Manuscript: “OX40 signaling is involved in the autoactivation of $CD4^+CD28^-$ T cells and contributes to pathogenesis of autoimmune arthritis”

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Rheumatology JC

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Disclosures

- NIH
  - 2 T32 HL007457-36A1
  - UAB Division of Clinical Immunology and Rheumatology for pre and post-doctoral trainees
- Illumina
  - In-kind contribution
  - study epistasis between HLA risk alleles in RA

Rationale for selection of article:

- Next talk is on Phase III trial of abatacept in PsA.

- "Regarding [synovial inflammation in PsA] agents targeting IL-23/IL-17 can achieve complete clearing of psoriatic skin lesions without a similar level of efficacy in the skin. We speculate that T-cell subsets driving pathology in the skin differ with respect to their expression of CD28 and hence their abatacept susceptibility"

Rationale, continued

- So, I wanted to focus on a recent study involving CD4+ cells with
  - variable CD28 expression
  - related to an immune phenotype
  - Uses flow & other data
- Not expert on methods used in this manuscript
  - E.g. flow cytometry
  - Would love a dialectic on how data quality may impact the findings in this study.

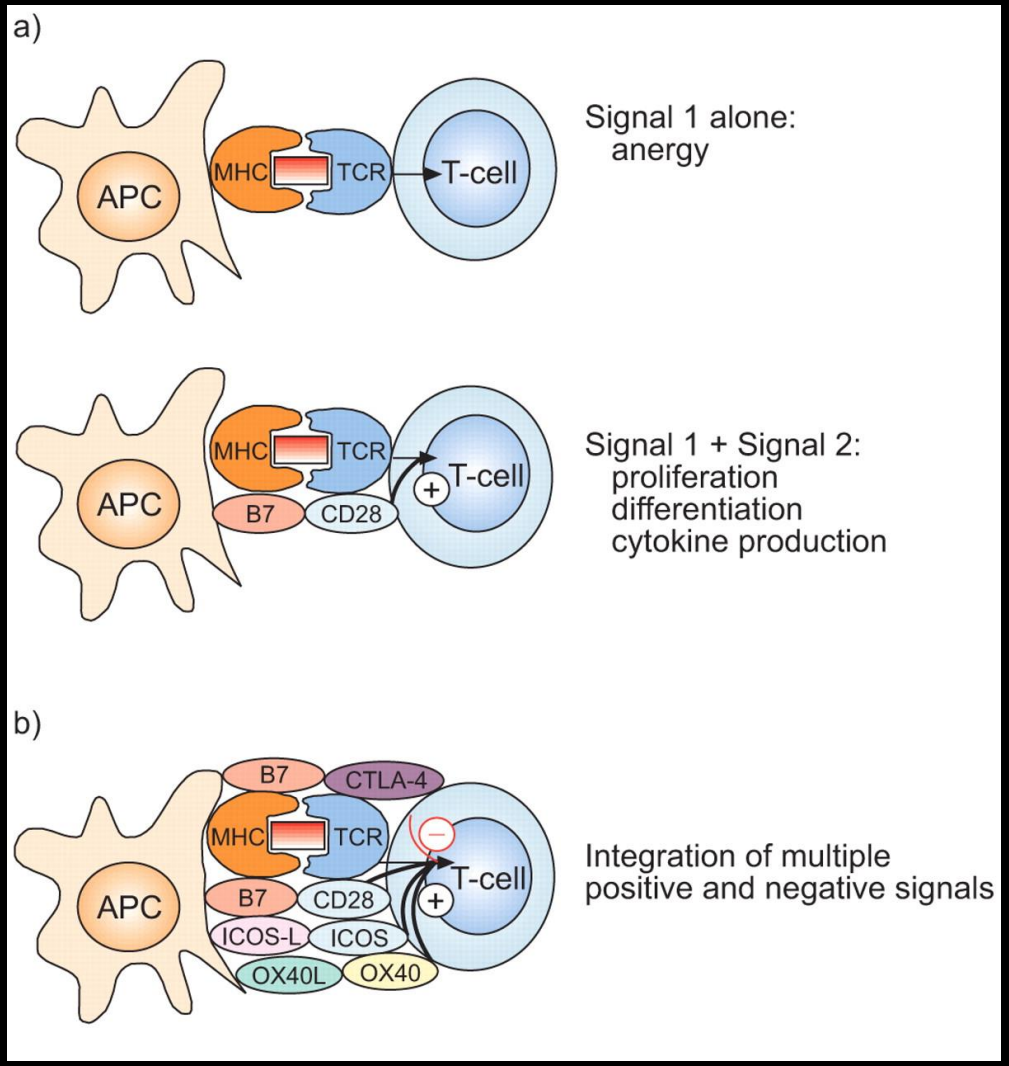
Background

T-cell co-stimulation

Signal I:
TCR – MHC

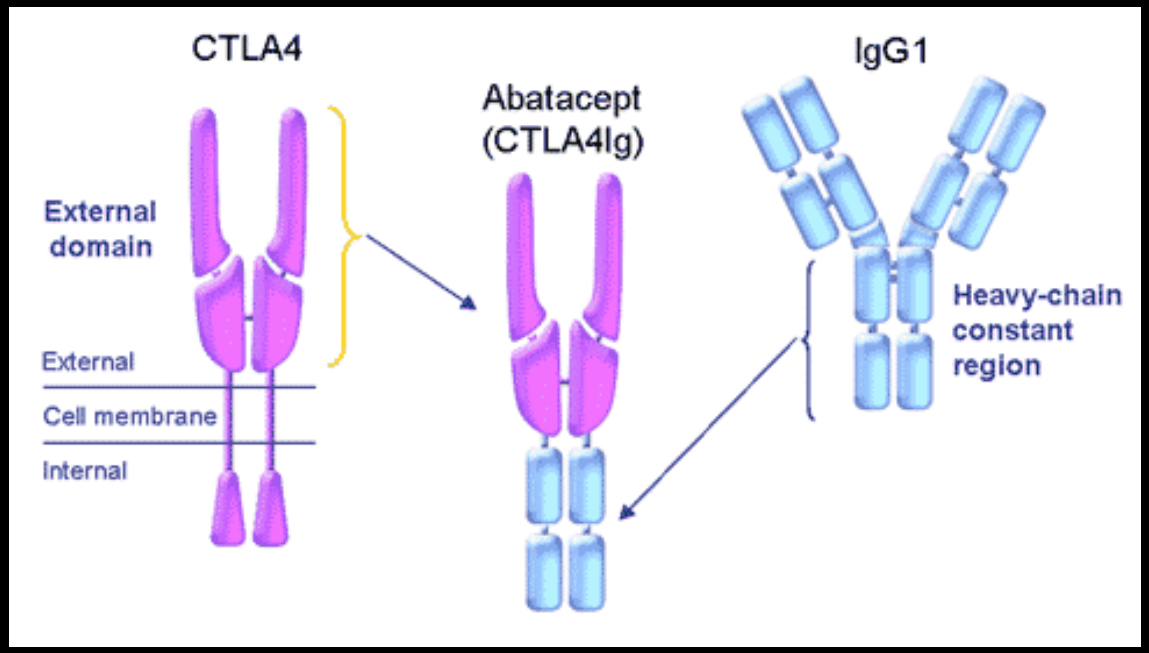
Factors influencing Signal II:

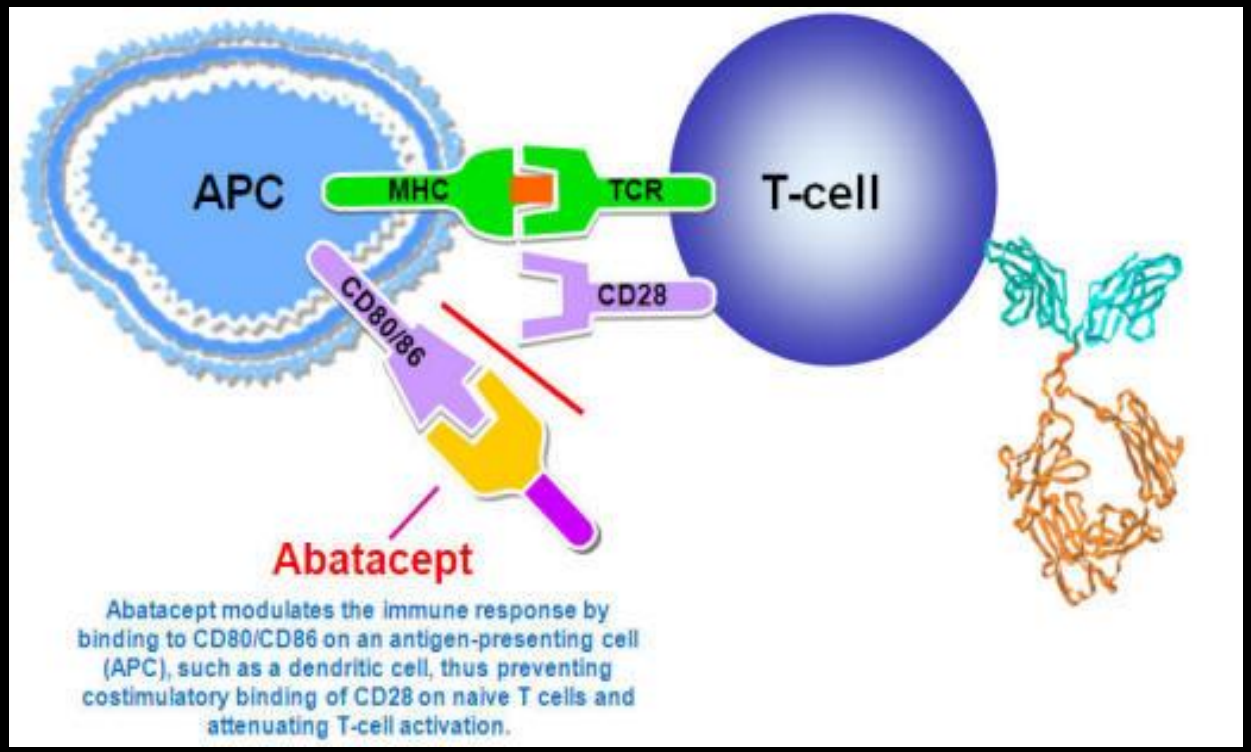
CD28 – B7
CTLA4 – B7
CD40L – CD40
OX40 – OX40L
4BB-1 – CD137L



CD28

- Binds B7 (CD80/86) → costimulation
  - Survival
  - IL-2 production
  - Metabolic Activity
  - Clonal Expansion





Abatacept (Orencia)

- CTLA4Ig …
- Extracellular domain of CTLA4
- Fc portion of Ig
- Binds CD80/86, preventing CD28 binding

$CD4^{+}CD28^{-}$ T-cell characteristics

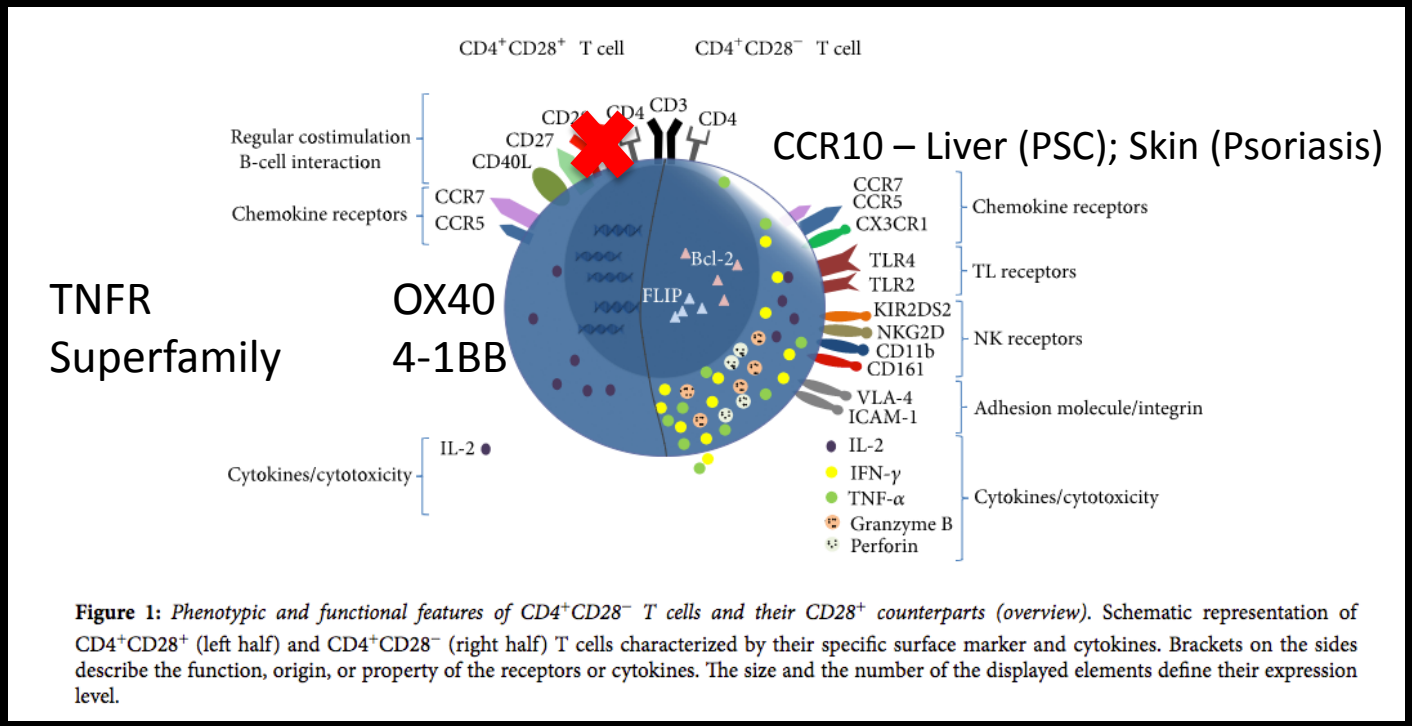


Figure 1: *Phenotypic and functional features of $CD4^{+}CD28^{-}$ T cells and their $CD28^{+}$ counterparts (overview).* Schematic representation of $CD4^{+}CD28^{+}$ (left half) and $CD4^{+}CD28^{-}$ (right half) T cells characterized by their specific surface marker and cytokines. Brackets on the sides describe the function, origin, or property of the receptors or cytokines. The size and the number of the displayed elements define their expression level.

Described in RA ~20YA

More common in >65YOA; chronic disease states

Express variety of markers not often found tog.

Chemokine markers, including those related to tissue invasion (differ by disease state)

IL-2 secretors, *but apparently independently of CD28*

High amounts of IFNy, perforin, granzyme ("cytotoxic Thelper 1")

Produce high amounts of TNFalpha → may help keep CD28lo.

