The Role of Altered Cytokine Signaling in Autoimmunity

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Approaches to understand failed tolerance in T1D and Autoimmunity

- Genetic Risk Variants
- Cytokine Signaling
- T cell regulation
- T cell specificity and function
Approaches to understand failed tolerance in T1D….. and Autoimmunity

- Genetic Risk Variants
- Cytokine Signaling
- Role of IL-6 signaling in T1D
- T cell regulation
- T cell specificity and function
Checkpoints in Autoimmune Disease

T1D as a model
- Clearly defined criteria for diagnosis.
- Known genetic risks.
- Patients are not on immunosuppressive therapies.
- Young age of onset.
- Clearly defined criteria for disease risk.
- Cohorts of at-risk available

Can we then extend these observations to other autoimmune diseases?
T1D is a complex disease. Loss of tolerance occurs through multiple mechanisms and at different stages of disease.
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A translational approach to defining disease mechanisms in autoimmunity

Define functional immune phenotypes in disease
1. Use genetic variants associated with an autoimmune disease to identify pathways that are common to the disease.
2. Examine a pathway implicated by the genetics of autoimmune diseases.
3. Assess the function of cell type known to participate in the development of disease in models of autoimmunity.

Assess how perturbation of a pathway alters the immune response in disease.

Determine when a pathway is altered in the at risk population
A translational approach to defining disease mechanisms in autoimmunity

Three approaches:

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The IL-2/IL-2R pathway is implicated by the genetic studies of autoimmunity.

- IL-2 Polymorphisms: T1D, Graves’ disease, RA, celiac disease and multiple sclerosis.
- CD25 SNPs: T1D, Grave’s disease, MS and RA.
- PTPN2-snps: T1D, Crohn’s disease, Celiac Disease, UC.

Reviewed in: Wang, Seminars Immunology 2009; Rai E, Seminars in Immunology 2011.)
How do the T1D genetic risk variants alter the IL/IL-2R response?

- We first looked at the impact of these variants in healthy subjects

![Graph showing % pSTAT5 of CD4+CD25+ for different genotypes.](image)

IL-2/pSTAT5 phenotype is stable over time

Long et al Genes & Imm. 2010 and Cerosaletti Plos One 2013
Blunting of IL-2/IL-2R signaling extends to subjects with T1D

pSTAT5 is diminished in response to IL-2 in total CD4+ T cells

pSTAT5 in response to IL-2 is a consistent feature over time in

T1D risk variant

PTPN2 contributes to altered pSTAT5

Factors

PTPN2 variant

Long et al. Diabetes 2010
IL-2 alter lineage commitment contributing to early failure of T and B cell tolerance

Blunted CD4 T cell responses to IL-2 leads to early failure of T and B cell tolerance.

Blunted CD4 T cell responses to IL-2 leads to increased Tfh cells.

↓FOXP3 induction in T1D

↑FOXP3 in T1D Treg

↓FOXP3 in T1D Treg

↓IL2/pSTAT5 →↑ Tfh cells

Kenefeck R. et al JCI 2014

Long et al Diabetes 2010
What is the relevance of blunted IL-2 signaling in CD4 T cells to T1D development and progression?

- Does it predate clinical disease?
- Does it predict progression to T1D?
- Does it occur at onset of disease?

Why Ask?
- Hints about its role in the failure of tolerance early vs late.
- Type and timing of therapeutic intervention.
What is the relevance of blunted IL-2 signaling in CD4 T cells to T1D development and progression?

- Genetic Risk
- Pre-clinical Autoimmunity
  - Studies of at Risk Populations (FDR)
    - Ab- 1 Ab 2Ab >2Ab Clinical Disease
- Progressive Autoimmunity
- Clinical Disease

Ab- Relatives Ab+ non-progressors Ab+ progressors

Therapeutic Intervention
IL-2/ Rapa

% pSTAT5 of CD4+CD25+

Patients
- 001
- 002
- 003
- 004
- 005
- 006
- 007
- 008
- 009

Days
- 0
- 28
- 56
- 84
- 168
- 364

% pSTAT5 of CD4+CD25+

***  

% pSTAT5 of CD4+CD25+

***  

% pSTAT5 of CD4+CD25+

*p = 0.0156

*p = 0.0090

ns

% pSTAT5 of CD4+CD25+

% pSTAT5 of CD4+CD25+

% pSTAT5 of CD4+CD25+ 
Is blunted IL-2R signaling reproducible? Is it seen in other diseases?

