Immune considerations relevant to DMD and dystrophin replacement/correction therapies

(not all immune responses are bad)

M. Carrie Miceli, Ph.D.
Professor of Microbiology, Immunology and Molecular Genetics
Co-Director, Center for Duchenne Muscular Dystrophy
David Geffen School of Medicine and College of Letters and Sciences at UCLA
277B Biomedical Sciences Research Building
Basic Immunology: Self Non-Self Discrimination for Defense, Self-Tolerance, and Regeneration

**Innate Immune Response**
- 1st line of defense
- Looks for Danger
- Associated Molecular Pattern = DAMPS
- bacterial sugars
- structure
- Viral capsid, viral DNA/RNA
- Stressed or dying cells
- Alerts Adaptive Immune Cells to DANGER

**Adaptive Immune Response**
- high specificity
- (viral AAV capsid or micro-dystrophin peptides)

**Memory**
- B cell antibodies
- viral neutralizing

**T cells**
- Aggressive (Cytotoxic Lymphocytes CTL)
- Tolerizing
  - (Treg + others)
- Regenerative
  - Treg, M2 + others

Figure 2. Innate and adaptive immunity time line. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and require the activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and may vary in different infections.
Immune response to muscle damage guides regeneration

Acute injury

Macrophage depletion
In response to acute injury waves of infiltrating cells coordinate patching, stem cell activation, muscle repair.

- Innate immunity
- Adaptive immunity

In DMD,
- Chronic damage
- Asynchronous repair
- Improper resolution
- Ineffective regeneration
- Profibrotic

Can we reset?
Can we intervene with drugs:
- Antifibrotics or immune modulators
- Dystrophin replacement

Activates complement to create a fibrin/platelet patch (clot) at the lesion site.
Clear debris, pro-inflammatory cytokines cells.
Repair and resolution

Muscle Regeneration
In Duchenne Muscular Dystrophy chronic injury prevents resolution or immune response, drives muscle damage and fibrosis.
A Special Population of Regulatory T Cells Potentiates Muscle Repair and Inhibits Fibrosis

T-Reg’s
Suppress specific inflammatory immune responses
Blocks fibrosis (IL-10)
Promote muscle regeneration (amphiregulin)

Upregulation improves mdx DMD mouse
Downregulation worsens

Cell, Volume 155, Issue 6, 2013, 1282 - 1295

Dalia Burzyn, Wilson Kuswanto, Dmitriy Kolodin, Jennifer L. Shadrach, Massimiliano Cerletti, Young Jang... Diane Mathis...
**Can immune modifiers limit fibrosis/promote regeneration?**

Rosenberg and Woodcock, *Nature Immunology*

---

### Immunomodulators in DMD

NFAT, nuclear factor of activated T cells; PDE, phosphodiesterase.

<table>
<thead>
<tr>
<th>Drug/compound</th>
<th>Target</th>
<th>Pathological process</th>
<th>Preclinical trials</th>
<th>Clinical trials/use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone, deflazacort</td>
<td>NF-κB, others</td>
<td>Anti-inflammatory</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>VBP15</td>
<td>NF-κB, membrane protection</td>
<td>Anti-inflammatory, sarcolemma stability</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>NFAT</td>
<td>Anti-inflammatory</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>Purine synthesis</td>
<td>Anti-inflammatory</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Poloxamer</td>
<td>Membrane synthesis</td>
<td>Sarcolemma stability</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gene therapy</td>
<td>Dystrophin replacement</td>
<td>Sarcolemma stability</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Exon skipping</td>
<td>Dystrophin replacement</td>
<td>Sarcolemma stability</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>TLR7/8/9 antagonists</td>
<td>TLR7/8/9</td>
<td>Anti-inflammatory</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>NEMO peptide</td>
<td>NF-κB</td>
<td>Anti-inflammatory</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Infliximab</td>
<td>TNF-α</td>
<td>Anti-inflammatory</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>IL-2/anti-IL-2 complex</td>
<td>Tregs</td>
<td>Anti-inflammatory</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pentoxifyline</td>
<td>PDE inhibitor</td>
<td>Anti-fibrotic</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pirfenidone</td>
<td>TGF-β signaling</td>
<td>Anti-fibrotic</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Losartan</td>
<td>Angiotensin type 1 receptor inhibitor</td>
<td>Anti-fibrotic</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>Angiotensin-converting enzyme inhibitor</td>
<td>Anti-fibrotic</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Anti-IL-6</td>
<td>IL-6</td>
<td>Anti-inflammatory</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Anti-myostatin antibodies</td>
<td>Myostatin</td>
<td>Anti-fibrotic, hypertrophy</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cromolyn</td>
<td>Mast cells</td>
<td>Membrane stability</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

