Approaches to the Cure: Nanotechnology and Immunology

Type 1 Diabetes Research Forum
Bethesda, MD
March 1, 2014

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Department of Microbiology-Immunology and The Interdepartmental Immunobiology Center Northwestern University Medical School Chicago, IL
**Immune Tolerance Strategies for Treatment of Immune-Mediated Diseases**

- **Immunologic Tolerance** - antigen-specific inhibition of selective immune responses is the ‘holy grail’ and the ‘future’ for the treatment of autoimmune diseases, allergy and tissue transplantation.

- **Question** - can ‘induced’ peripheral immune tolerance be used to treat Th1/17-mediated autoimmune disease, Th2-mediated allergic responses or prevent graft rejection in the absence of the application of immune subset depleting agents or prolonged use of immunosuppressive drugs/Abs (i.e. ‘cure’ rather than continuously ‘suppress’ disease with biologics/small molecules).

*(Scalpel vs. the Big Hammer)*

**Former Lab People**
- Carol Vanderlugt
- Todd Eagar
- Cassandra Smith
- Emma Feeney
- Danielle Turley
- Aaron Martin
- Adam Kohm

**Current Lab People**
- Daniel Getts
- Joe Podojil
- Suchitra Prasad
- Charles Smarr
- Derrick McCarthy
- Chris Harp
- Zoe Hunter
- Rachael Terry

**FSM Collaborators**
- Xunrong Luo – Islet Transplant
  - Taba Kheramand
  - Sushen Wang
- Paul Bryce – Allergy
  - Chia-Lin Hsu
- Lonnie Shea – Nanoparticles
  - Woon Teck Yap
  - Kelan Hlavaty
Model of Epitope Spreading in the Pathogenesis of Type 1 Diabetes

Luo, Herold & Miller, Immunity 32:488, 2010
Model of Epitope Spreading and Tolerance in T1D

Composition of the Immune Repertoire in a Healthy Individual vs. a T1D Patient

**Healthy Individual**

<table>
<thead>
<tr>
<th>Clone</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-Influenza Virus</td>
</tr>
<tr>
<td>2</td>
<td>Anti-Salmonella</td>
</tr>
<tr>
<td>3</td>
<td>Anti-Hepatitis Virus</td>
</tr>
<tr>
<td>4</td>
<td>Anti-Lung Cancer Protein</td>
</tr>
<tr>
<td>5</td>
<td>Anti-β cell</td>
</tr>
</tbody>
</table>

... 10-100x10^6

**T1D Patient**

<table>
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... 10-100x10^6

↓ T1D
Comparison of Immunosuppressive vs. Tolerance Therapies for Treatment of T1D

**Immunosuppressive Therapy**

**Clone 1**
- Anti-Influenza Virus
- Anti-Salmonella
- Anti-Hepatitis Virus
- Anti-Lung Cancer Protein
- Anti-β cell

**Clone 2**
- Anti-Influenza Virus
- Anti-Salmonella
- Anti-Hepatitis Virus
- Anti-Lung Cancer Protein
- Anti-β cell

**Clone 3**
- Anti-Influenza Virus
- Anti-Salmonella
- Anti-Hepatitis Virus
- Anti-Lung Cancer Protein
- Anti-β cell

**Clone 4**
- Anti-Influenza Virus
- Anti-Salmonella
- Anti-Hepatitis Virus
- Anti-Lung Cancer Protein
- Anti-β cell

**Clone 5**
- Anti-Influenza Virus
- Anti-Salmonella
- Anti-Hepatitis Virus
- Anti-Lung Cancer Protein
- Anti-β cell

**T1D**

**Tolerance Therapy**

**Clone 1**
- Anti-Influenza Virus
- Anti-Salmonella
- Anti-Hepatitis Virus
- Anti-Lung Cancer Protein
- Anti-β cell

**Clone 2**
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- Anti-Salmonella
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- Anti-β cell

**Clone 4**
- Anti-Influenza Virus
- Anti-Salmonella
- Anti-Hepatitis Virus
- Anti-Lung Cancer Protein
- Anti-β cell

**Clone 5**
- Anti-Influenza Virus
- Anti-Salmonella
- Anti-Hepatitis Virus
- Anti-Lung Cancer Protein
- Anti-β cell

**T1D**

10-100x10^6
Major Questions Regarding Tolerogenic Therapy of Type 1 Diabetes

1. Can tolerance be effectively induced to pancreatic β cell antigens – to ameliorate autoimmune disease progression?

2. Can tolerance be effectively induced to allo- or xeno-antigens – to promote indefinite acceptance of foreign islet grafts?