A second large dataset confirms blunted IL-2/pSTAT5 in T1D memory cells.

Impaired Treg responses to IL-2 extend to MS but not SLE.

Alice Long  BRI Human Immune phenotyping core
A translational approach to defining disease mechanisms in autoimmunity

Three approaches:

1. Use genetic variants associated with an autoimmune disease to identify pathways that are common to the disease.
2. Examine a pathway implicated by the genetics of autoimmune diseases.
3. Assess the function of cytokine or cell type known to participate in the development of disease in models of autoimmunity.
IL-6 is associated with multiple autoimmune diseases

Rheumatoid arthritis
Elevated IL-6 in serum and synovial fluid
Correlation with disease activity

Psoriasis
Elevated IL-6 in psoriatic plaques
Teff escape from Treg suppression

Multiple sclerosis
Increased IL-6 in CNS of MS patients
Enhanced IL-6 signaling/Teff resistance in RRMS

Systemic Lupus Erythematosus
Elevated IL-6 and activated STAT3 in serum and lesions
Evidence that IL-6 may be driving T1D

- Increased IL-6 serum levels
- Increased IL-6 production by monocytes
- Th17 cells have been implicated
- Decrease in Treg function and numbers
- Increased resistance of T_{eff} to suppression by T_{reg}
- IL-6R variant associated with protection from T1D

1 Tharger G et al., 2001. *Diabetes Care*
2 Bradshaw EM et al., 2009. *J Immunol*
3 Marhawa AK et al., 2010. *J Immunol*
4 Arif S et al., 2011. *Diabetes*
5 Long A et al., 2010. *Diabetes*
6 Lindley S et al., 2005. *Diabetes*
7 Schneider A et al., 2008. *J Immunol*
8 Ferreira R et al., 2013. *PLOS Genetics*
Hypothesis

Enhanced T cell responses to IL-6 are present in T1D and contribute to the cascade of inflammatory events that result in beta cell destruction.

Classical IL-6 signaling

Adapted from: Calabrese, L.H. & Rose-John, S. Nat. Rev. Rheumatol. 10, 2014
Effector CD4 T cells in T1D are resistant to Treg suppression

A biostastical model of the factors which influenced the outcome of suppression demonstrates that the source of Teff alone is adequate to explain all differences in % inhibition. (p<0.0001)

Effector CD4 T cells in RRMS are resistant to Treg suppression.

Resistance correlates with disease activity.
IL-6/pSTAT3 is enhanced in RRMS CD4 T cells mediated through increased IL6R expression

Schneider A, et al. Sci Trans Medicine 2013 Jan 15
Enhanced pSTAT3 in RRMS is unique to IL-6 and its receptor

Gp130 expression is not increased

No increase in pSTAT3 with IL-27

pSTAT3 in response to IL-10 is not increased

Schneider A, et al. Sci Trans Medicine 2013 Jan 15
Teff resistance correlates with level of pSTAT3 in response to IL-6

Impaired suppression correlates with ↑ pSTAT3

Resistance is reversed by a STAT3 inhibitor

Schneider A, et al. Sci Trans Medicine 2013 Jan 15
Enhanced T cell responses to IL-6 in type 1 diabetes are associated with early clinical disease and increased IL-6 receptor expression

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Study cohort

Screened healthy controls (n = 58)¹

- Age matched adults (Ctrl: 35.2 ± 13.2y, T1D: 32.7 ± 14.8y)
- Gender matched
- Controls: no autoimmune disease or family history of autoimmunity