Maly and Schirmer. The Story of CD4+CD28– T Cells Revisited: Solved or Still Ongoing? J Immun. Rsch. 2015

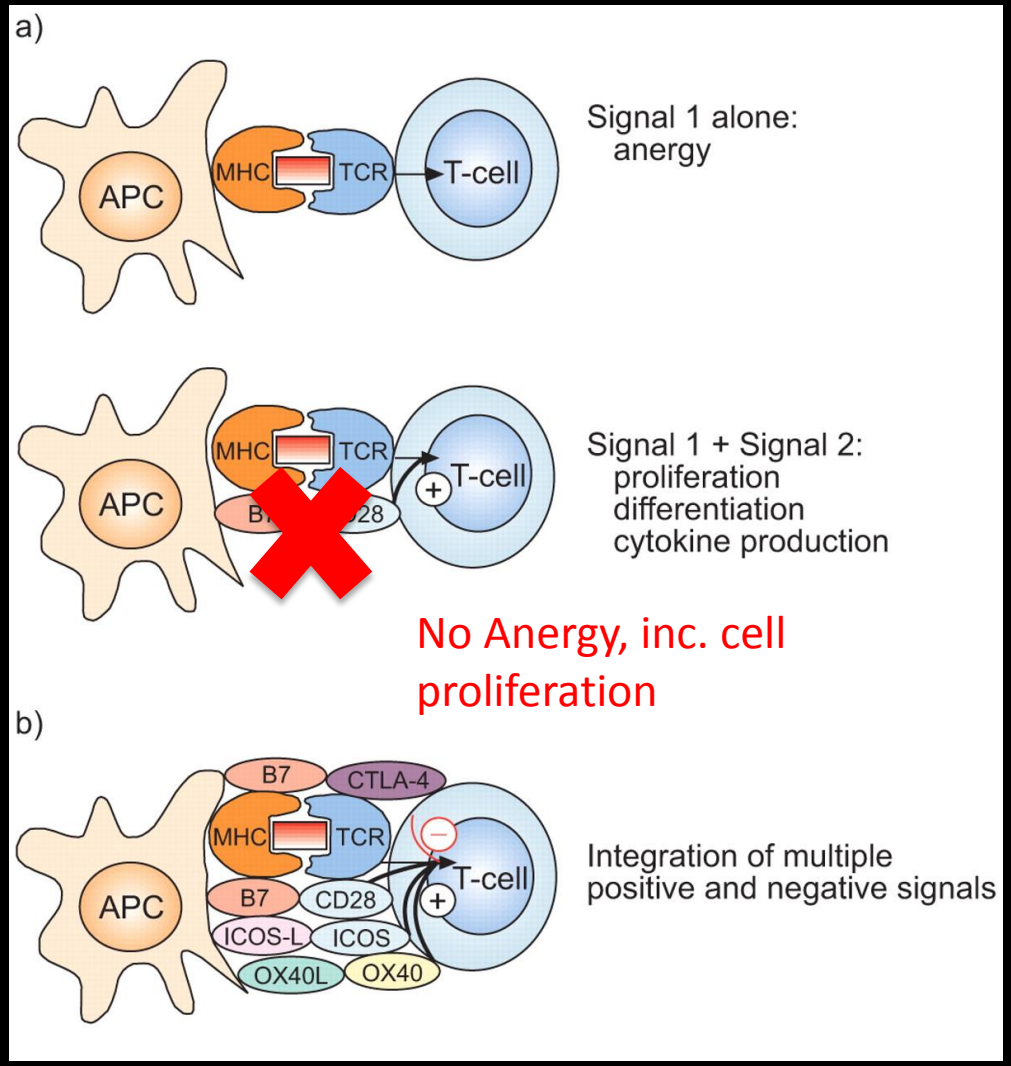
CD4+CD28- cells show *increased* proliferation?!

Shortened telomere length
No CD28 / abnormal Lack of costimulation, yet

Increased cell proliferation, and *decreased* apoptosis

CD28 → IL-2 production, but …

??? → high IL-2 → FLIP → Fas-FasL inhibition → interacts with Caspase8 and 10 → decreased apoptosis.



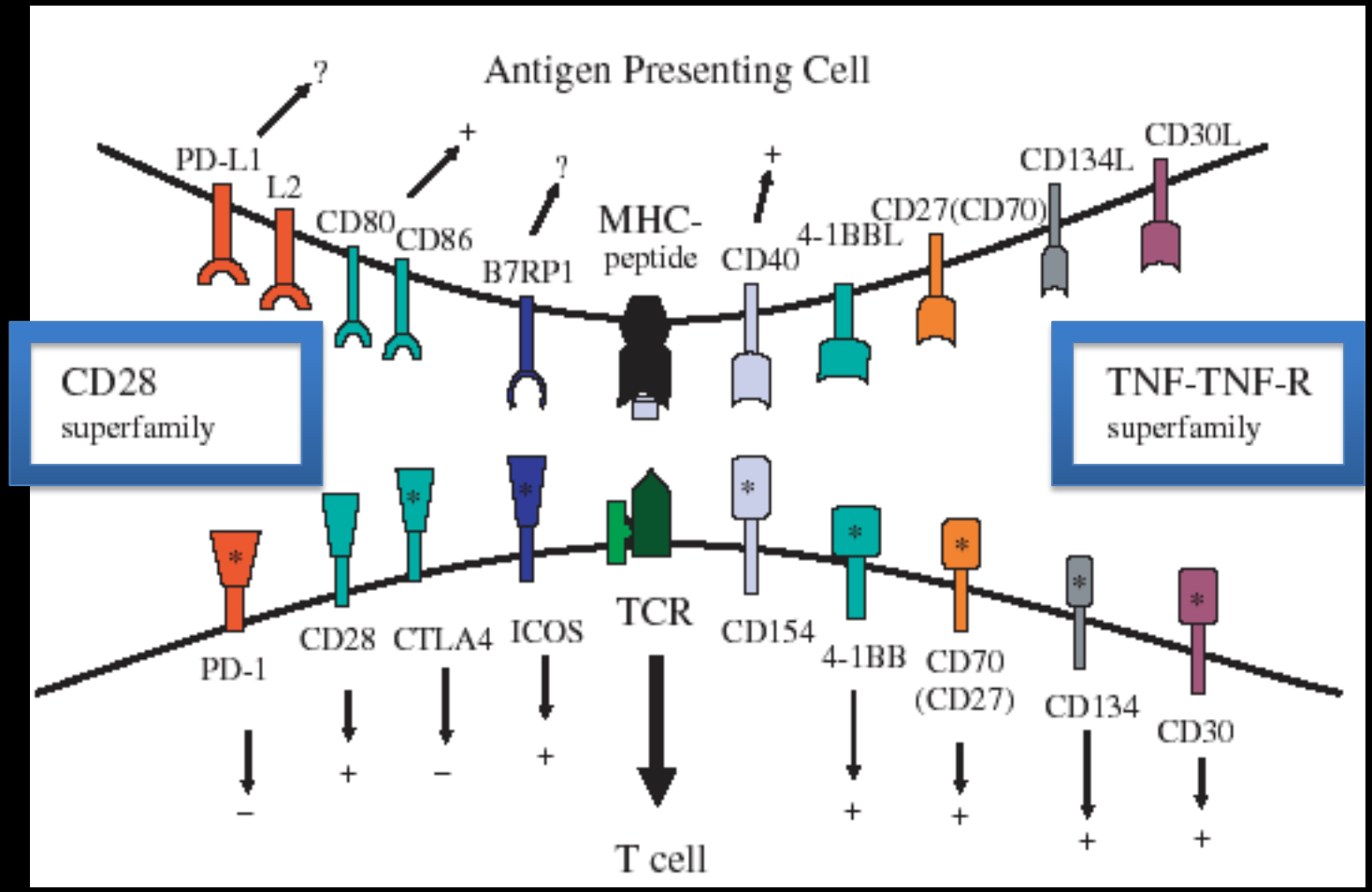
Costimulation independent T cell activation??