3. Can tolerance be effectively induced to both to autoantigens and foreign transplant antigens – to allow for the reversal of overt T1D?
Protocol for Inducing T Cell-Mediated Immunity and Antigen-Specific Tolerance Using ECDI-Fixed Splenocytes (Ag-SP)

Spleens removed from naïve syngeneic mice

RBCs lysed

Single Cell Suspension

1 hour, 4° C

Peptide

Covalent attachment of peptide

Apoptosis

Antigen-coupled Splenocytes (Ag-SP)

Tolerance

Intra- and Intermolecular Epitope Spreading in R-EAE in SJL Mice: A Model of Relapsing-Remitting Multiple Sclerosis

• Autoimmune diseases are moving targets due to epitope spreading
• Tolerance-based immunotherapy can be used to treat ongoing autoimmune disease
• Inhibition of relapses by peptide-specific tolerance administered during disease remission is only induced by the spread epitope
Can Ag-SP coupled with diabetogenic peptides be used to prevent or treat type 1 diabetes in the NOD mouse and to demonstrate that epitope spreading occurs?

Adam Kohm, Ph.D.
Suchitra Prasad
Tolerance in T1D in the NOD Mouse can be Employed to both Prevent and Treat New Onset Disease

Proposed Mechanisms of Ag-Leukocyte Tolerance
(Recapitulates how tolerance is maintained in the hematopoietic compartment)

BLOOD

I.V. Infusion of Apoptotic Autoantigen-Coupled Host Leukocytes (Ag-SP) – divert to spleen

SPLEEN

Control of Autoreactive T cells
T cell deletion or anergy from:
• negative co-stimulation
• lack of positive stimulation
IL-10 and TGFβ induce Tregs
• maintain tolerance

BLOOD

Peripheral T cell regulation

Apoptosis
• Ag-decorated apoptotic splenocytes travel to the spleen and liver and are uptaken by host APCs

Antigen Presentation
Autoantigen is:
• processed by regulatory APCs
• presented to naïve and activated autoreactive T cells
T cells get:
• negative co-stimulation
• anti-inflammatory milieu (IL-10 / TGF-β)

Immune Tolerance

Disease specific autoreactive antigen

Deletion
Anergy
Establish Tolerance In MS (ETIMS) - MRI-Controlled Phase I*/IIA Dose-Escalation Trial for Treatment of Relapsing MS Using Peptide-Coupled PBL Tolerance

Stephen Miller, Ph.D. – Northwestern Univ. Medical School
Roland Martin, M.D. & Andreas Lutterotti, M.D. – Univ. of Hamburg/Zürich

Early R-R MS Patient

Leukocytapheresis

i.v. re-infusion of autologous Ag-PBLs (3x10^9)

Antigen-coupled PBLs (Ag-PBL)

Cocktail of Immunodominant Myelin Peptides

MBP13-32
MBP83-99
MBP111-129
MBP146-170
MOG1-20
MOG35-55
PLP139-154

Funding:
German Government
Cumming Foundation
Myelin Repair Foundation

*Phase I - successfully completed 6/2012, Phase IIa – in development
Antigen-Specific Tolerance by Autologous Myelin Peptide–Coupled Cells: A Phase 1 Trial in Multiple Sclerosis

Andreas Lutterotti,¹,² Sara Yousef,¹ Andreas Sputtek,³ Klarissa H. Stürner,¹ Jan-Patrick Stellmann,¹ Petra Breiden,³ Stefanie Reinhardt,¹ Christian Schulze,⁴ Maxim Bester,⁵ Christoph Heesen,¹ Sven Schippling,¹,⁶ Stephen D. Miller,⁷⁎ Mireia Sospedra,¹,⁶⁎ Roland Martin¹,⁶⁎