**Current treatments**

**Future options**

- Chloroquine
- Eculizumab
- Rapamycin
- Plerixafor
- IL-10
- Anti-osteopontin antibodies
- Candesartan

Lysosomal pH
Complement C5
Tregs + Akt/mTOR
CXCR4
Alternatively activated macrophages
Osteopontin
Angiotensin type 2 receptor inhibitor
Anti-inflammatory
Anti-inflammatory
Anti-inflammatory, regeneration
Anti-inflammatory
Anti-inflammatory, anti-fibrotic
Anti-fibrotic

No
No
No
No
No
No
No
No
No
Dystrophin replacement strategies
self/non-self discrimination

• Will there be an immune response that limits safety or efficacy?
• -exon skipping, NS read-through and micro-dystrophin gene therapy all strive to make an altered dystrophin protein in boys who lack dystrophin.
  • Will this dystrophin proteins be seen as non-self threat?
• Micro-dystrophin gene therapy has additional potential immune challenge to AAV vector
• Can we induce specific self-tolerance to AAV/dystrophin?
• Will dystrophin replacement prevent/reverse immune pathology?
  • Reverse tissue damage and fibrosis, while promoting regeneration
Gene therapy for DMD

Full-length dystrophin (Hoffman et al 1987)

ΔDysM3 (Yuasa et al 1997)

Δ3990 (Wang et al 2000)

ΔR4-23/ΔC (Harper et al 2002) (also called ΔCS1, MD1, H2μDys)

μDys-5R (Hakim et al 2017)

*slide courtesy of Dongsheng Duan

Sarepta
We don’t know if there will be a immune response to AAV micro-dystrophin gene therapies that limits/tempers efficacy or safety in AAV sero-negative DMD patients? Complement activation?

Why are AAV seropositive boys currently excluded from trials?
Exposure to AAV in the wild induces production of AAV specific neutralizing antibodies that can block GT delivery.

80% neutralizing
Immune Response to AAV: pre-existing antibodies
### Potential Solutions for Pre-formed Antibodies

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Pros</th>
<th>Cons</th>
<th>Clinical feasibility</th>
</tr>
</thead>
</table>
| Select patients with low or no NAbs          | - No need for intervention  
- Simple to implement (128)                                                                                                                  | - Can result in exclusion of several candidates (125)                                                                                                                                            | Currently broadly adopted in gene therapy trials                                                                                                    |
| Use less-seroprevalent capsids or switch serotype | - No need for pharmacological intervention  
- Proof-of-concept studies in monkeys and humans promising                                                                                   | - Almost all serotypes are cross-neutralized (125)  
- Each new serotype is a new product to be developed                                                                                           | Hard to implement due to the high costs associated with bringing multiple serotypes to the clinic                                                |
| Plasmapheresis (134, 135)                     | - Safe and effective in reducing antibody titers  
- Proof-of-concept studies in monkeys and humans promising                                                                                   | - Requires multiple cycles of plasma absorption  
- Less efficient with high-titer NAbs  
- Nonspecific, depletes all immunoglobulins                                                                                                     | Likely feasible, technology already available in hospitals                                                                                         |
| Immunosuppression                             | - Some technologies seem promising (136-138)                                                                                                                                                    | - Most drugs ineffective at eradicating antibodies (138)  
- Global immunosuppression associated with side effects and can interfere with gene transfer (30, 139)                                      | Feasible, granted a favorable risk/benefit ratio; most likely effective in the prevention setting (to allow for vector readministration) (140) |
| Isolated organ perfusion                      | - Proof-of-concept results promising in liver gene transfer (141)  
- Does not require immunosuppression                                                                                                        | - Does not work well in the presence of high-titer NAbs  
- Not useful in the setting of systemic diseases                                                                                                  | Procedure not currently in use in the clinic; invasive                                                                                              |
| Increase the capsid dose or use capsid decoys | - Proof-of-concept results promising in liver gene transfer (66)  
- Does not require immunosuppression                                                                                                        | - Higher vector doses may pose a constraint in terms of manufacturing  
- Unlikely to be effective with NAb titers >1:100 (66)                                                                                          | Feasible, but may contribute to vector antigen load                                                                                               |
Pre-existing AAV or dystrophin reactive T cells in DMD?

