38

Replenishing intracellular ROS in RA T cells

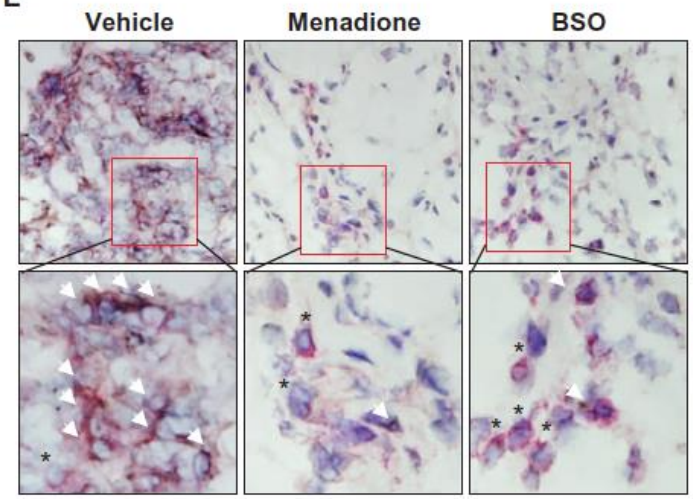
1. **Corrects** ATM insufficiency,
2. **Reverses** T cell mal-differentiation and arthritogenic effector functions

RA CD4 treated with ROS inducers (Menadione) resulted in ATM dimerization and pATM formation



ROS "generators"
- ✓ Menadione (Me)
- ✓ BSO

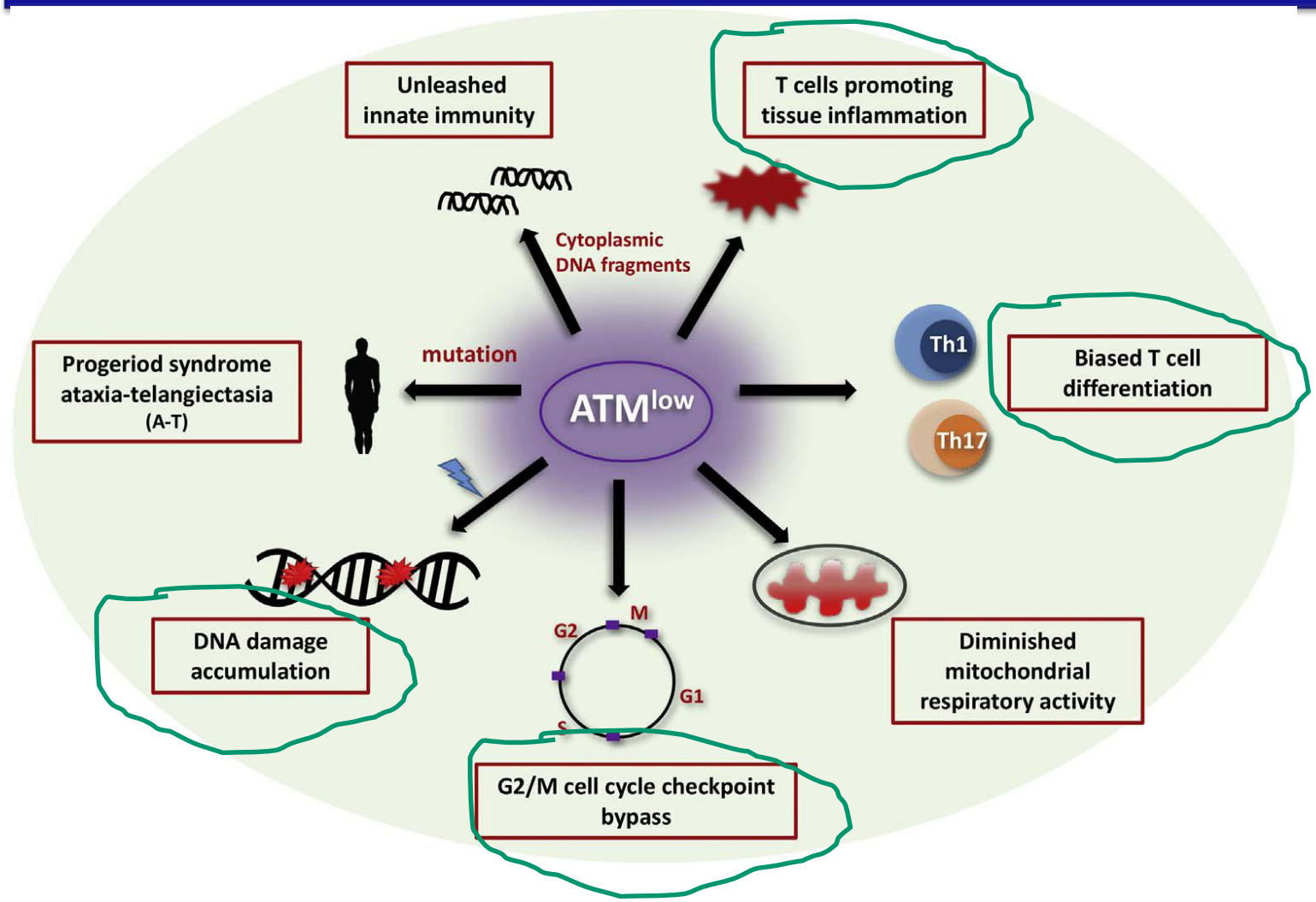
E Human CD3 (pink) and RANKL (brown)



- ✓ Treatment with both ROS inducers had a beneficial effect on synovitis
- ✓ Menadione and BSO reduced RANKL+ CD3+ infiltrates of engrafted synovium

Sci Transl Med. 2016 Mar 23;8(331):331ra38

ATM^{low} T cells in RA patients promote tissue inflammation



Experimental Gerontology (2017)

General concept for RA pathogenesis

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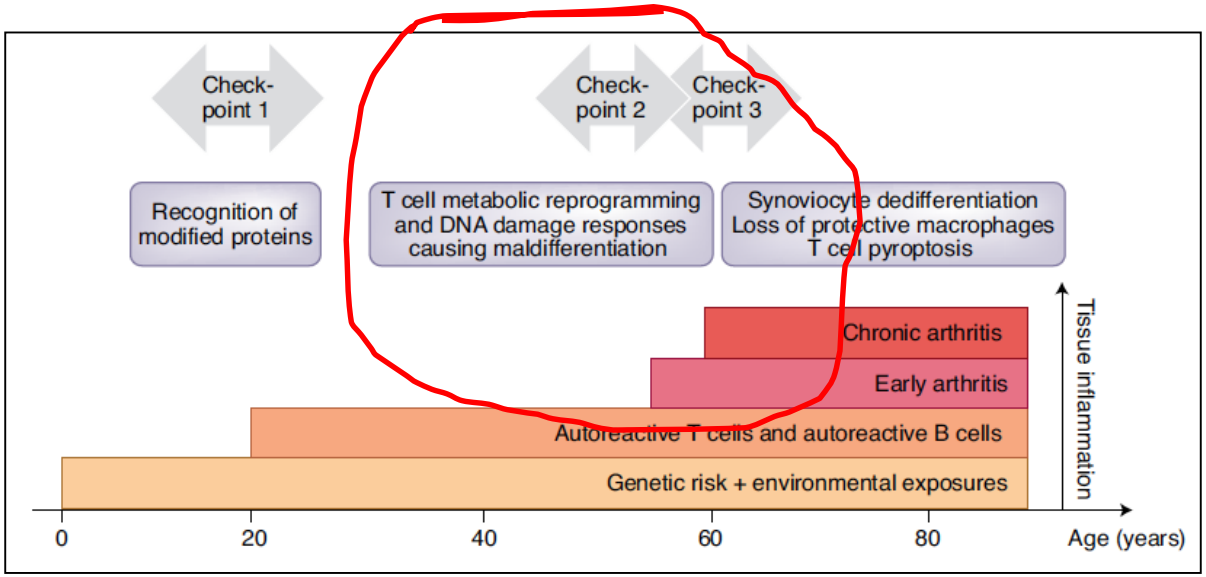


Nat Immunol. 2021 Jan;22(1):10-18

Immunity

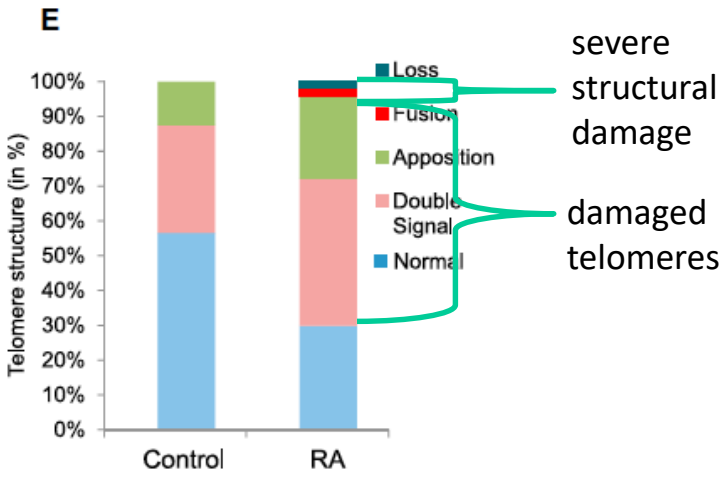
Deficient Activity of the Nuclease MRE11A Induces T Cell Aging and Promotes Arthritogenic Effector Functions in Patients with Rheumatoid Arthritis

Li et al., 2016, Immunity 45, 903–916

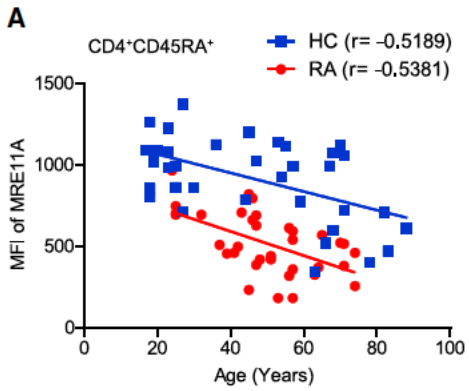
Immunity

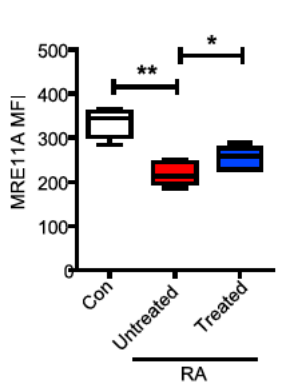
Deficient Activity of the Nuclease MRE11A Induces T Cell Aging and Promotes Arthritogenic Effector Functions in Patients with Rheumatoid Arthritis

✓ RA T cells, age-related telomeric defects manifested not only as shortening, but also as damage accumulation.



