Immune considerations in DMD relevant to gene therapy

Not all immune responses are bad
- inconsequential
- clearance/rejection
- active tolerance induction
- memory responses

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SUMMARY POINTS

1. AAV vector-mediated gene transfer has resulted in long-term therapeutic efficacy in humans affected by a variety of diseases. However, preclinical and clinical experience indicates that components of AAV vectors can be recognized by the host immune system.

2. Thus far, no serious or permanent consequences of immune responses, other than a transient, asymptomatic elevation of liver enzymes, have resulted from AAV vector administration in humans, reflecting the poorly inflammatory profile of these vectors.

We don’t know if there will be a significant immune response to AAV micro-dystrophin Gene Therapies.
Basic Immunology: Self Non-Self Discrimination for Defense

Innate Immune Response
1st line of defense
DAMPs (capsid, vector)
TLR2, 7, 9

Adaptive Immune Response
specificity
viral AAV capsid or micro-dystrophin
memory

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.
Dystrophin expressed in satellite cells is required for regeneration
Immune response drives regeneration and/or fibrosis

Immune response can exacerbate membrane damage
CTL, ROS

Immune response required for regeneration

Chronic assault drives fibrosis (DMD) and inefficient regeneration.

In Duchenne Muscular Dystrophy chronic injury prevents resolution, drives muscle damage and fibrosis.
Activates complement to create a fibrin/platelet patch (clot) at the lesion site.

Clear debris, pro-inflammatory cytokines and cells.

Repair and resolution

Waves of infiltrating cells coordinate patching, stem cell activation, muscle repair

IN DMD, Chronic damage, Asynchronous repair, Improper resolution, Ineffective regeneration, Profibrotic

Can we reset?

Can we intervene with drugs antifibrotics biologics (cytokines/factors) maintain tolerance
A Special Population of Regulatory T Cells Potentiates Muscle Repair and Inhibits Fibrosis

T-Reg’s
- Suppress specific immune response
- Promote muscle regeneration

Upregulation improves mdx
Downregulation worsens
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)
First exposure activates effectors for the fight and memories that can respond faster and better next exposure.
Immune Response to AAV: pre-existing antibodies
Exposure to AAV in the wild induces production of AAV specific neutralizing antibodies that can and render GT ineffective.
## Potential Solutions for Pre-formed Antibodies

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

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

- Pro-inflammatory
- Cytokines/Chemokines
- IL-6 and IL-1\textsubscript{b} and others

- Phagocytes
- Complement Activation

Candidate GT triggers:

- TLR2 AAV capsid
- TLR9
- AAV vector

In DMD
- TRL7
Immune Response to AAV: Adaptive Immunity Specific for AAV-vector or for micro-dystrophin transgene?

CD4

Micro-dystrophin
Muscle in DMD is not “normal”

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

Local tissue microenvironment and /or route of delivery matter?

<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>
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</table>
Pre-exposure to dystrophin
Tolerizing or activating and memory?

• Revertant fibers?
• Exon skipping pre-treatment prime/vaccinate/tolerance
• Ataluren pretreatment

• **Tolerance**
  • Low levels of chronic activation can lead to clonal T cell exhaustion/anergy

• **Regeneration**
  • Targeting satellite cells
  • Replacing defect with cytokines/growth factors
## Potential Problems and Solutions

### AAV Immune Response

<table>
<thead>
<tr>
<th>Immune Responses in the Human Host</th>
<th>Possible Solutions</th>
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<tbody>
<tr>
<td>Anti-capsid Immunity</td>
<td>selection of patients with low or no neutralizing antibodies, plasmapheresis, use of less seroprevalent capsids, capsid serotype switching, not-cross-reactive engineered capsids, exo-AAV, capsid decoy, prevention of NAb induction by using immunosuppressive drugs to allow AAV re-administration (if required), reduction of AAV capsid antigen load by decreasing therapeutic doses and/or removal of empty capsids from vector preparations, 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)</td>
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### Dystrophin Immune Response

