Immune considerations relevant to DMD and dystrophin replacement/correction therapies

(not all immune responses are bad)

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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 CT

Tolerizing

(Treg + others)

Regenerative
Treg, M2 + others

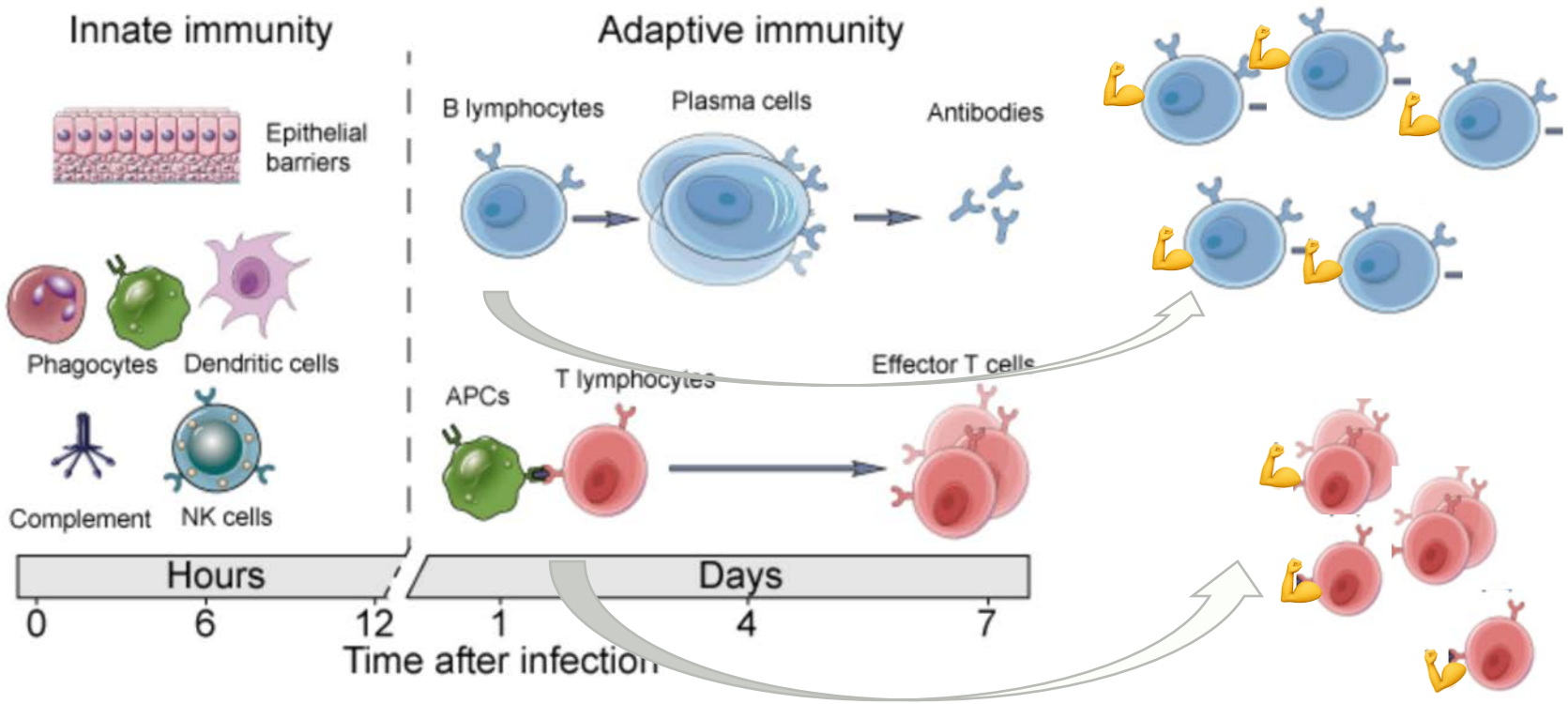
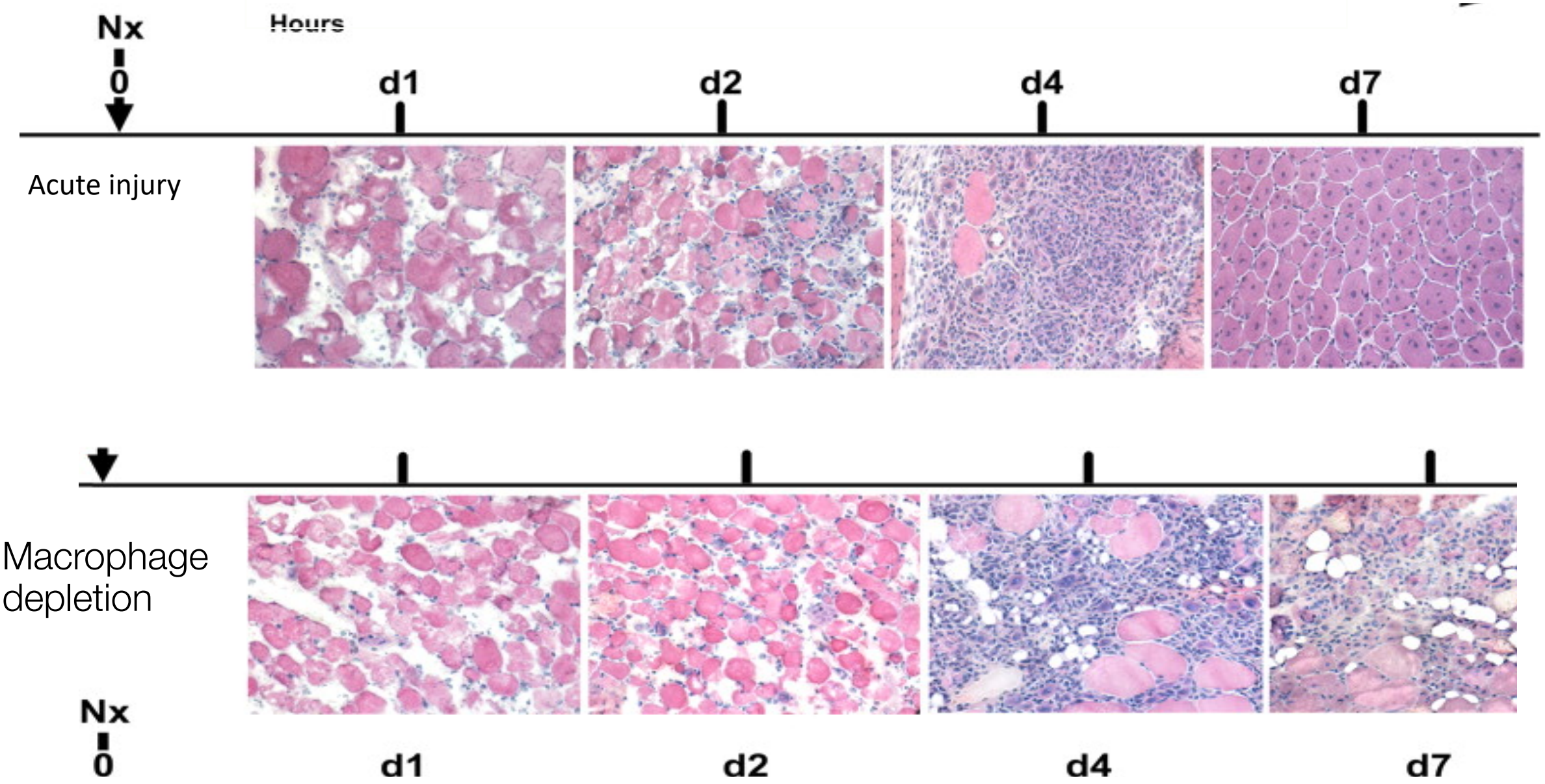