✓ Aged RA T cells were low-expressers for the DNA repair nuclease MRE11A irrespective of immunosuppresion



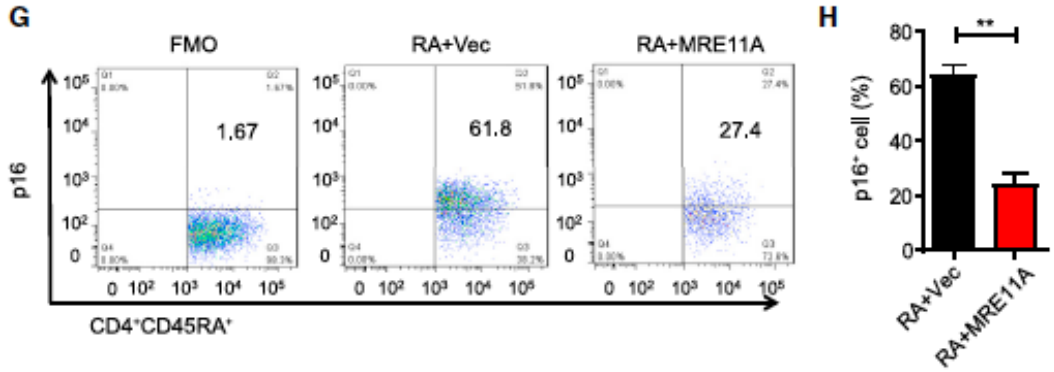


Li et al., 2016, Immunity 45, 903–916

Immunity

Deficient Activity of the Nuclease MRE11A Induces T Cell Aging and Promotes Arthritogenic Effector Functions in Patients with Rheumatoid Arthritis

✓ Restoring MRE11A Expression Repairs Telomeric Damage and Prevents T Cell Aging



Li et al., 2016, Immunity 45, 903–916

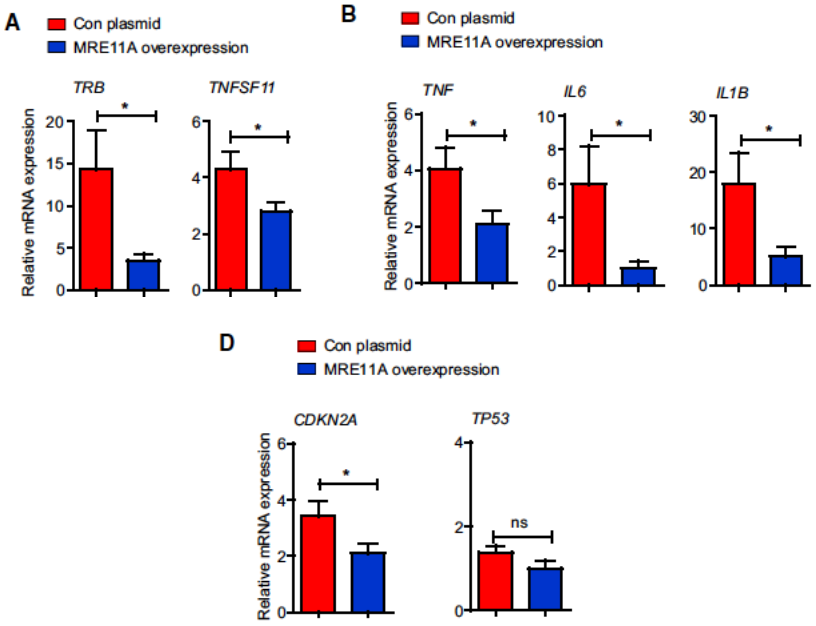
Restoring MRE11A Expression in RA T Cells Prevents Pro-arthritogenic Effector Function

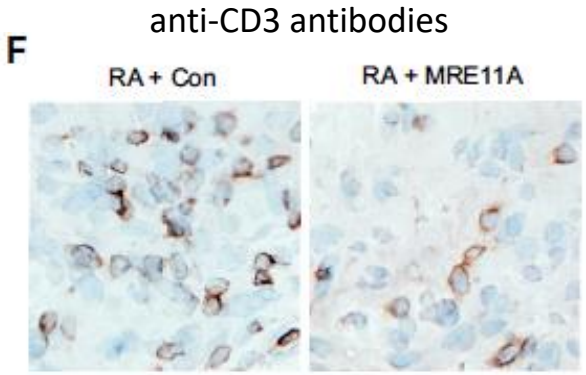
Setting: NSG mice were engrafted with human synovial tissue and adoptively transferred PBMC from RA patients transfected with:

- ✓ either control plasmid or
- ✓ plasmid expressing MRE11A

Transfer of MRE11A ^{high} RA T cells **reduced**:

- ✓ tissue T Cell infiltrate (TRB transcript levels)
- ✓ TNF, IL6, and IL1B expression,
- ✓ Senescent markers





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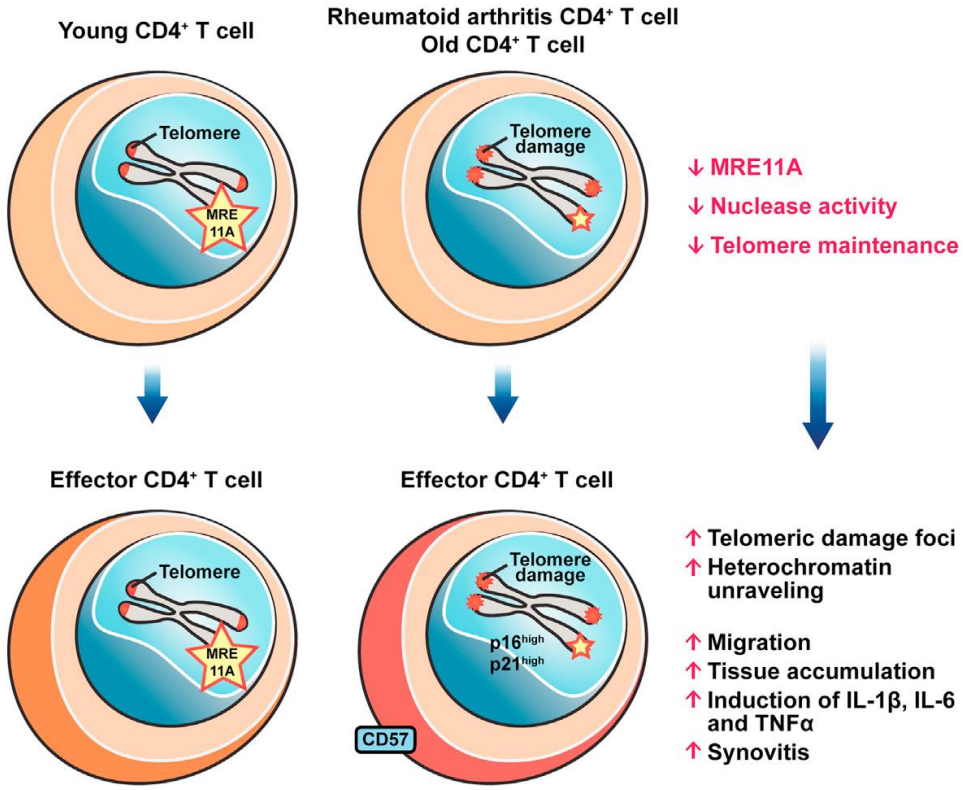


Li et al., 2016, Immunity 45, 903–916

Cell Metabolism

The DNA Repair Nuclease MRE11A Functions as a Mitochondrial Protector and Prevents T Cell Pyroptosis and Tissue Inflammation