<table>
<thead>
<tr>
<th>Anti-transgene Immunity</th>
<th>Development of antibodies toward the transgene product</th>
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<tr>
<td>selection of subjects having low risk of developing anti-transgene immune responses (e.g., subjects bearing missense rather than null disease causative mutations)</td>
<td>use of immune suppression, use of strategies to induce immune tolerance</td>
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</tbody>
</table>

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<tr>
<th>CD8$^+$ T cell-mediated cytotoxicity toward the transgene-expressing cells</th>
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<tr>
<td>de-targeting transgene expression from antigen-presenting cells</td>
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*Include strategies at different stages of development (preclinical and clinical settings).
*Observed in animal models, not observed so far in human clinical trials.
*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).

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.
Immune suppression

• Immune suppression to prevent reactivity to capsid protein and/or transgene
  • What type is best?
    • Goal to block activation of immune response to capsid and dystrophin and induce capsid and dystrophin tolerance.
    • Block T conv response
    • Enhanced Tregs will promote tolerance and regeneration
    • Promote anergy/exhaustion
    • Reprogram dendritic or tolerogenicDC to Treg and
    • Block Neutralizing antibodies
    • Enable re-administration
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
T cell activation versus tolerance dictated by:

(generalizations)

• Status of the APC and local cytokines

• Upregulation of inhibitory proteins on the T cell surface

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

<table>
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<tr>
<th>Type of approach</th>
<th>Modality</th>
<th>Institutions supporting the concept</th>
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<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>
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<td>With DNA vaccines</td>
<td>Tolerion</td>
</tr>
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<td></td>
<td>With autoantigenic peptides containing thioredoxin motifs</td>
<td>Incyse</td>
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<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>
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<td>Transferring in vitro inactivated autologous autoantigen-specific T cells to expose ergotypic antigens</td>
<td>Opexa Therapeutics</td>
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<td></td>
<td>Transferring autologous regulatory chimeric antigen receptor-T (CAR-T reg) cells</td>
<td>Tcell/Sangamo</td>
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<td>Administering engineered bacteria expressing host autoantigens together with host immune modulators</td>
<td>ActoBio/Intrexon, Allero Therapeutics</td>
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<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>
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</tbody>
</table>

J Exp Med
Exposure to wild-type AAV drives distinct capsid immunity profiles in humans


INSERM U794, Sorbonne Université, Paris, France; Genethon, Evry, France; INSERM 5951, Université Evry, Université Paris Saclay, EPHÉ, Evry, France.

ARTICLE
DOI: 10.1038/s41467-018-06621-3
OPEN

Antigen-selective modulation of AAV immunogenicity with tolerogenic rapamycin nanoparticles enables successful vector re-administration

Amine Meliani, Florence Boisgerault, Romain Hardet, Solenne Marmier, Fanny Collaud, Giuseppe Ronzitti, Christian Leborgne, Helena Costa Verdera, Marcelo Simon Sola, Severine Charles, Alban Vignaud, Laetitia van Wittenbergh, Giorgia Manni, Olivier Christophe, Francesca Fallarino, Christopher Roy, Alicia Michaud, Petr Ilyinskii, Takashi Kei Kishimoto & Federico Mingozzi

NOT tested in DMD models

A unique population identified and blocked IL-6 and IL1b

AAV2 and AAV8
How do we monitor response?

- Muscle biopsy - limited number
  - how many; and when
  - Needle biopsies vs open mu
- MRI/MRS - Imaging DMD muscle biopsy
- 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
Same issues with pre-formed AAV or Cas9 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

Dimitrios L. Wagner, Leila Amini, Desiree J. Wendinger, Lisa-Marie Burkhardt, Levent Akyüz, Petra Reinke, Hans-Dieter Volk & Michael Schmuck-Rehnersse

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

0% had T cells against Cas9
65% had antibodies against Cas9

96% had T cells against Cas9
85% had antibodies against Cas9

Same issues with pre-formed immunity
Disclosures: Myself or a member of my family has received compensation and/or travel from the above. I am a member of SOLID SAB.