- Early paper
- Markovic-Plese, Martin. *$CD4^{+}CD28^{-}$ costimulation-independent T cells in multiple sclerosis.* J Clin Invest.

Lessons from transplant bio

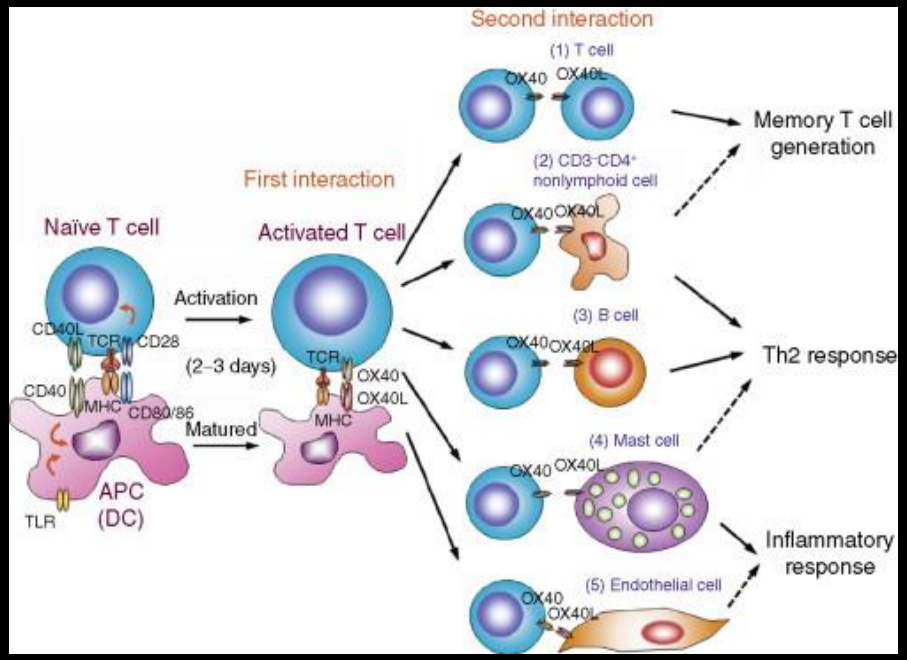
- Not necessarily costimulation independent
- Acute graft rejection
  - There are definitely ways of communicating with NK, effector T, memory cells independently of CD28/B7 … can still get AGR
  - CD154/CD40L – CD40 another major route
  - 4-1BB – 4-1BBL underpin cytotoxic T cell production in graft rejection.
  - OX40 – CD134L

Redundant (or hierachical?) pathways subserve costimulation



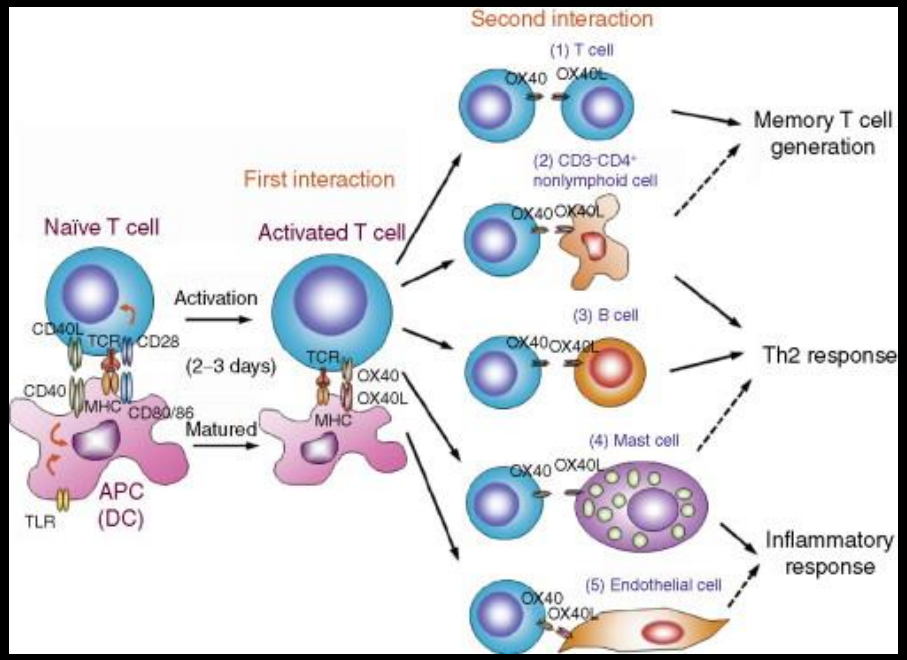
OX40 & T-cell activation

- *CD134*; *TNFRSF4*
- TNFR family protein
- Expressed predominantly on **activated** T-cells
  - CD44+
- Ligand is *OX40L* (*CD252*) - APCs
- Blockade of *OX40-OX40L* binding improves CIA mice



OX40 & T-cell activation, continued

- Seems to be able to drive Th1 or Th2 response
- Cytokine production, including signals to NK and NKT
- Effector T expansion and survival
- Appear to drive alloimmune (nonself from same species) Tcell stimulation



Part II: study

Jiang et al. OX40 signaling is involved in the autoactivation of CD4+CD28− T cells and contributes to the pathogenesis of autoimmune arthritis. Arthritis Research & Therapy. 2017.

https://doi.org/10.1186/s13075-017-1261-9

Methods (Human Subjects)

- 71 RA (2010 ACR/EULAR)
- 44 sex & age matched OA patients
- 47 healthy volunteers
- DAS28 for disease activity – separated into 3 strata or in remission.
- 9 had been given MTX
- No other DMARDS w/in 1 year prior
- PB & SF collected after IC

Methods (CIA mice)

- Male DBA/1 mice 8-10 wk
- D0 - Given 200ug bovine collagen type 2 & CFA
- D14 – reimmunized with CFA
- Scored 0-4 for degree of swelling in paw/wrist
- Grouped into Acute or Chronic CIA (A-CIA vs C-CIA).
  - Comments?
- D28 - Adoptive transfer and blocking studies
- D35 – dexamethasone given IP (0.5mg; 2mg; PBS)