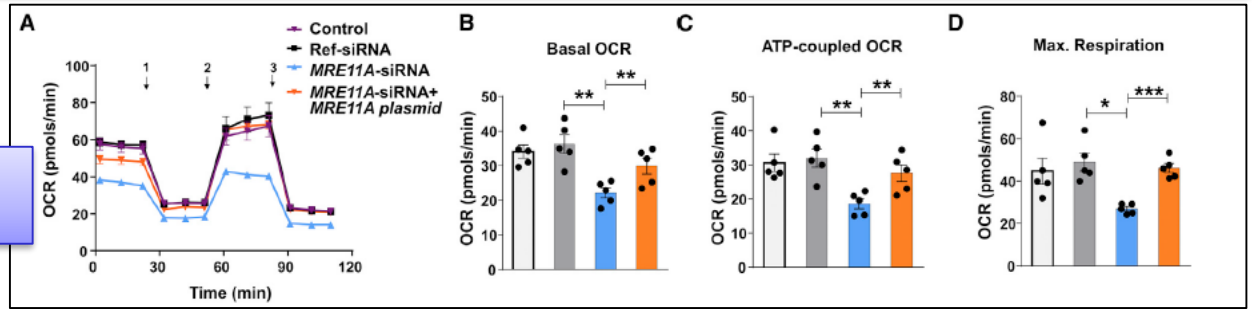
Li et al., 2019, Cell Metabolism 30, 1–16

Cell Metabolism

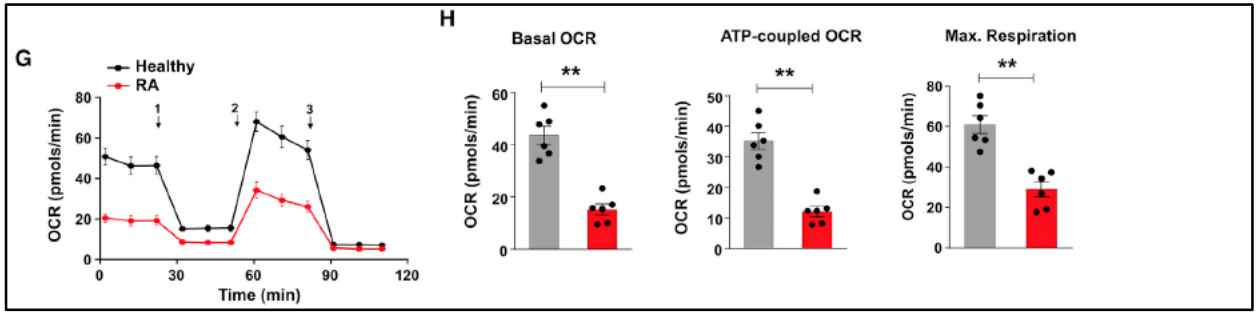
The DNA Repair Nuclease MRE11A Functions as a Mitochondrial Protector and Prevents T Cell Pyroptosis and Tissue Inflammation

✓ MRE11A knockdown in healthy T cells markedly **reduced mitochondrial oxygen consumption**, **comparable** to impaired mitochondrial function and ATP^{low} state of RA T cells.

MRE11A Knockdown

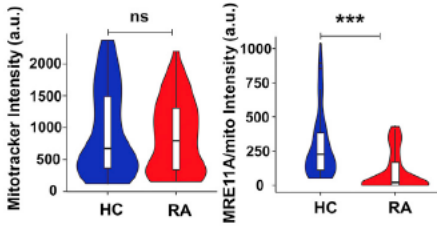


RA CD4



"MRE11A^{low} status of RA T cells extended to mitochondria, resulting in low mitochondrial respiration and ATP production."

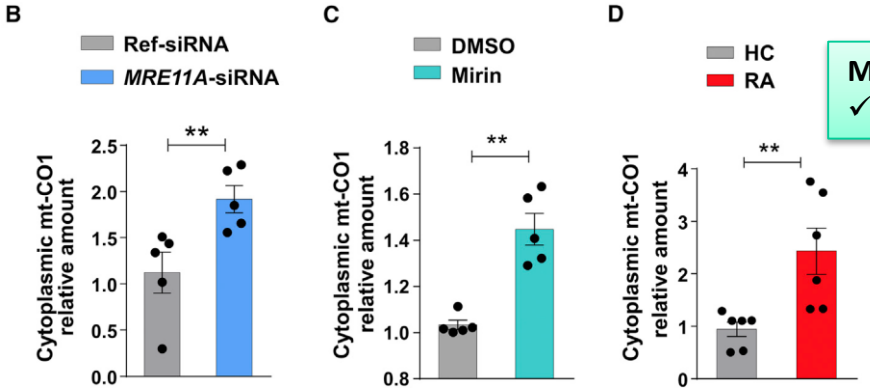
✓ **Low mitochondrial MRE11A** in RA T cells while
✓ mitochondrial mass was similar in control and RA T cells



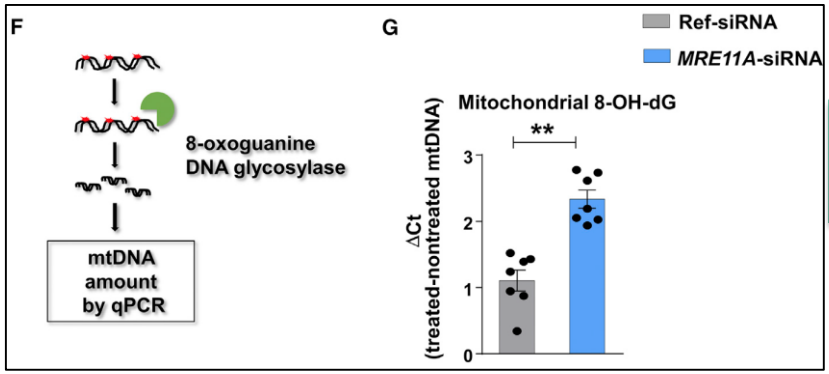
Li et al., 2019, Cell Metabolism 30, 1–16

MRE11A **protects** mtDNA from oxidative damage and leakage into the cytosol

MRE11A protects mtDNA leakage into the cytosol

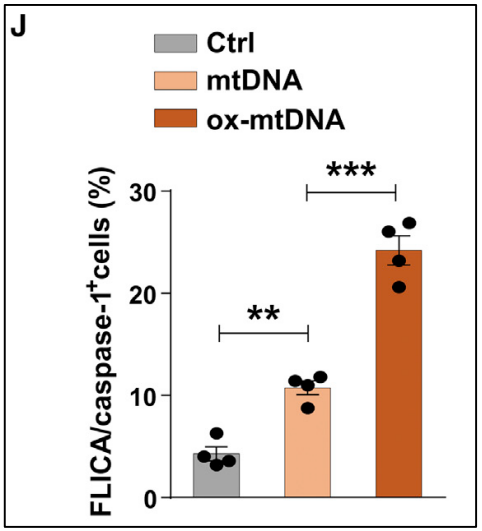


MRE11A inhibitor
✓ Mirin



MRE11A protects mtDNA oxidative lesion

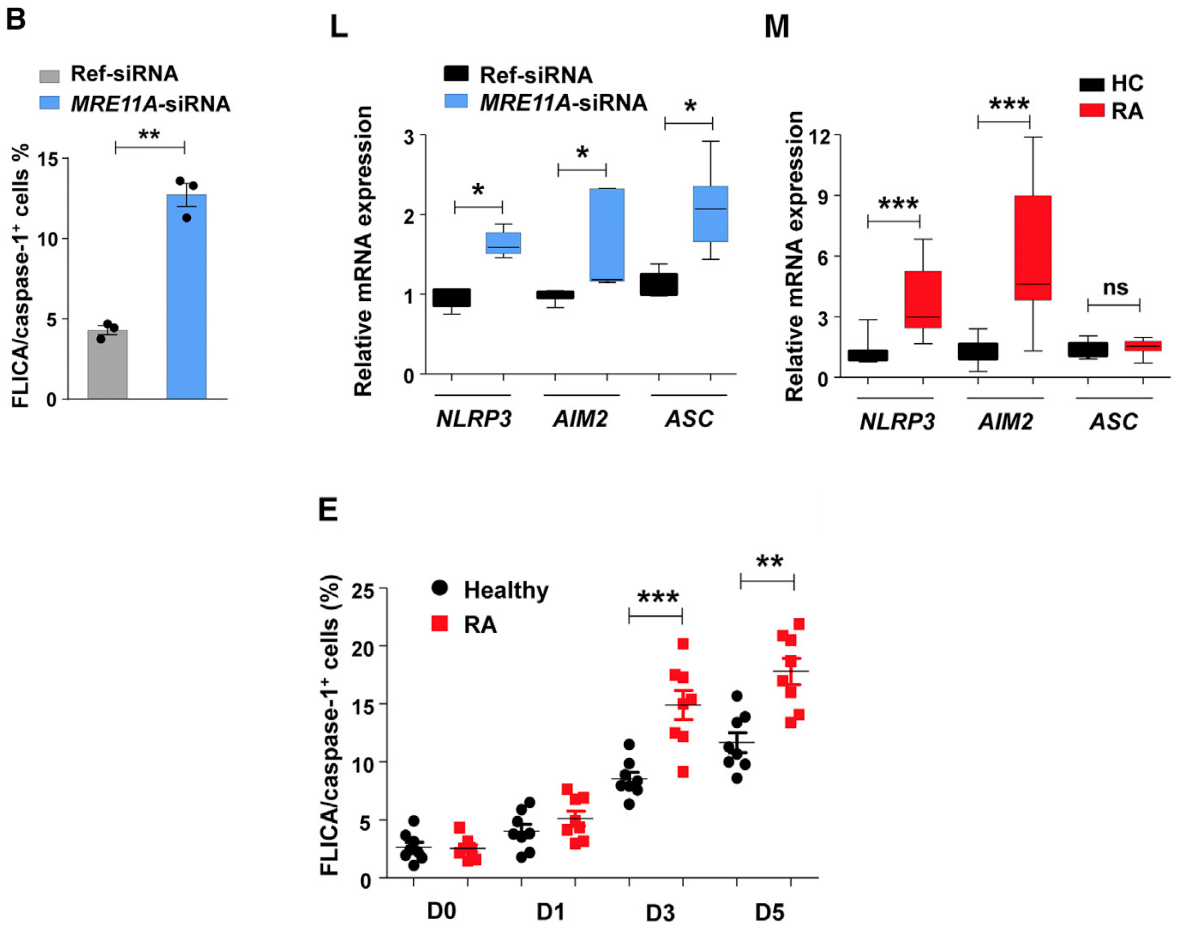
Oxidized mtDNA induced caspase-1 activation



Li et al., 2019, Cell Metabolism 30, 1–16

MRE11A protects against inflammasome assembly on T cells

MRE11A loss of function induces aberrant caspase-1 activation in RA T cells.