Muscle in DMD is not “normal”

Chronic Immune Activation
upregulation of
class I MHC
class II MHC
TLR7
cytokines

Screen for and exclude individuals with pre-existing AAV or dystrophin reactive T cells (g-IFN)

immunosuppress

<table>
<thead>
<tr>
<th>Muscle environment</th>
<th>Normal</th>
<th>Inflamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of delivery</td>
<td>Intravascular</td>
<td>Intramuscular</td>
</tr>
<tr>
<td>Genetic background</td>
<td>Presence of nonfunctional endogenous protein</td>
<td>Complete lack of endogenous protein</td>
</tr>
<tr>
<td>Expression cassette</td>
<td>Muscle specific or detargeted from antigen-presenting cells</td>
<td>Constitutive expression cassette</td>
</tr>
<tr>
<td>AAV vector genome</td>
<td>Single-stranded</td>
<td>Self-complementary</td>
</tr>
</tbody>
</table>
Exposure to wild-type AAV drives distinct capsid immunity profiles in humans


NOT tested in DMD

Deep immune profiling

A unique moDendritic cell population identified which produces IL-6 and IL-1b; blocking IL-1b with antibodies prevented AAV antibody production.

(AAV2 and AAV8)
Immune Response to AAV: Innate Immune Response

Mingozzi et al
DAMPS-Danger associated molecular pattern receptors
1\textsuperscript{st} line of defense; alert adaptive response
Can we identify players and modulate?

- Pro-inflammatory Cytokines/Chemokines
  - IL-6 and IL-1\textbeta and others

- Phagocytes
  - Complement Activation

- Upregulation of T cell co-stimulators on antigen presenting cells

Broad exposure to DAMER=
- Capsid proteins
- ssDNA
- dsDNA
- Bacterial sugars
- Cell stress
- Cell damage

Candidate AAV GT triggers:
- AAV capsid
- TLR2
- AAV vector/ssDNA
- TLR9

In DMD
- TLR7
- ssRNA

`Figure 3. Toll-like receptor (TLR) leukocyte expression patterns and PAMP specificities.`
Adaptive immunity: B cells and T cells each have surface receptors that recognize specific antigens, triggering the activation and development of immune effectors and memory cells.
Diverse CD4 and CD8 T cells subsets regulate immune activation and self tolerance

Subset distinguished by co-expression of surface antigens and functional output; plasticity and intermediates observed
Immune Response to AAV: Adaptive Immunity Specific for AAV-vector or for dystrophin transgene?

- Neutralizing anti-capsid antibodies
- CD4 T cells (B helpers)
- CD8 CTL
- Anti-capsid CD8 T-cell
- CD4 T cells (CD8 helpers)
- Tregs and others

Issues in AAV gene therapy:
1. Immunogenicity
2. Potency & Efficacy
3. Genotoxicity
4. Persistence

Innate immune response
- Endocytosis
- Endosomal escape
- Nuclear localization
- Uncoating
- SS
- SC

Episomal AAV genome
- Integrated AAV genome

Micro-dystrophin or AAV capsid

CD8 CTL

CD4 T cells (CD8 helpers)