Overall study design

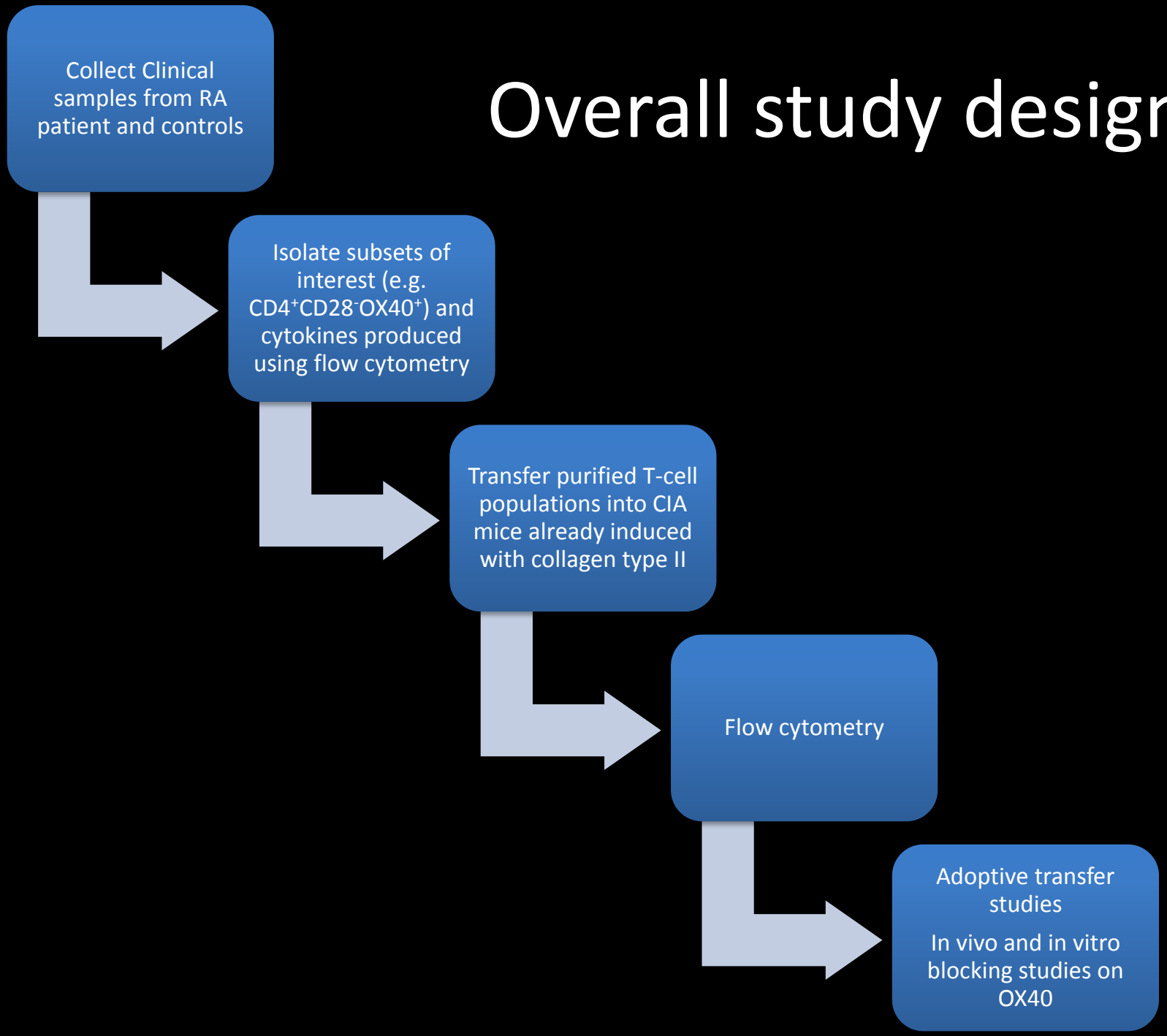
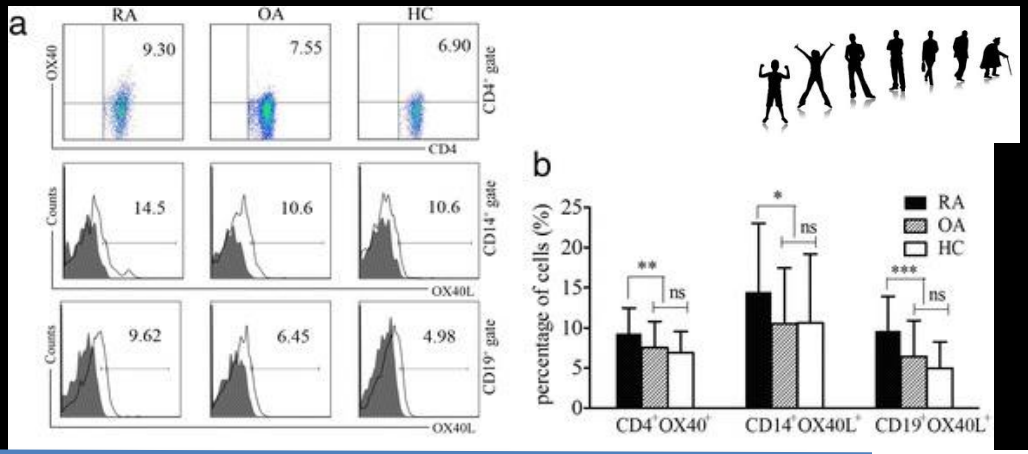


Fig 1: Differential expression of OX40 and OX40L in RA (1a, 1c) & CIA mice (1b)

A - PB of RA vs HC

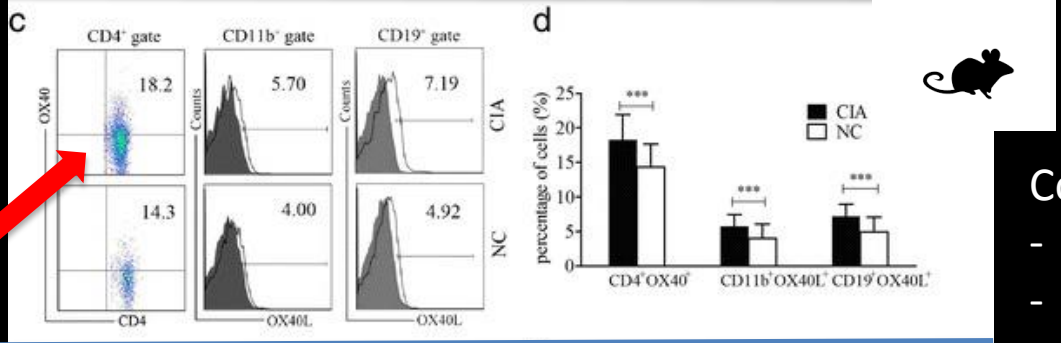
Top $CD4^+OX40^+$,
Mid $CD14^+OX40L^+$
Bot $CD19^+$+$OX40L^+$



CD4 - T
CD11b – NK, Macrophage, PMN
CD14 - monocytes
CD19 – B cells

C – Spleen in CIA

Top CIA
Bot HC



Comments
- Gates Non-spec?
- Gating (CD4 vs 44)
- Decades
- Naïve T (CD28-)

E – Synovial Tissue
Top RA SF
Bot OA SF

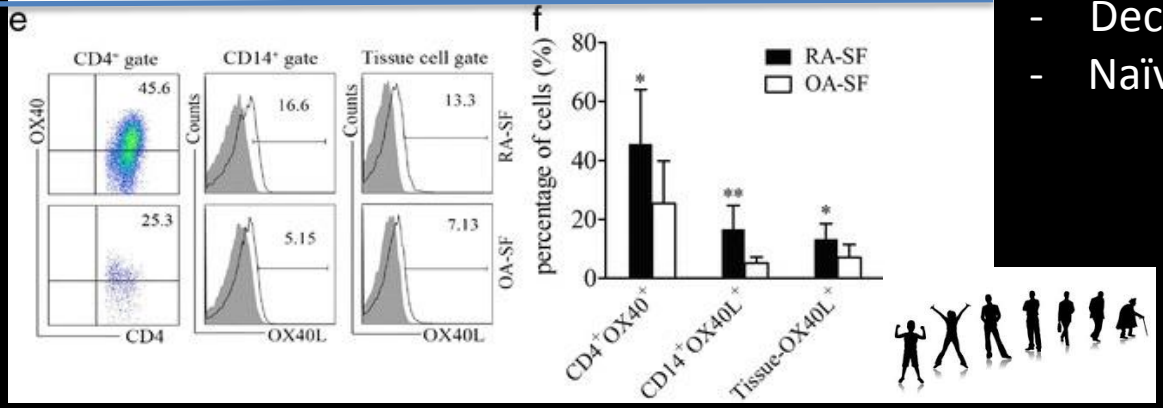


Figure 2

A,B,C - % CD4+ T cells in RA PB:
A – CD28-
B – CD28-OX40+
C - CD28+OX40+
Flow panels?