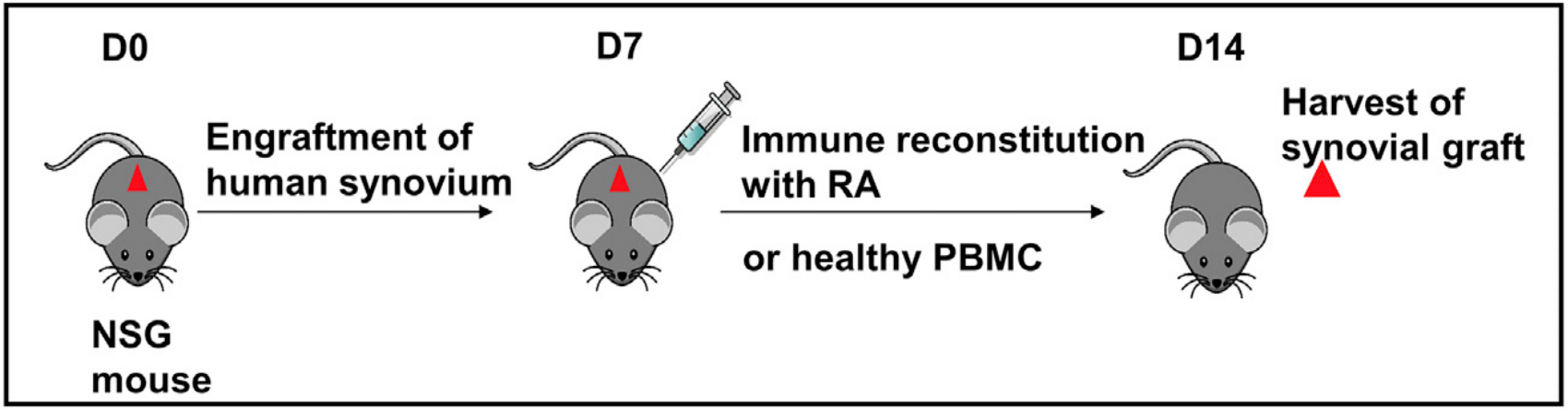


Li et al., 2019, Cell Metabolism 30, 1–16

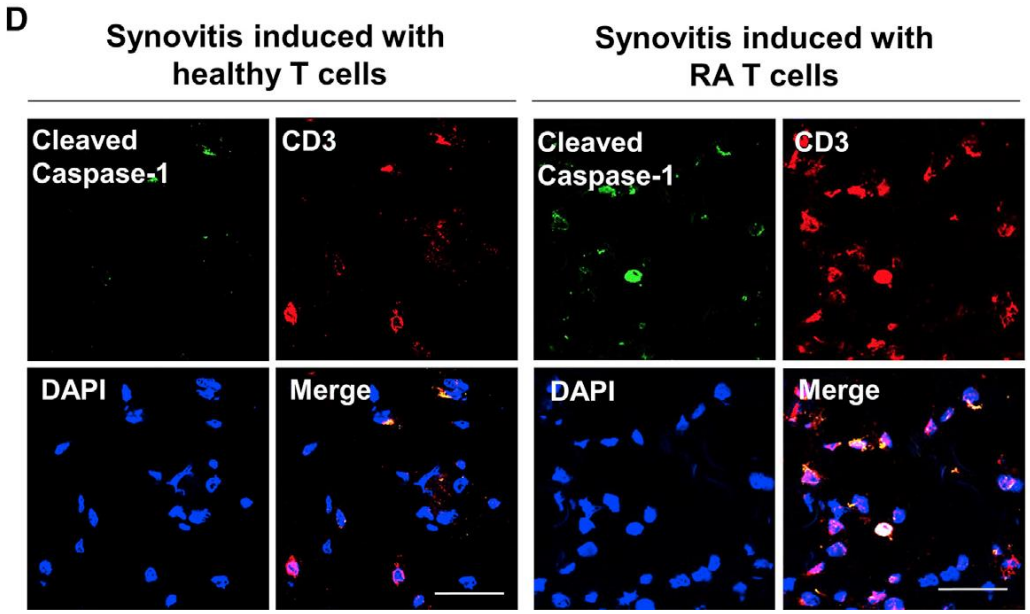
✓ We exploited a chimeric mouse model to test the in vivo relevance of MRE11A in mtDNA leakage, caspase-1 activation, pyroptosis, and tissue inflammation.

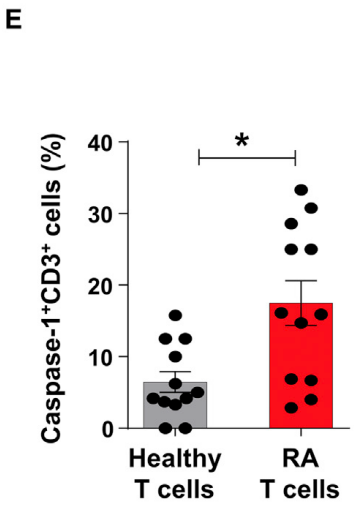
C

Synovial tissue was engrafted into NSG mice, which were reconstituted with either healthy or RA-derived peripheral blood mononuclear cells (PMBCs) and synovial grafts were explanted 7 to 10 days later



RA T cells in inflammatory lesions are predisposed to cleave caspase-1, shortening their survival and providing a trigger for tissue inflammation



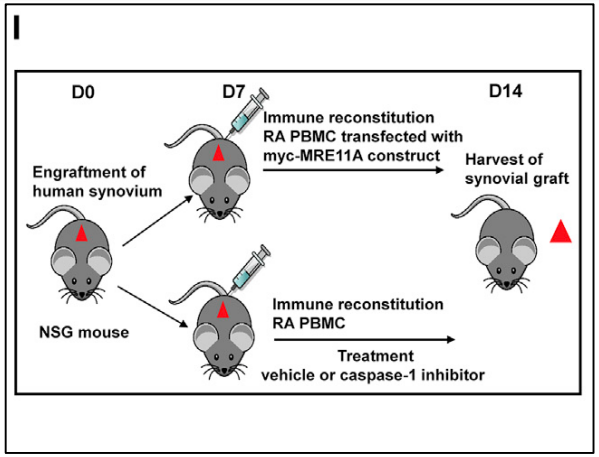


Li et al., 2019, Cell Metabolism 30, 1–16

MRE11A Protects Synovial Tissue from Inflammation

MRE11A overexpression

- ✓ suppressed tissue inflammation,
- ✓ reduced transcripts of innate (IL1B, IL6, and TNFA) and adaptive markers (TRB and TNFSF11),
- ✓ maintained tissue protective molecules (IL10 and TGFB1)



Li et al., 2019, Cell Metabolism 30, 1–16

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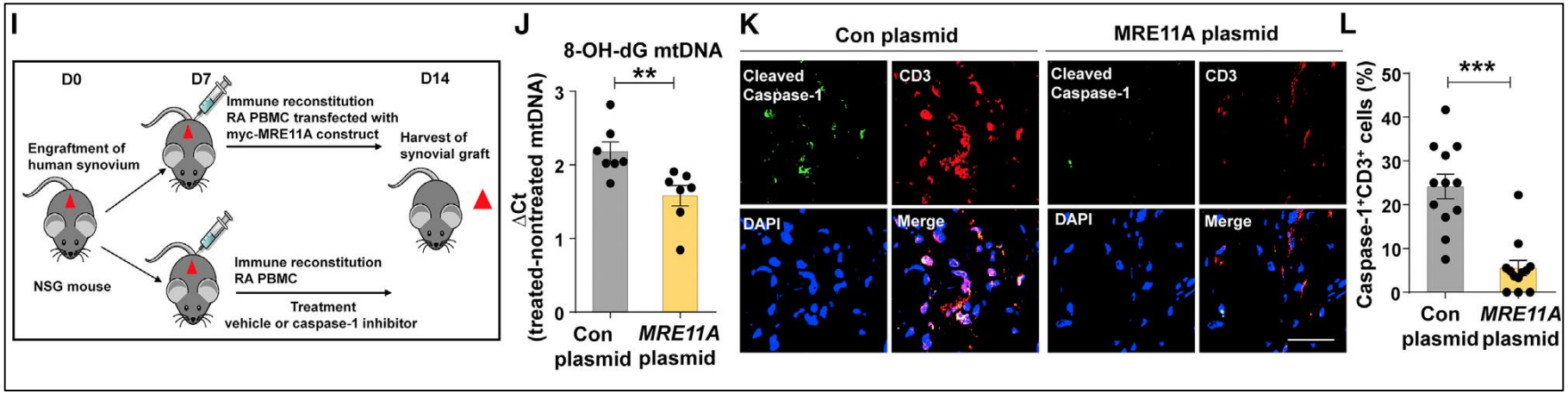

