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

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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 

Abatacept modulates the immune response by binding to CD80/CD86 on an antigen-presenting cell (APC), such as a dendritic cell, thus preventing costimulatory binding of CD28 on naive T cells and attenuating T-cell activation.
CD4\(^{+}\)CD28\(^{-}\) T-cell characteristics

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 \(\rightarrow\) may help keep CD28lo.

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.

No Anergy, inc. cell proliferation
Costimulation independent T cell activation?

- Early paper

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 costimuation
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) T cell stimulation
Part II: study

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

1. Collect Clinical samples from RA patient and controls
2. Isolate subsets of interest (e.g. CD4⁺CD28⁻OX40⁺) and cytokines produced using flow cytometry
3. Transfer purified T-cell populations into CIA mice already induced with collagen type II
4. Flow cytometry
5. Adoptive transfer studies
6. In vivo and in vitro blocking studies on OX40
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^+

C - Spleen in CIA
- Top CIA
- Bot HC

E - Synovial Tissue
- Top RA SF
- Bot OA SF

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

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

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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. 2\textsuperscript{nd} 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 idiootype binding
    • Could account for such high cytokine production levels
  – Too many cells per well → influences MIF
  – Etc.
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!