(Tregs and others)
Potential Problems and Solutions

AAV Immune Response

<table>
<thead>
<tr>
<th>Immune Responses in the Human Host</th>
<th>Possible Solutions$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-capсид Immunity</td>
<td></td>
</tr>
<tr>
<td>Pre-existing neutralizing antibodies (NAbS) toward the capsid proteins$^{16,181}$</td>
<td>selection of patients with low or no neutralizing antibodies$^{82}$</td>
</tr>
<tr>
<td></td>
<td>plasmapheresis$^{196,197}$</td>
</tr>
<tr>
<td></td>
<td>use of less seroprevalent capsids$^{62}$</td>
</tr>
<tr>
<td></td>
<td>not-cross-reactive engineered capsids$^{79}$</td>
</tr>
<tr>
<td></td>
<td>capsid decoy$^{67}$</td>
</tr>
<tr>
<td>CD8$^+$ T cell-mediated cytotoxic immune response toward transduced cells presenting AAV capsid antigens</td>
<td>prevention of NAb induction by using immunosuppressive drugs to allow AAV re-administration (if required)$^{195,198}$</td>
</tr>
<tr>
<td></td>
<td>reduction of AAV capsid antigen load by decreasing therapeutic doses$^{199}$ and/or removal of empty capsids from vector preparations</td>
</tr>
<tr>
<td></td>
<td>use of immune suppression (on demand or up front depending on the availability of biomarkers and endpoints, e.g., elevation of liver enzyme upon intravenous AAV administration)$^{204,208,40}$</td>
</tr>
</tbody>
</table>

Dystrophin Immune Response

- Anti-transgene Immunity
  - selection of subjects having low risk of developing anti-transgene immune responses (e.g., subjects bearing missense rather than null disease causative mutations)
  - use of immune suppression$^{198}$
  - use of strategies to induce immune tolerance$^{51,89-93,199}$
- Development of antibodies toward the transgene product$^b$
  - use of immune suppression (on demand or up front depending on the availability of biomarkers and endpoints)
  - use strategies to induce immune tolerance$^{202}$
  - de-targeting transgene expression from antigen-presenting cells$^{203}$

$^a$Include strategies at different stages of development (preclinical and clinical settings).
$^b$Observed in animal models, not observed so far in human clinical trials.
$^c$Observed so far in human clinical trials of AAV-muscle gene transfer.
The goal of T cell immunosuppression for gene therapy is to block Teff and induce tolerance (Tregs + other).

Can we borrow from fields of transplantation or autoimmunity where the goal is also to dampen inflammation and tolerize?

Impact of Immune-Modulatory Drugs on Regulatory T Cell
Furukawa, Wisel, MD, and Tang, Transplantation 2016;100: 2288–2300

All T/B cell responses
Vs novel mechanism/drugs for inducing antigen specific tolerance.

Barry B
Tacromilus /rituximab (rapamycin)
T cell activation versus tolerance dictated by:
(generalizations)

• Status of the APC and local cytokines

• Upregulation of inhibitory proteins on the T cell surface

• Low level chronic exposure to antigen often tolerogenic

Front. Immunol., 09 November 2015
| https://doi.org/10.3389/fimmu.2015.00569
# Ongoing approaches to induce antigen specific tolerance