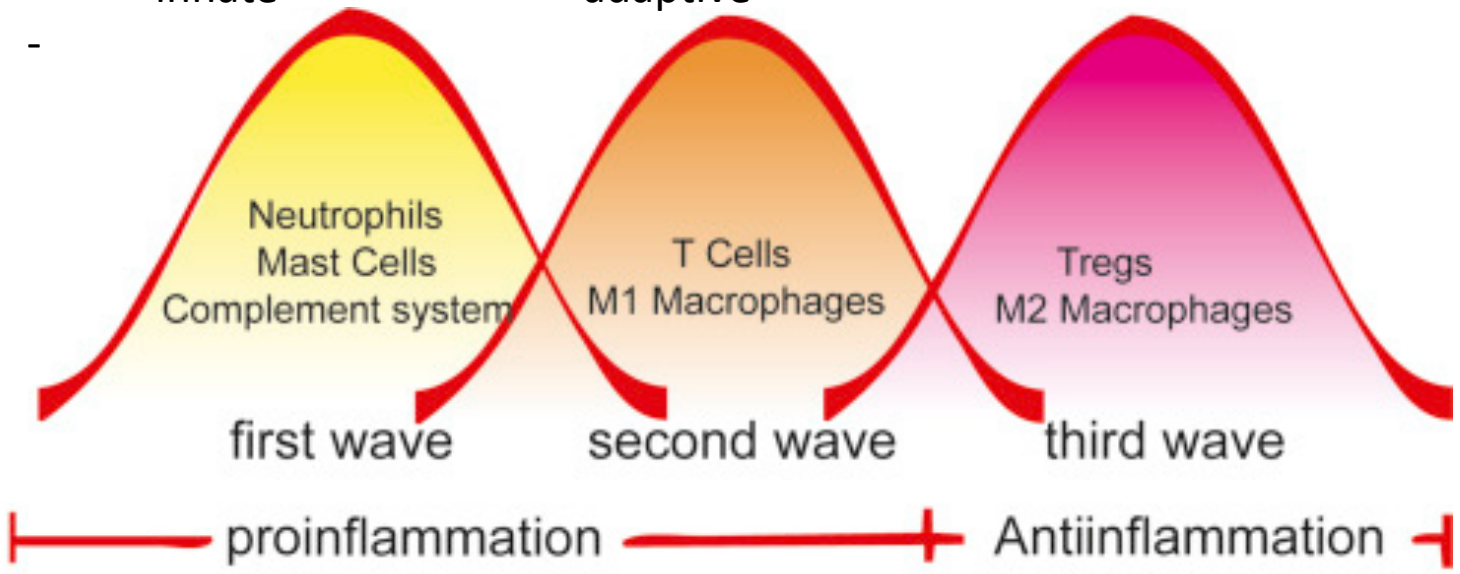
Figure 2. Innate and adaptive immunity time line. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and require the activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and may vary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