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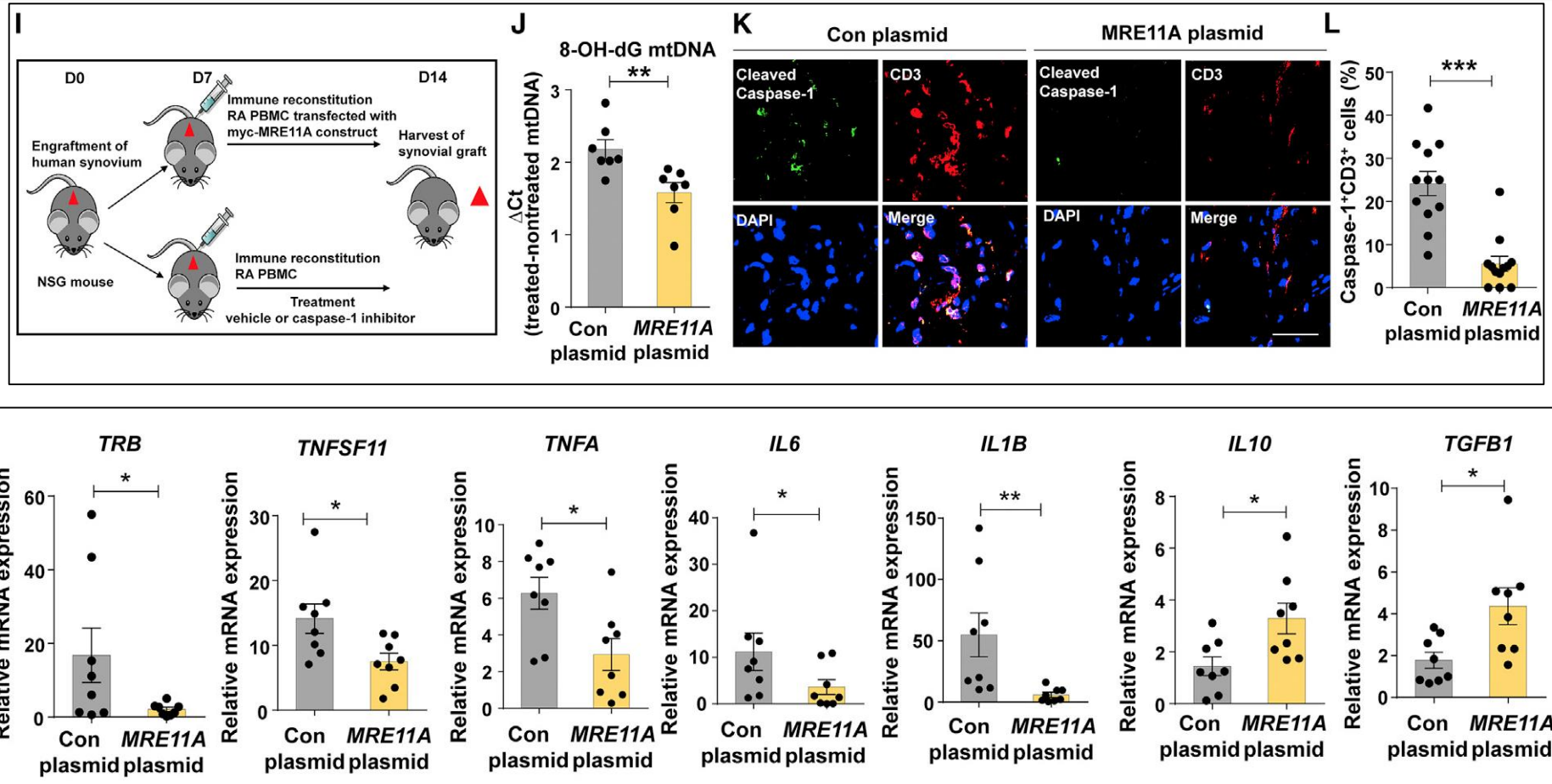
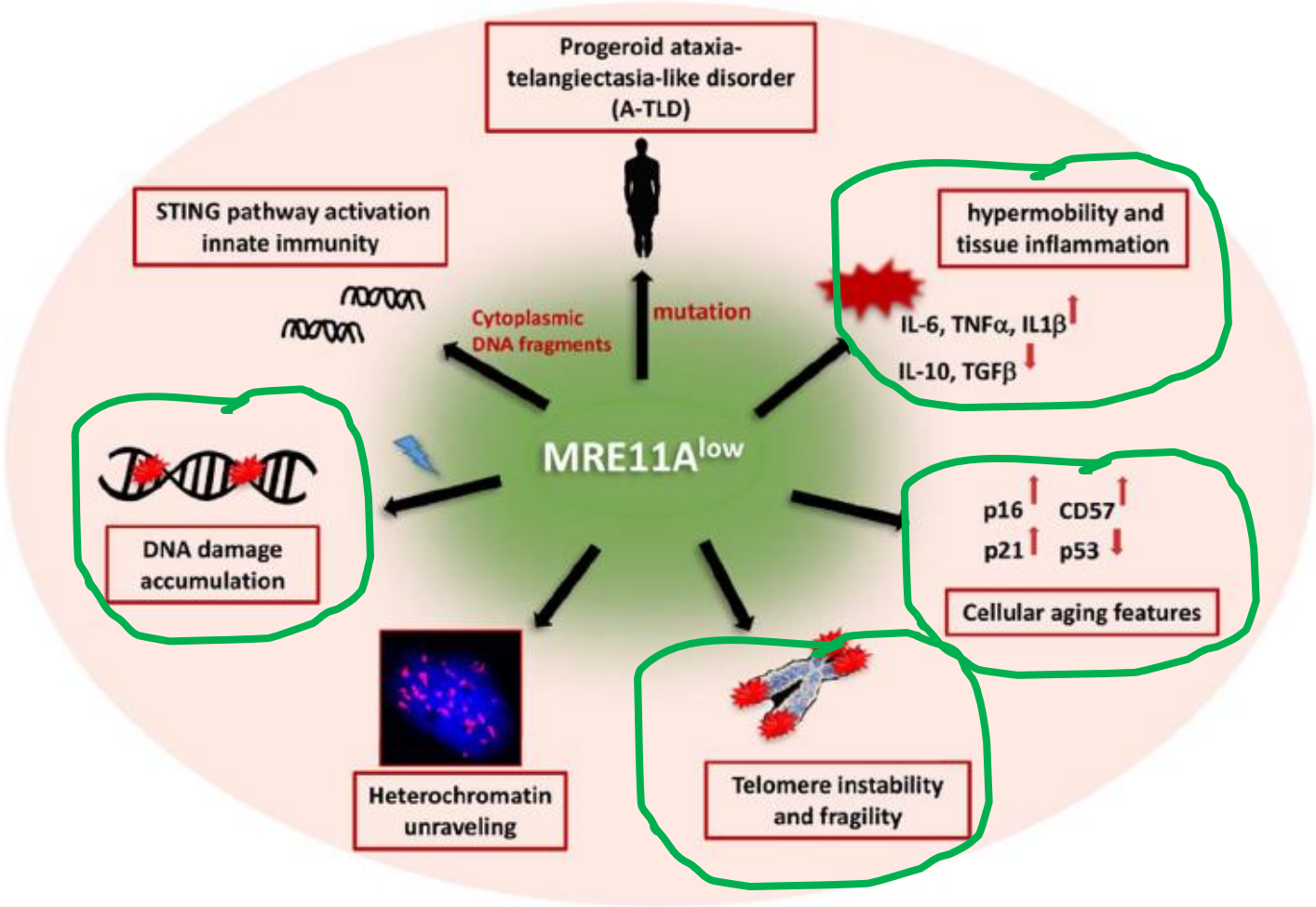