Multiple sclerosis (MS) is a devastating inflammatory disease of the brain and spinal cord that is thought to result from an autoimmune attack directed against antigens in the central nervous system. The aim of this first-in-man trial was to assess the feasibility, safety, and tolerability of a tolerization regimen in MS patients that uses a single infusion of autologous peripheral blood mononuclear cells chemically coupled with seven myelin peptides (MOG1–20, MOG35–55, MBP13–32, MBP83–99, MBP111–129, MBP146–170, and PLP139–154). An open-label, single-center, dose-escalation study was performed in seven relapsing-remitting and two secondary progressive MS patients who were off-treatment for standard therapies. All patients had to show T cell reactivity against at least one of the myelin peptides used in the trial. Neurological, magnetic resonance imaging, laboratory, and immunological examinations were performed to assess the safety, tolerability, and in vivo mechanisms of action of this regimen. Administration of antigen-coupled cells was feasible, had a favorable safety profile, and was well tolerated in MS patients. Patients receiving the higher doses (>1 × 10⁹) of peptide-coupled cells had a decrease in antigen-specific T cell responses after peptide-coupled cell therapy. In summary, this first-in-man clinical trial of autologous peptide-coupled cells in MS patients establishes the feasibility and indicates good tolerability and safety of this therapeutic approach.
Effect of Tolerance on Myelin Peptide-Specific T Cell Responses in Patients Receiving Hi vs. Low Dose Ag-PBMC

Ag-coupled biodegradable PLG nanoparticles serve as surrogates for apoptotic membranes for efficient induction of tolerance

Antigen-coupled apoptotic cells 1979
Antigen-coupled carboxylated PLG Nanoparticle 2008
Tolerogenic Immune Modifying Particle (TIMP) 2012

Next Generation

Miller Lab – Northwestern University
Daniel Getts, Ph.D.
Aaron Martin, Ph.D.
Chris Harp, Ph.D.
Derrick McCarthy, B.S.
Zoe Hunter, Ph.D.
Rachael Terry

Lonnie Shea – Northwestern University
Woon Teck Yap
Kelan Hlavaty
**Tolerance Induced by PLP\textsubscript{139-151}-Coupled PLG Nanoparticles Inhibits Induction and Progression of PLP\textsubscript{139-151} R-EAE**

Microparticles bearing encephalitogenic peptides induce T-cell tolerance and ameliorate experimental autoimmune encephalomyelitis

Daniel R Getts\textsuperscript{1,2,7}, Aaron J Martin\textsuperscript{1,2,7}, Derrick P McCarthy\textsuperscript{1,2}, Rachael L Terry\textsuperscript{3}, Zoe N Hunter\textsuperscript{1,2}, Woon Teck Yap\textsuperscript{4}, Meghann Teague Getts\textsuperscript{1,2}, Michael Pleiss\textsuperscript{5}, Xunrong Luo\textsuperscript{1,6}, Nicholas JC King\textsuperscript{3}, Lonnie D Shea\textsuperscript{4} & Stephen D Miller\textsuperscript{1,2}

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### C. Active EAE Day -7: Prevention

![Graph showing prevention of disease progression](image)

- **Rx**: Prevention strategy
- **Mean Clinical Score** vs. **Day Post Immunization**
- **OVA\textsubscript{323-339}PLG\textsubscript{PEMA} (n=5)**
- **PLP\textsubscript{139-151}PLG\textsubscript{PEMA} (n=5)**

### D. Active EAE Onset: Treatment

![Graph showing treatment strategy](image)

- **Rx**: Treatment strategy
- **Mean Clinical Score** vs. **Day Post Immunization**
- **OVA\textsubscript{323}-PLG (n=5)**
- **PLP\textsubscript{139}-PLG (n=5)**

### E. Active PLP\textsubscript{178-191} EAE Remission: Reversal

![Graph showing remission](image)