D – SF (RA vs OA) (same markers as A-C)
E – Quantification in RA vs OA.

F, G, H % splenic T cells in CIA and NC (same markers as A-C)
Flow panels?
I – CD45RA and CD45RO expression differs between CD4+CD28- and CD4+CD28+ populations in RA PB
- CD45RA – naïve; CD45RO – memory
- % look inherently incorrect to me

J – Cytokine production for gamma, IL-4, and IL-17A in PB samples from RA and control
- IFNy and IL4 with naïve cells?

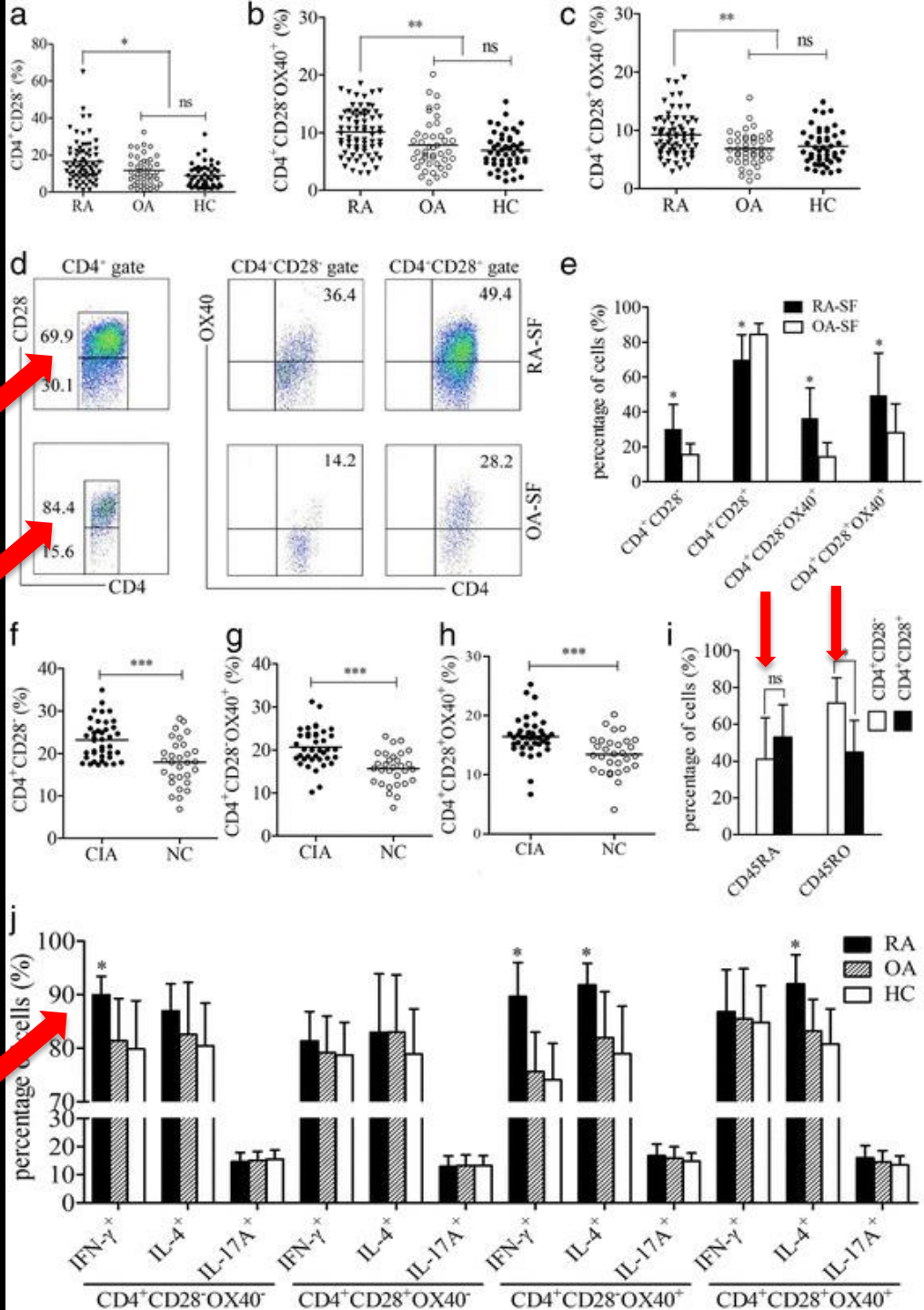


Figure 3 – $CD4^+CD28^-OX40^+$ T-cells correlate with RA clinical indicators

A – DAS28
B – RA Sev (EULAR)
C – MTX tx response
D – RA Stage (Huizinga 2002)
E – RF titer
F – CIA mouse arthritis score
G – A-CIA and C-CIA % (Thornton 2000)
H – Dex dose or PBS (DD)
I – Change in subset frequency over time (NS)

R values range 0.29 – 0.53

Citations found in Jiang et al.