Subjects with T1D (n = 60)¹

- Patients: disease duration: 0.2-51y
- Study material: cryopreserved PBMC and serum

¹ Participants in the Benaroya Research Institute’s Immune Mediated Disease Registry and Repository
T cells from T1D subjects show increased pSTAT3 in response to IL-6

Red = T1D
T cells from T1D subjects show increased in IL-6R

IL-6Ra cell surface expression

IL-6Ra is increased in T1D and correlates with pSTAT3 response.
IL-6/pSTAT3 is reproducible and stable

A

Reproducibility
CD4 T cells

B

Stability
CD4 T cells

$ r = 0.95$

$ r = 0.91$
IL-6/pSTAT3 decreases with disease duration

**CD4 RA**
- $r = -0.37$
- $p = 0.06$ (ns)

**CD4 RO**
- $r = -0.45$
- $p = 0.02$

**CD8 RA**
- $r = -0.42$
- $p = 0.03$

**CD8 RO**
- $r = 0.03$
- $p = 0.9$ (ns)
IL-6/pSTAT1 T cell responses are weaker but also increased in T1D

Positive correlation between IL-6/pSTAT3 and IL-6/pSTAT1

IL-6 stim: 2 ng/ml for 10 min

controls

patients
T cell responses to IL-10 and IL-27 are similar in patients and controls

- IL-10 stim: 5 ng/ml for 20 min
- IL-27 stim: 10 ng/ml for 20 min

controls
patients

IL-6
IL-6Ra
IL-6
IL-10
IL-10R1
IL-27
IL-27Ra
IL-27
gp130
gp130
STAG3
pSTAT3
MFI gp130
mRNA expression of IL6R expression and pathway components are not altered in this T1D cohort.

IL6R358asp risk variant not a factor in IL6R expression in this T1D cohort.

qRT-PCR, CD4+CD25− T cells

- **IL-6R**
- **TYK2**
- **JAK1**
- **JAK2**
- **SOCS1**
- **SOCS3**

controls

patients
Reduced expression of IL-6R sheddase ADAM17 in subjects with T1D

Adapted from: Calabrese, L.H. & Rose-John, S. Nat. Rev. Rheumatol. 10, 2014

qRT-PCR, CD4⁺CD25⁻ T cells
IL-6 regulated gene expression in T1D: the transcriptome of CD4 T cells

- CD4^+CD25^- T cells from patients with T1D (n=7)
- untreated or stimulated with 10 ng/ml IL-6 for 24 hours
- RNA extraction
- Construction of sequencing libraries and single-read RNA sequencing
KEGG pathway analysis identifies cluster of IL-6 induced chemokines and chemokine receptors

KEGG pathway: Cytokine-cytokine receptor interaction
Fold enrichment: 1.42
P = 0.019
IL-6 upregulates genes associated with T cell trafficking and extracellular matrix degradation

Gene ontology (GO) term enrichment analysis

- locomotion
- cell chemotaxis
- regulation of cell adhesion
- inflammatory response
- regulation of cell proliferation
- positive regulation of immune system process

GO term enrichment score
Summary and Conclusions

- Enhanced CD4 and CD8 T cell responses to IL-6 in T1D:
  - correlate with increased surface IL-6R (role of ADAM17)
  - are associated with early clinical disease

- The IL-6 molecular signature of CD4 T cells from patients with T1D is characterized by genes involved in T cell trafficking and ECM remodeling

- Does the enhanced response to IL-6 in T1D result in alterations in lineage commitment Th17, Tfh, Treg? Or altered function Teff resistance?

- Does intervention in the IL-6 pathway influence T1D progression?
Conclusions

• Multiple regulatory defects may be at play in T1D.
• Each defect may impact a different stage of disease.
• Genetics can be a guide to identify pathways involved with disease.
• Studies across autoimmune disease will benefit all diseases.
• Heterogeneity among subjects may be present & help target future interventions.
A translational approach to defining disease mechanisms in autoimmunity

Develop models that mimic human disease to address mechanism and new therapeutics.
Disclosures

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