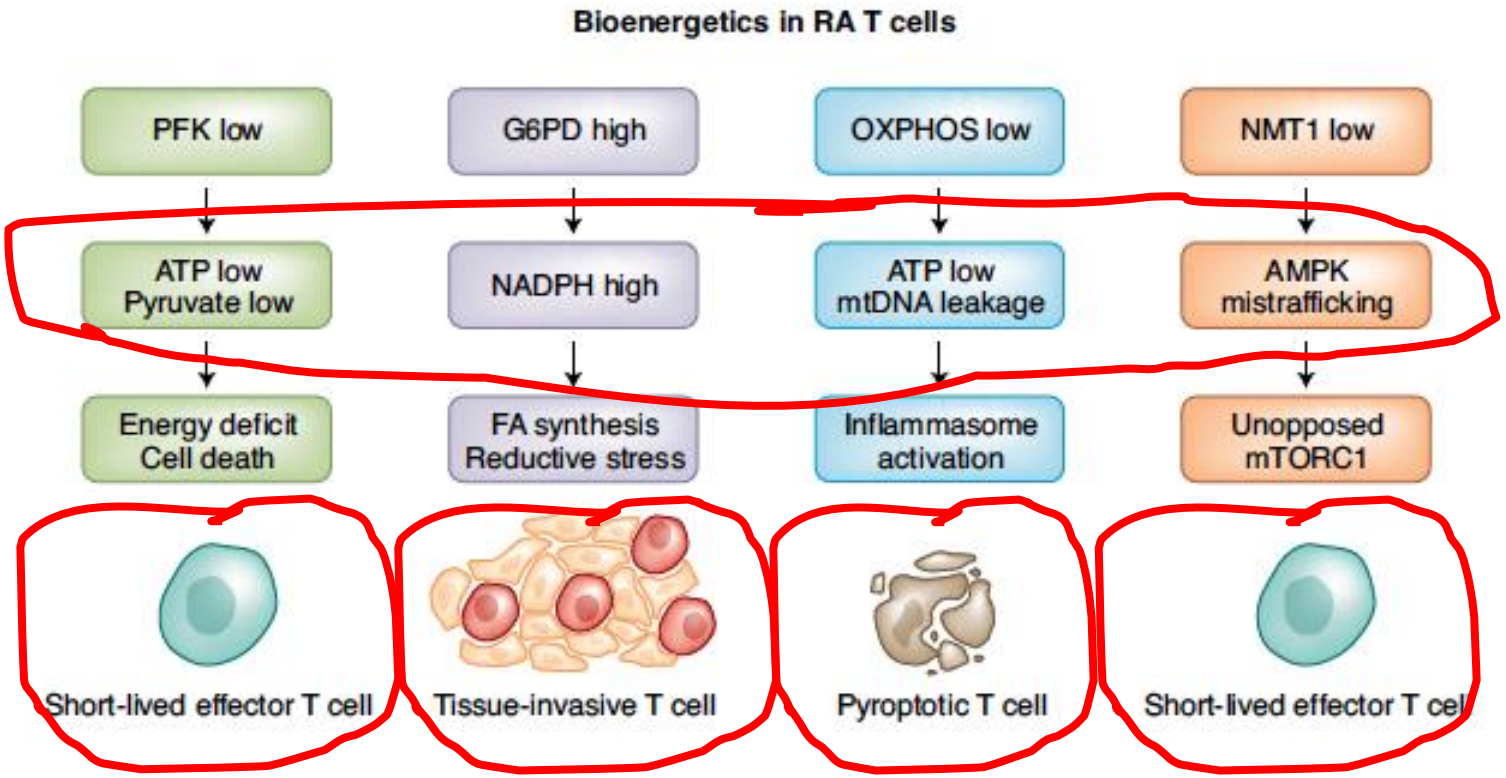
Li et al., 2019, Cell Metabolism 30, 1–16



Experimental Gerontology (2017)

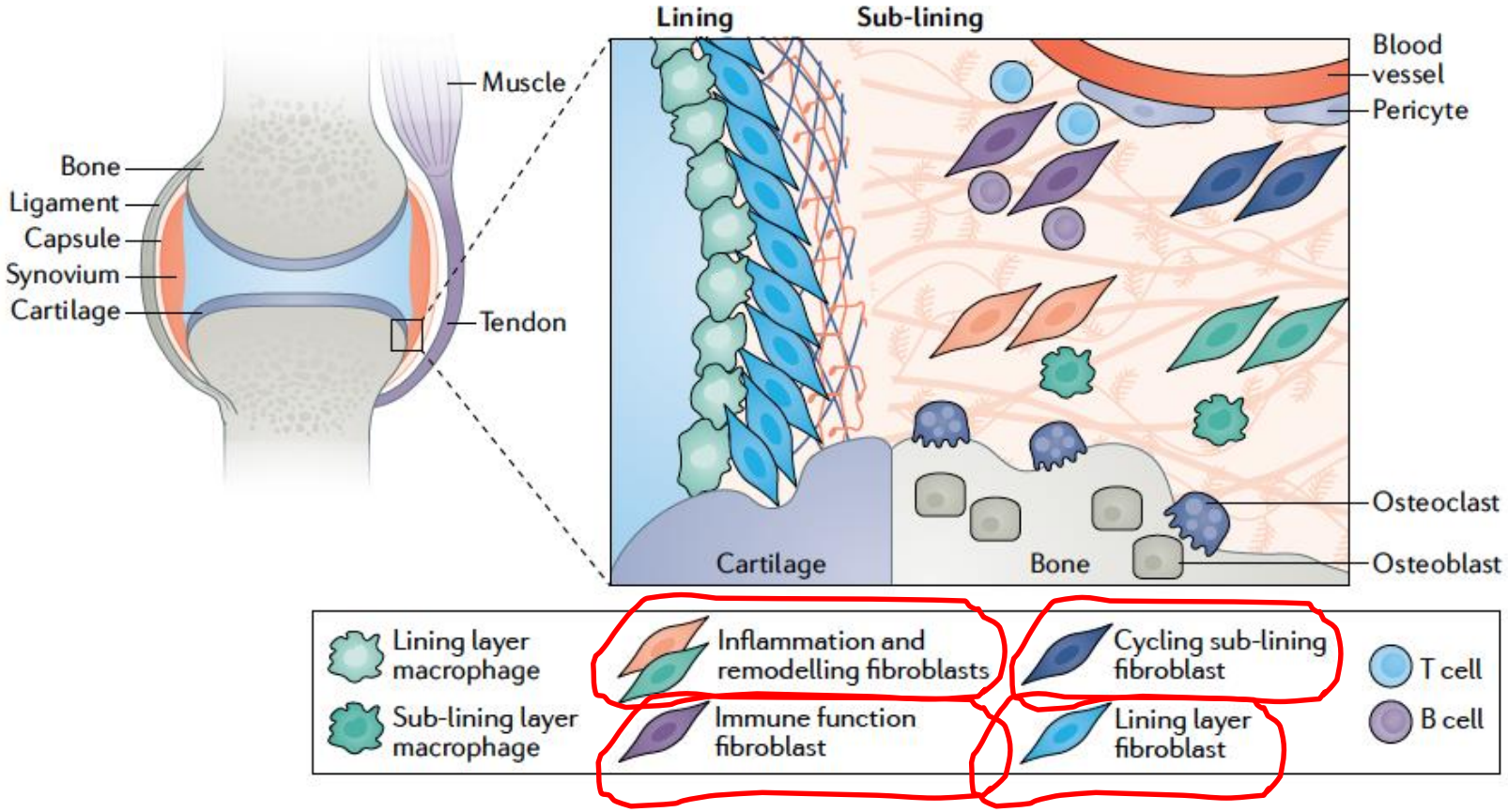
Metabolic abnormalities identified in **PA-derived naive CD4+** T cells undergoing activation:

link to inflammation and tissue invasiveness



Nat Immunol. 2021 Jan;22(1):10-18

The multiple faces of fibroblasts in RA



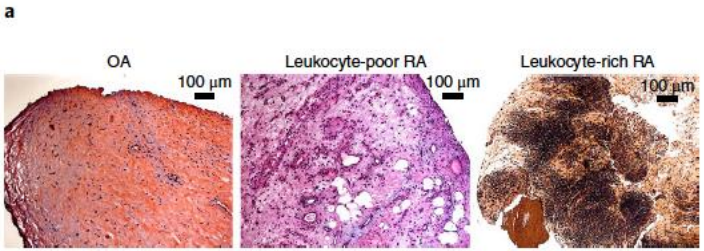
Nat Rev Rheumatol. 2021 Apr;17(4):195-212

nature immunology

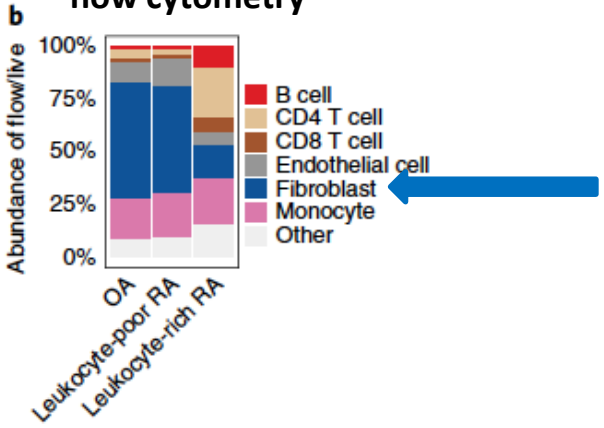
Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry