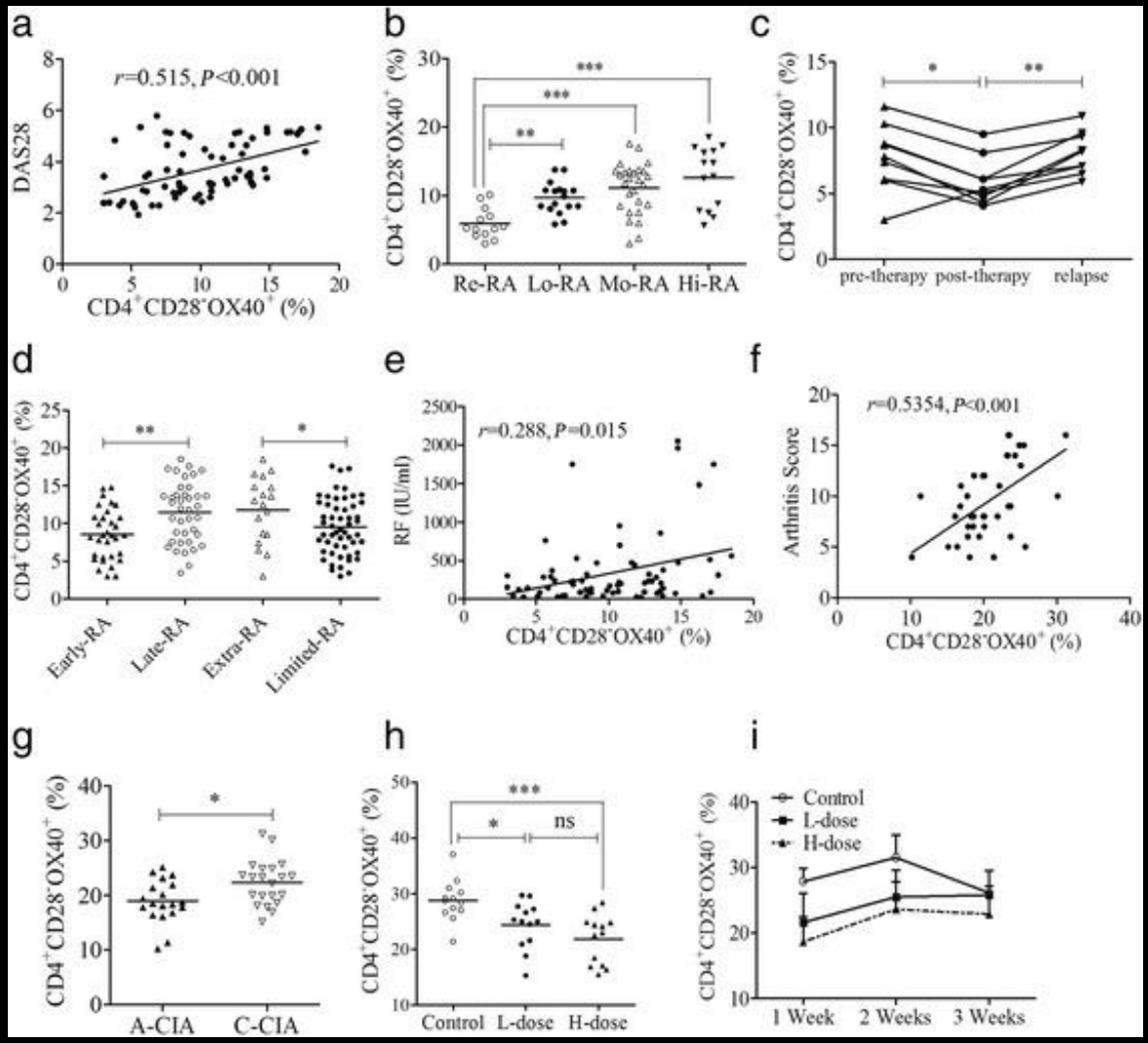


Fig 4 – Adoptive Transfer of T cell subsets into CIA mice

D28 → sac → spleen → purify → AT into D0

A – CD28+OX40-

B – CD28-OX40+

C – CD28+OX40+

D – CD28-OX40-

Arthritis began earlier and had much much higher arthritis scores in CD4+ CD28- OX40+

compared to "control"

E – (top) H&E @ 200x of ankle sections

(bot) micro-computed tomographic analysis of ankles of animals from A-D.

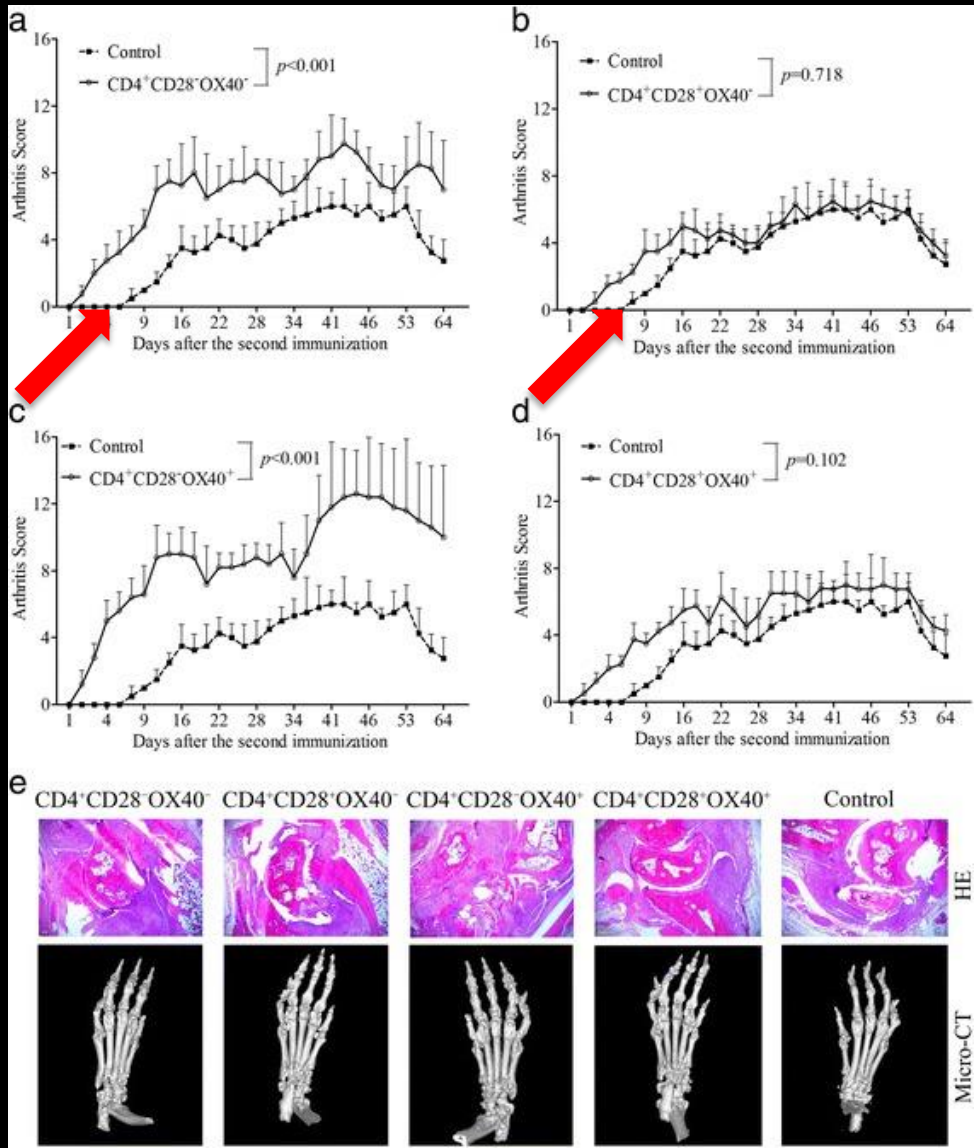


Figure 5 – *In vitro* blocking of OX40L

A OX40L blocking Ab **reduces** cell proliferation (fold change compared to IgG controls) on splenocytes

B,C OX40L blocking Ab **reduces** cytokine secretion of CII or CD3-stimulated splenocytes, respectively (pg/ml)

D28
Sac
Spleen
96 well plate
Anti-CII or anti-CD3 mAb
Stimulate
Rat IgG used for control

AT studies vs blocking studies (see next slide)