<table>
<thead>
<tr>
<th>Type of approach</th>
<th>Modality</th>
<th>Institutions supporting the concept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal deletion using pre-apoptotic cells</td>
<td>With autologous peripheral blood mononuclear cells; in vitro coupled to a cocktail of autoantigen-derived peptides prior to cell transfer</td>
<td>Cellerys</td>
</tr>
<tr>
<td></td>
<td>With autologous RBCs; in vitro coupled or loaded with autoantigens/autoantigen-peptides</td>
<td>Rubius Therapeutics, SQZ Biotechnologies</td>
</tr>
<tr>
<td></td>
<td>With autologous RBCs; in vivo targeted with RBC-binding molecules fused to autoantigens/autoantigen-peptides</td>
<td>Anokion/Celgene, Kanyos (Anokion/Astellas)</td>
</tr>
<tr>
<td>Therapeutic immunization</td>
<td>With peptide or whole autoantigen proteins, alone or as cocktails, with or without adjuvants</td>
<td>Apitope, Diamyd Medical, Immusant, Orban Biotech, UCB Pharma</td>
</tr>
<tr>
<td></td>
<td>With DNA vaccines</td>
<td>Tolerion</td>
</tr>
<tr>
<td></td>
<td>With autoantigenic peptides containing thioredoxin motifs</td>
<td>Incyte</td>
</tr>
<tr>
<td>Cell-based approaches</td>
<td>Transferring autologous dendritic cells differentiated in vitro using cytokines, vitamin D3, dexamethasone, or genetically engineered to downregulate costimulatory molecules</td>
<td>Baylor Research Institute, Diavacs, Leiden University</td>
</tr>
<tr>
<td></td>
<td>Transferring in vitro inactivated autologous autoantigen-specific T cells to expose ergotypic antigens</td>
<td>Opeka Therapeutics</td>
</tr>
<tr>
<td></td>
<td>Transferring autologous regulatory chimeric antigen receptor-T (CAR-T reg) cells</td>
<td>Tcell/Sangamo</td>
</tr>
<tr>
<td></td>
<td>Administering engineered bacteria expressing host autoantigens together with host immune modulators</td>
<td>ActoBio/Intrexon, Allero Therapeutics</td>
</tr>
<tr>
<td>Engineered nanomedicines</td>
<td>Delivering autoantigenic peptides/proteins, alone or in combination with immunomodulatory agents, to APCs using nanoparticle vehicles</td>
<td>AntolRx/Pfizer, Cour Pharmaceuticals, Dendright/Janssen Biotech, Midatech Pharma, Regimmune, Selecta Biosciences, Toleranzia, Topas Therapeutics, Toralgen</td>
</tr>
<tr>
<td></td>
<td>Directly targeting autoantigen-specific T cells with pMHC proteins coated onto nanoparticles, to reprogram and expand cognate T reg cells</td>
<td>Parvus Therapeutics/Novartis</td>
</tr>
</tbody>
</table>
Is pre-exposure to dystrophin inconsequential, tolerizing, or activating?

• Revertant fibers?
• Exon skipping pre-treatment?
• Ataluren pretreatment?

• Priming (vaccination)
• Tolerance?
  • Low levels of chronic activation can lead to clonal T cell exhaustion/anergy
  • Combination therapy?
Co-administration of rapamycin nanoparticles with AAV prevents activation of AAV specific B and T cell and induction of memory responses in mice and non-human primates (Not tested in DMD).

Likely through induction of antigen specific T regulatory cells
Cas9 is a bacterial protein; pre-formed antibody T cell immunity immunity blocking efficacy

Identification of preexisting adaptive immunity to Cas9 proteins in humans


Nature Medicine 25, 249–254 (2019) | Download Citation

High prevalence of Streptococcus pyogenes Cas9-reactive T cells within the adult human population


Nature Medicine 25, 242-248 (2019) | Download Citation

0% had T cells specific for Cas9
65% had antibodies specific for Cas9
96% had Cas9 specific T cells
85% had antibodies specific for Cas9

Exclude patients with preformed immunity? Immunosuppress? Identify Cas9-like protein with lo/no immunogeneicity.
How do we monitor response?

• Muscle biopsy- limited number
  • how many; and when
  • Needle biopsies vs open muscle biopsy (infiltrate and regeneration evaluation)

• MRI/MRS- Imaging DMD

• Peripheral blood –
  • Standards for human immune monitoring of subsets evolving with improved ability to characterize subpopulations and functionality
  • Can detect AAV/dystrophin reactive T cells in blood
  • Can better characterize T cell subsets using multi-parameters
    • Deep immune profiling using CyTOF and single cell RNAseq
  • Perhaps a signature can serve as a biomarker for efficacy or tolerance
Disclosures: Myself or a member of my family has received compensation and/or travel from the above.
Adaptive Immunity: First exposure activates B and T cells for defense and memories that can respond faster and better upon re-dosing.