- **Rx**: Reversal strategy
- **Mean Clinical Score** vs. **Days Post Immunization**
- **OVA323-PLG**
- **PLP139-PLG**

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[Diameter (nm)](image)

- **Intensity** vs. **Diameter**
- **PLS**
- **PLG**
Ag-PLG Tolerance Regulates Development of T1D in NOD Mice and Works via Activation of Tregs
Tolerance Induced by Insulin-Coupled PLG Nanoparticles Inhibits Development of T1D in NOD Mice

PLG-BSA or PLG-Ins i.v. at 6, 8 and 10 weeks of age

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**Tolerance Induced Ag-PLG Nanoparticles Inhibits Spontaneous T1D and T1D Induced by Transfer of Activated BDC2.5 Tg T Cells**

A

- BDC2.5 (3 wks) spleen, axillary, brachial, inguinal and pancreatic LN.
- Activate in vitro
  - $2 \times 10^6$ cells/mL with 0.5µM p31 peptide for 96 hr
- $5 \times 10^6$ cells Transferred i.v. to NOD.SCID (6-8wks)
- Tolerized i.v. p31- or MOG$_{35-55}$-PLG 2h later

B

- Blood Glucose (mg/dL)
- Days Post Transfer
- CG p31-PLG, n=5
- MOG$_{35-55}$-PLG, n=5

C

- Spleen
  - CG p31-SP: 5.34
  - MOG$_{35-55}$-SP: 8.21
- Pancreatic LN
  - CG p31-SP: 20.50
  - MOG$_{35-55}$-SP: 2.20
- Pancreas
  - CG p31-SP: 1.76
  - MOG$_{35-55}$-SP: 22.00
- Isotype
  - CD4
    - IFN-γ
      - 0.69
      - 0.03

D

- NOD
- PLG-BSA or PLG-Ins i.v. at 6, 8 and 10 weeks of age
- Percent Non-Diabetic
- Age (days)
- PLG-insulin (n=23): 16/23 (69.6%)*
- PLG-BSA (n=22): 5/22 (22.7%)*
- *p=0.0027

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*Prasad, et al., unpublished.*
Tregs are Required for Tolerance Induction Using Ag-PLG NP in Adoptive Transfer T1D Mediated by BDC2.5 Tg T Cells

- BDC2.5 Mice - TCR specific for Chromogranin A (Vasostatin-1)

BDC2.5 (3 wks) spleen, axillary, brachial, inguinal and pancreatic LN.

Activate in vitro
2x10^6 cells/mL with 0.5μM p31 peptide for 96 hr

5x10^6 cells Transferred i.v. to NOD.SCID (6-8wks) at Time 0

Tolerized i.v. with p31- or MOG35-55-PLG 2h later

Pre-Sort

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<thead>
<tr>
<th>CD25</th>
<th>Foxp3</th>
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Post-Sort

<table>
<thead>
<tr>
<th>CD25</th>
<th>Foxp3</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.5</td>
<td>0.241</td>
</tr>
</tbody>
</table>

MOG35-PLGA N=5
p31-PLGA N=5
Treg depleted p31-PLGA, N=3
Treg depleted MOG35-55-PLGA, N=4

Proposed Mechanisms of Ag-Nanoparticle Tolerance
(Recapitulates how tolerance is maintained in the hematopoietic compartment)

**Apoptosis**
- Apoptosis of TIMP-monocytes in the spleen
- TIMP and apoptotic debris picked up by antigen presenting cells (APCs)

**Antigen Presentation**
Autoreactive antigen is:
- processed by APCs
- presented to T cells

T cells get:
- negative co-stimulation
- anti-inflammatory milieu (IL-10 / TGF-β)

**Immune Tolerance**
T cell deletion or anergy from:
- negative co-stimulation
- lack of positive stimulation

IL-10 and TGF-β induce Tregs
- maintain tolerance

**Proprietary surface modification**

**Proposed Mechanisms of Ag-Nanoparticle Tolerance (Recapitulates how tolerance is maintained in the hematopoietic compartment)**