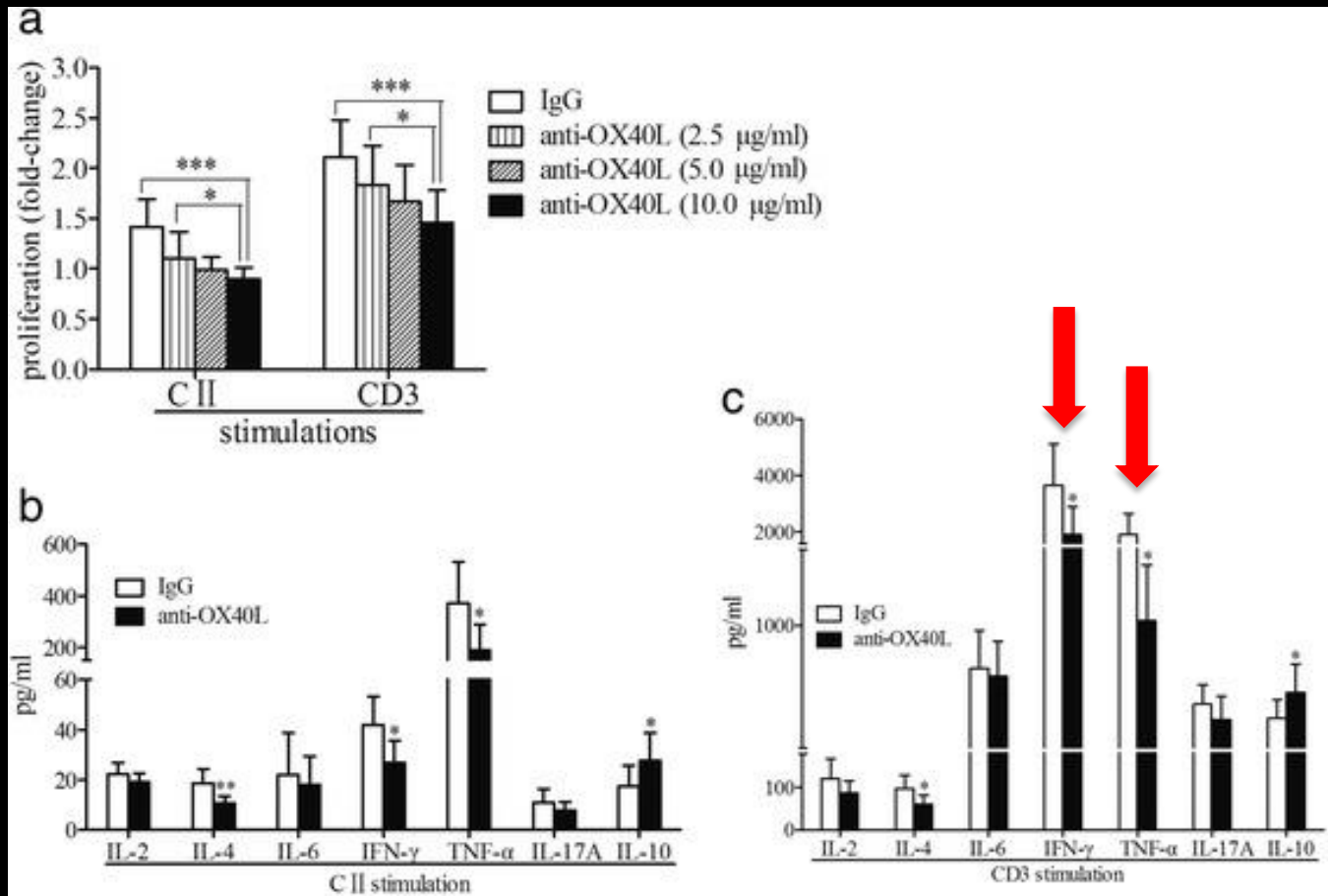


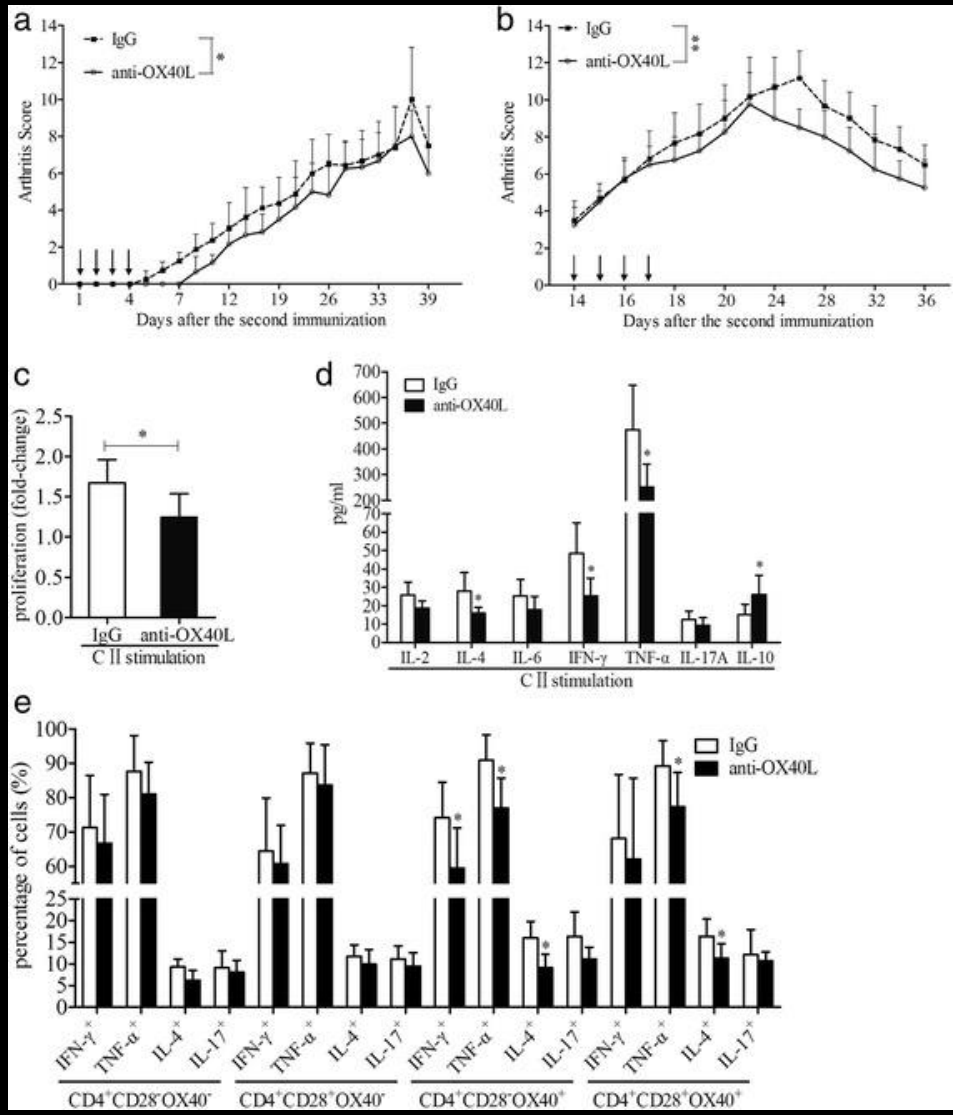
Figure 6 – OX40/40L blockade *in vivo* s.p. 2nd immunization

A,B OX40 blockade on D1 and D14 delays arthritis onset and scores of CIA mice compared with IgG controls, *in vivo*.

C Decreases proliferative index after CII stimulation

D Decreases cytokine Secretion a/f CII stimulation

E Decreases cytokine secretion in CD4+CD28-OX40+ cells, but not others.



Blocking studies on unpurified populations

- In Blocking studies (in vitro and in vivo) T cells were not purified from mononuclear splenocytes (cf. adoptive transfer studies).

- This raises a variety of technical issues.
  - Example: Mphage, DC, PMN, present
    - Have FcR
    - FcR binds heavy chain of Ab.
    - This type of interaction could be counted as a positive event rather than the desired idiotype binding
    - Could account for such high cytokine production levels
  - Too many cells per well → influences MIF
  - Etc.

Discussion

CD4+CD28-OX40+ cells are clinicopathologically significant cell type

OX40 expression level was independent of CD28 level, which fits with other studies; could also present some rationales as to why CD28 expression could be driven down

I'd like to see the findings replicated in particular a little more carefully w/ respect to the flow data and the blocking studies.

I'd also like to see cohort characteristics granted what we know of age-dependency of this T-cell subset from other studies

I'd like to see some different comparisons made than what they make in particular in Fig 4.

Thanks for your attention!