Nat Immunol. 2019 Jul;20(7):928-942

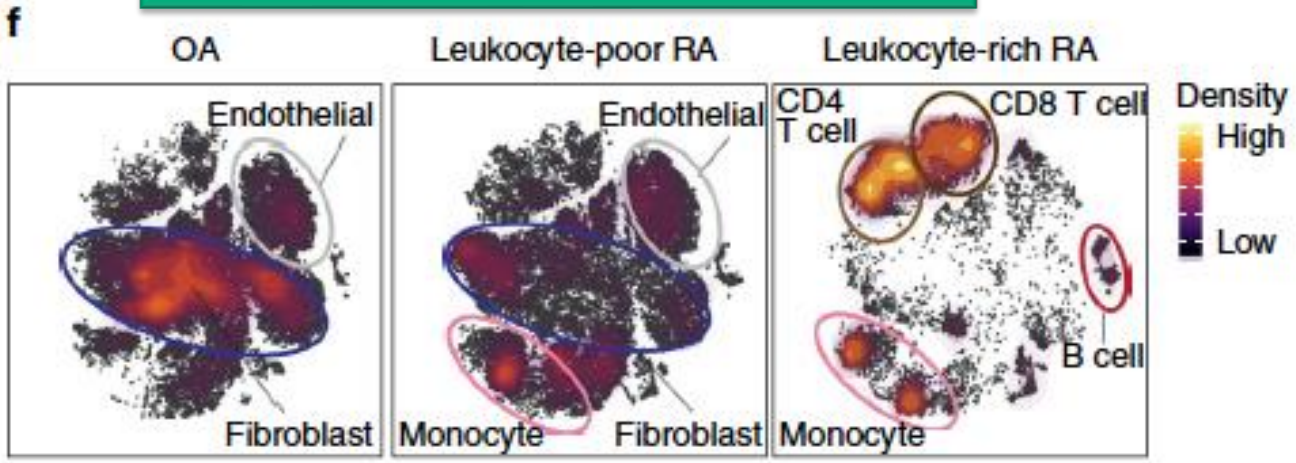
Histological assessment



Cellular composition by flow cytometry



tSNE visualization of synovial cell types in OA, leukocyte-poor RA, and leukocyte-rich RA **by mass cytometry density plot.**



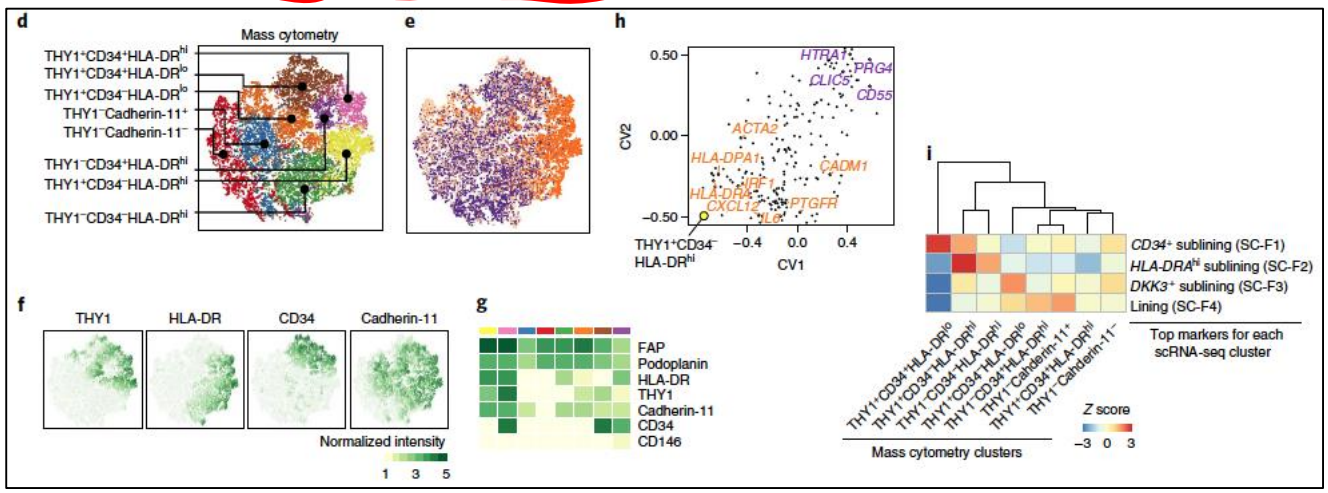
nature immunology

Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry

Nat Immunol. 2019 Jul;20(7):928-942

Table 1 | **Conserved cell populations in rheumatoid arthritis joints**

Cell subsets	Marker genes (human)	Marker genes (mouse)	Activation marker or effector genes
Fibroblasts			
Lining layer	Negative (*CD90*); positive (*CD55* and *PGR4*)	Negative (*Cd90*); positive (*Pgr4*)	*RANKL:OPG* ratio, *CCL9*, *CLIC5*, *MMP1*, *MMP2*, *MMP3*, *MMP9*, *MMP13*, *HAS1*, *HTRA4* and *DNASE1L3*
Sub-lining layer (immunomodulatory	Positive (*CD90* and *CD34*)	Positive (*Cd90* and *Cd34*)	*IL6*, *IL33*, *IL34*, *IFI30*, *LIF*, *CXCL9*, *CXCL12*, *CXCL13*, *CCL2*, *CCL19* and *CCL21*
	Negative (*CD34*); positive (*CD90* and *DKK*)		
Sub-lining layer (perivascular)	Negative (*CD34*); positive (*CD90* and *HLA-DRA*)	Negative (*Cd34*); positive (*Cd90*)	



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4-5 Functionally distinct fibroblast subgroups

Sublining fibroblasts as a potential therapeutic target in RA:

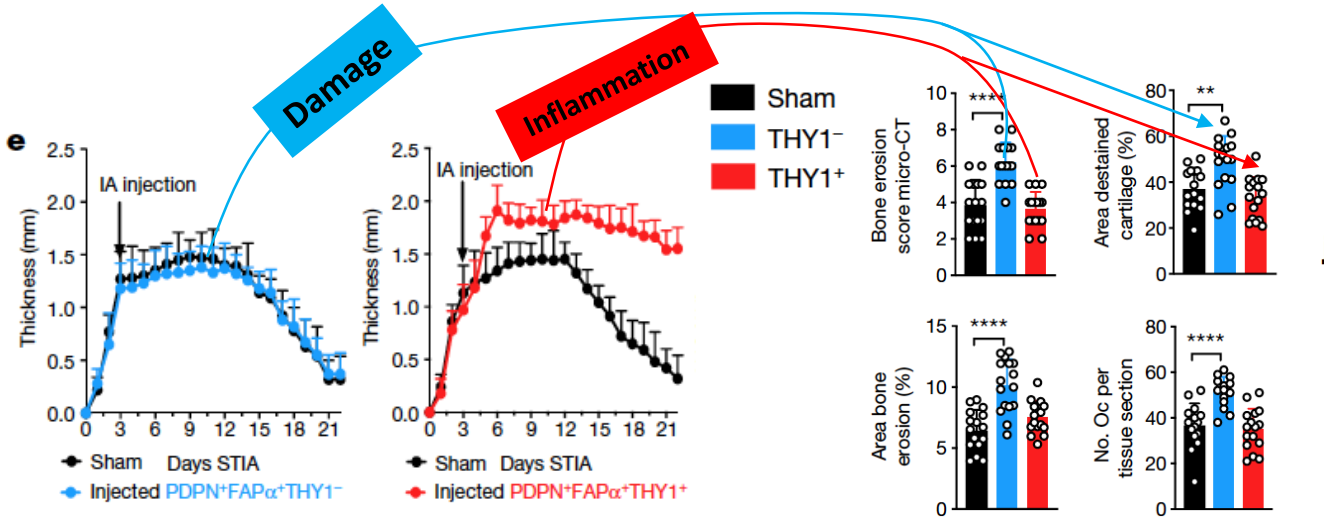
- Are a major source of pro-inflammatory cytokines such as IL6
- All SF subsets express TNF receptor 1, **but none is a TNF producer**
- Express MHC II (SC-F2, $THY1^{+}CD34–HLA\text{-}DR^{hi}$)

Further studies are needed to define molecular mechanisms that regulate sublining fibroblast expansion in RA.

Distinct fibroblast subsets drive inflammation and damage in arthritis

Adam P. Croft[1,2], Joana Campos[1], Kathrin Jansen[3], Jason D. Turner[1], Jennifer Marshall[1], Moustafa Attar[3], Loriane Savary[1], Corinna Wehmeyer[1,4], Amy J. Naylor[1], Samuel Kemble[1], Jenefa Begum[1], Kerstin Dürholz[1,5], Harris Perlman[6], Francesca Barone[1], Helen M. McGettrick[1], Douglas T. Fearon[7], Kevin Wei[8], Soumya Raychaudhuri[8], Ilya Korsunsky[8], Michael B. Brenner[8], Mark Coles[3], Stephen N. Sansom[3,11], Andrew Filer[1,2,9,10,11] & Christopher D. Buckley[1,2,3,10]*

Nature. 2019 Jun;570(7760):246-251



Adoptive transfer into the joint, different fibroblasts mediate distinct effects:

- ✓ FAPα+THY1− fibroblasts selectively mediate bone and cartilage damage with little effect on inflammation,
- ✓ FAPα+ THY1+ fibroblasts resulted in a more severe and persistent inflammatory arthritis, with minimal effect on bone/cartilage.