TIMPs bind to monocytes – divert to spleen

Antigen Presenting Cells (APC) take up & process TIMP

Control of Autoreactive T cells

Deletion

Anergy

Peripheral T cell regulation

BLOOD

SPLEEN

BLOOD

TIMPs bind to monocytes – divert to spleen

Disease specific autoreactive antigen

Apoptosis

Antigen Presentation

Immune Tolerance

Proposed Mechanisms of Ag-Nanoparticle Tolerance (Recapitulates how tolerance is maintained in the hematopoietic compartment)

TIMPs bind to monocytes – divert to spleen

Disease specific autoreactive antigen

Apoptosis

Antigen Presentation

Immune Tolerance

Proposed Mechanisms of Ag-Nanoparticle Tolerance (Recapitulates how tolerance is maintained in the hematopoietic compartment)
Advantages of Ag-PLG NPs for Treatment of (Auto)Immune Diseases vs. Ag-Pulsed/Ag-Directed Immature Tolerogenic DCs or Engineering of Ag-Specific Tregs

- Rapidity and simplicity of tolerogen preparation and induction using a GMP manufacturable off-the-shelf ‘universal’ tolerogenic carrier
- No need to isolate and expand immature DCs (or Tregs) ex vivo
- No need to be concerned with immature DCs being activated upon ex vivo manipulation and becoming ‘stimulatory’ rather than tolerogenic; or Tregs converting to Th1/17 after transfer
- Directs Ag to ‘tolerogenic DCs in splenic MZ and liver - since the ‘host’ immature APCs process and represent the antigen in a tolerogenic manner, host APCs can select the relevant immunodominant self epitopes from PLG NPs encapsulating intact auto-Ags or tissue extracts (e.g. OVA encapsulated PLG NPs prevent OVA/Alum-induced AAD)
- The protocol appears to be exquisitely Ag-specific (no apparent bystander suppression), non-toxic, safe, highly efficient and can induce unresponsiveness in both effector T cells (Th1, Th2, Th17 and CD8) and naïve T cells involved with epitope spreading
Can apoptotic allogeneic or xenogeneic leukocytes be used to induce allo/xenoantigen-specific tolerance to prevent rejection of transplanted islets?
Protocol for Inducing Alloantigen-Specific Tolerance Using ECDI-Fixed Splenocytes

Spleens removed from naïve donor mice

BALB/c

C57BL/6

RBCs lysed

Single Cell Suspension

1 hour, 4°C

ECDI

Apoptotic Allogeneic Donor Splenocytes

i.v.
Islet Transplant Tolerance by ECDI-Coupled Donor Splenocytes is Donor-Specific in SZ-Treated B6 Recipients of BALB/c Islets

- Tolerance is donor-specific and requires ECDI fixation (apoptosis)
- Tolerance protects islets transplanted i.p. on PLG scaffolds and islets transplanted intraportally

B Cell Depletion (Anti-CD20) Promotes Acceptance of Islet Xenografts

Xenogeneic Rat → B6

![Graph showing percent survival days post transplantation for different treatment groups: No Treatment n=5, ECDI-BALB SP, n=5, αCD20 Alone n=3, and αCD20 + ECDI-BALB SP n=5. The graph indicates significant differences with *p<.001 and *p=.003.](image-url)
Progress in Answering Major Questions Regarding Tolerogenic Therapy of Type 1 Diabetes

1. Can tolerance be effectively induced to pancreatic β cell antigens to ameliorate autoimmune disease progression - YES
2. Can tolerance be effectively induced to allo- or xeno-antigens to promote indefinite acceptance of foreign islet grafts - YES
3. Can tolerance be effectively induced to both to autoantigens and foreign transplant antigens to allow for the reversal of overt T1D? - ?UNDER INVESTIGATION?

The Path Forward to Clinical Translation of Ag-PLG Nanoparticle Therapy of Type 1 Diabetes

Potential Clinical Disease Indications Supported by Pre-Clinical Data:
  • Autoimmune diseases – MS; T1D; Celiac Disease
  • Allergic diseases – allergic asthma, food allergies
  • Protein replacement/gene therapy – hemophilia
  • Tissue and organ transplantation, BMT, GVHD – islet transplantation