"Our findings describing anatomically discrete, functionally distinct fibroblast subsets with non-overlapping functions have important implications for cell-based therapies aimed at modulating inflammation and tissue damage."

nature immunology

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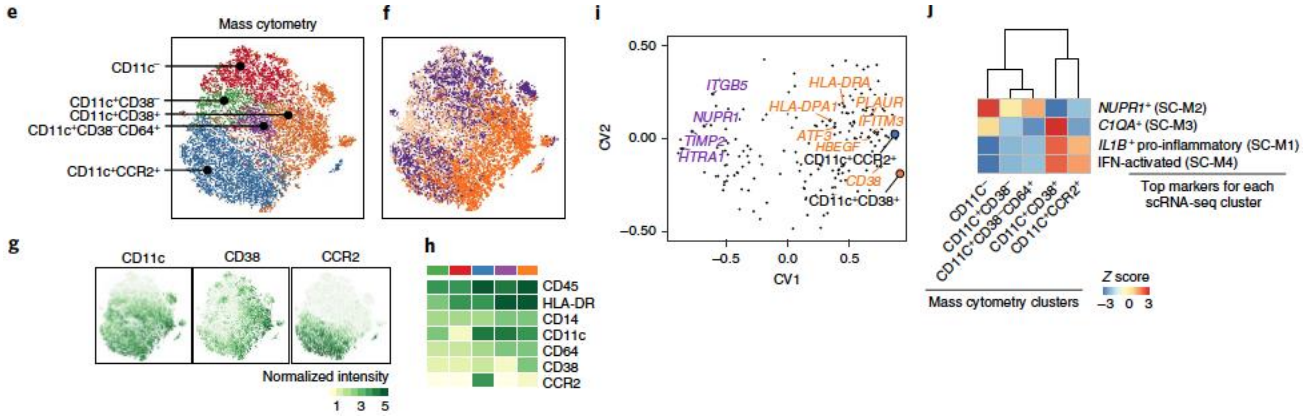
Cell subsets	Marker genes (human)	Marker genes (mouse)	Activation marker or effector genes
Macrophages			
Lining layer	Not reported	Negative (*Cfsr1*); positive (*Cx3cr1*)	*TREM2, VSIG4, AXL, MFGE8, JAM1, ZO1, CLDN5, FAT4* and *VANGL2*
Interstitial	Negative (*CD11C* and *CD38*); positive (*NURP1*)	Negative (*Cx3cr1*); positive (*Cfsr1*, MHC class II genes and *Aqp1*)	*MERTK, CTSK, HTRA1, GPNMB* and *ITGB5*
	Positive (*C1QA, CD11C* and *CD38*)	Negative (*Cx3cr1*); positive (*Cfsr1* and *Relma*)	*MRC1, CD163* and *MARCO*
Monocyte-derived (infiltrating)	Positive (*SPP1, CD11C, CCR2* and *CD38*) when activated by interferon	Negative (*Ly6c2*); positive (*Ccr2* and *Arg1*)	*ARG1, IFI6, IFI44L, LY6E* and *SPP1*
	Positive (*IL1B, CD11C, CCR2* and *CD38*)	Negative (*Ly6c2*); positive (*Ccr2* and *Il1b*)	*NR4A2, HBEGF, PLAUR, RGS2, IL1B, HTF3, CXCL2* and *EREG*



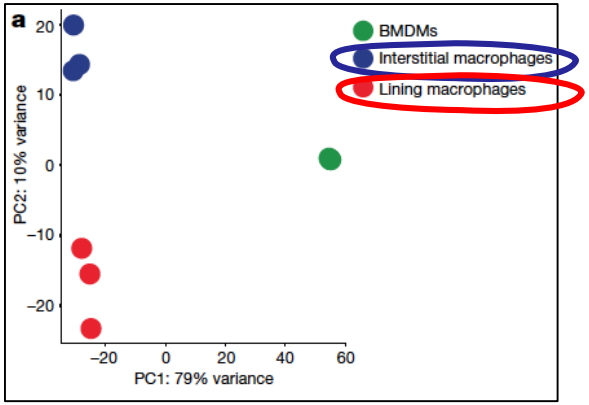
Nat Rev Rheumatol. 2021 Apr;17(4):195-212

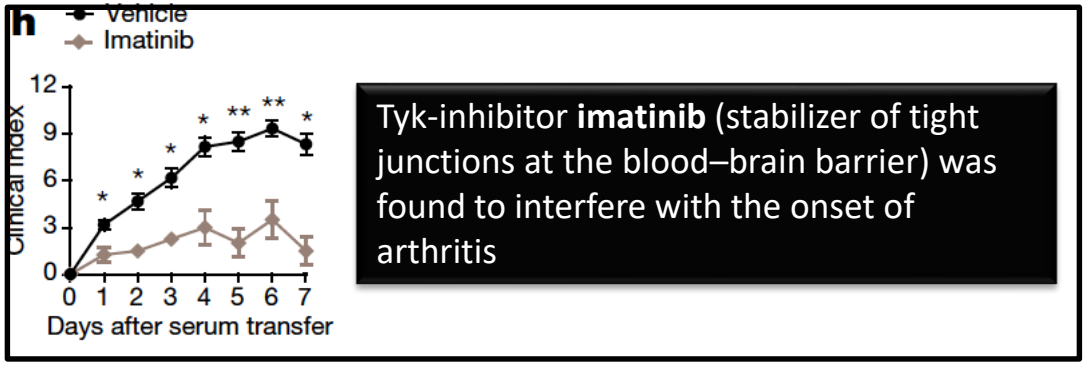
Locally renewing resident synovial macrophages provide a protective barrier for the joint

Stephan Culemann[1,2,11], Anika Grüneboom[1,2,11], José Ángel Nicolás-Ávila[3], Daniela Weidner[1,2], Katrin Franziska Lämmle[1,2], Tobias Rothe[1,2], Juan A. Quintana[3], Philipp Kirchner[4], Branislav Krljanac[5], Martin Eberhardt[6], Fulvia Ferrazzi[4], Elke Kretzschmar[7], Martin Schicht[7], Kim Fischer[1], Kolja Gelse[8], Maria Faas[1,2], René Pfeifle[1,2], Jochen A. Ackermann[1,2], Milena Pachowsky[8], Nina Renner[8], David Simon[1], Reiner F. Haseloff[9], Arif B. Ekici[4], Tobias Bäuerle[10], Ingolf E. Blasig[9], Julio Vera[6], David Voehringer[5], Arnd Kleyer[1], Friedrich Paulsen[7], Georg Schett[1], Andrés Hidalgo[3] & Gerhard Krönke[1,2*]

Nature 2019;572(7771):670-675

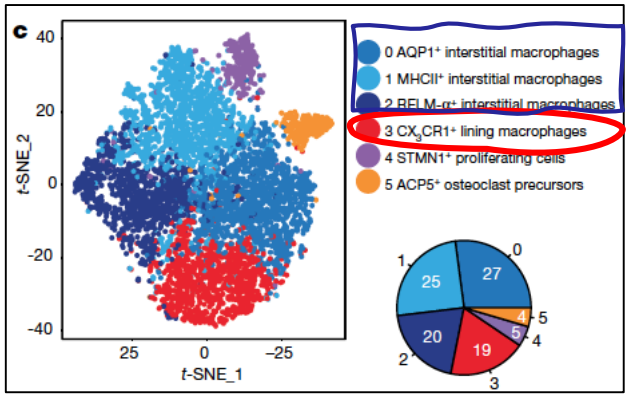
Bulk RNA sequencing of sorted steady-state CX3CR1$^+$ lining Mϕ

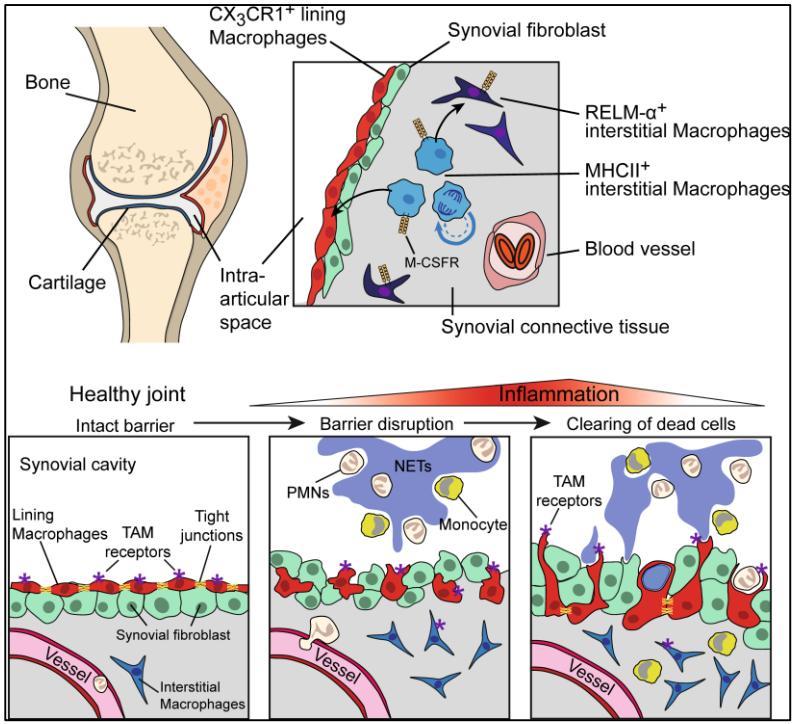




Tyk-inhibitor **imatinib** (stabilizer of tight junctions at the blood–brain barrier) was found to interfere with the onset of arthritis

Single-cell RNA sequencing of total synovial CD45+CD11b+Ly6G-mononuclear phagocytes





Key points

- **T cell-endogenous abnormalities** present in naive T cells drive the differentiation program to favor the generation effector/inflammatory cells with tissue-invading properties
  - DNA-repair failure & a metabolic shift
- Subsets of **highly activated synovial fibroblasts** adopt proinflammatory and tissue-invasive functionalities
- **Anti-inflammatory macrophages** in the synovium fail to protect the synovium
- Potential of Novel therapies:
  - The recognition of stable stages and the molecular characterization of the relevant transition points has the potential to identify targets that could re-engineer the immune system to halt the disease process prior to irreversible tissue damage.