Immune response to muscle damage guides regeneration



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

-------- innate---------- adaptive----------
-



death of injured muscle cells activation of muscle stem cells

Activates complement to create a fibrin/platelet patch (clot) at the lesion site.

activation and expansion of muscle stem cells

Clear debris, pro-inflammatory cytokines

differentiation of muscle stem cells

Repair and resolution

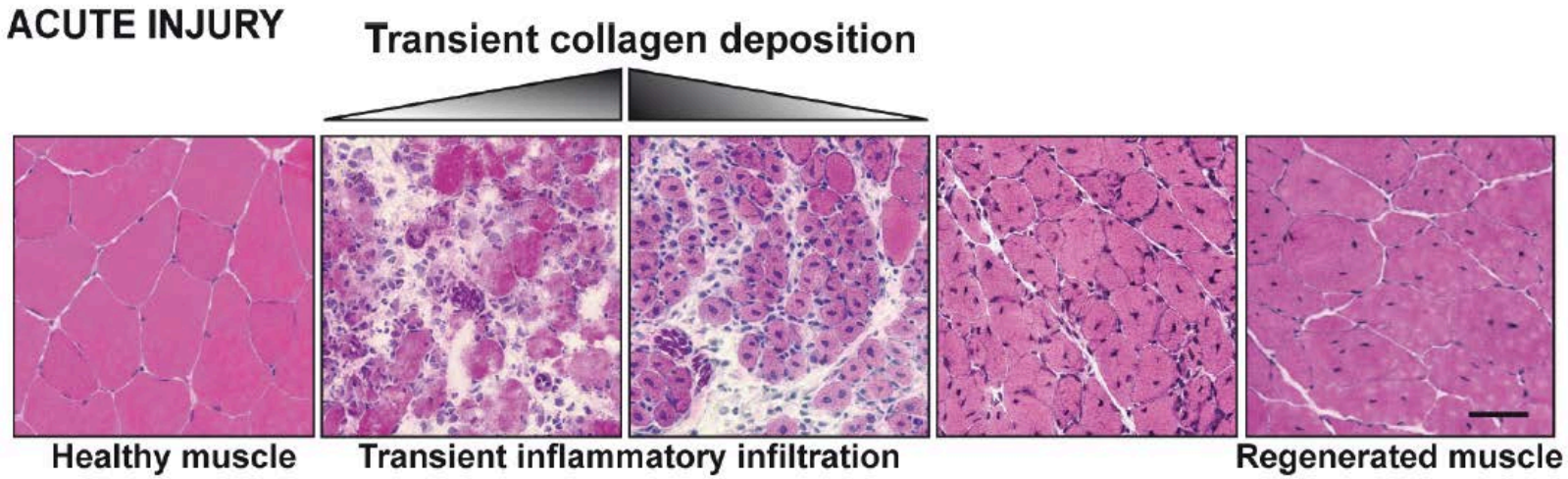
In DMD ,
Chronic damage
Asynchronous repair

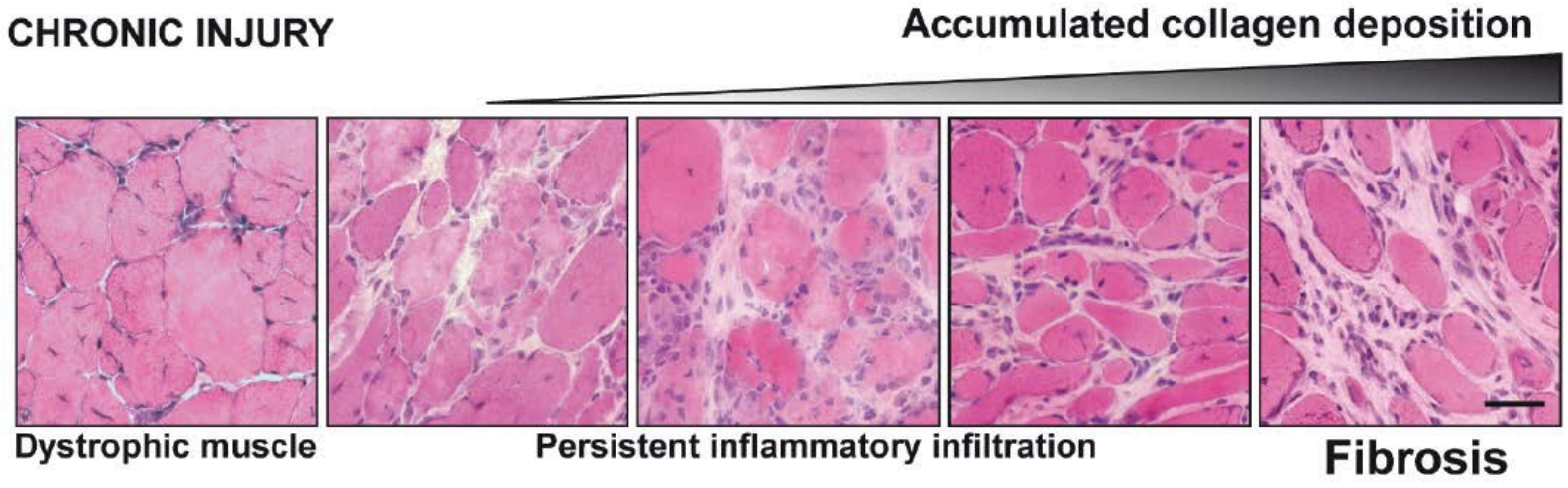
Improper resolution
Ineffective regeneration
Profibrotic

Can we reset?-

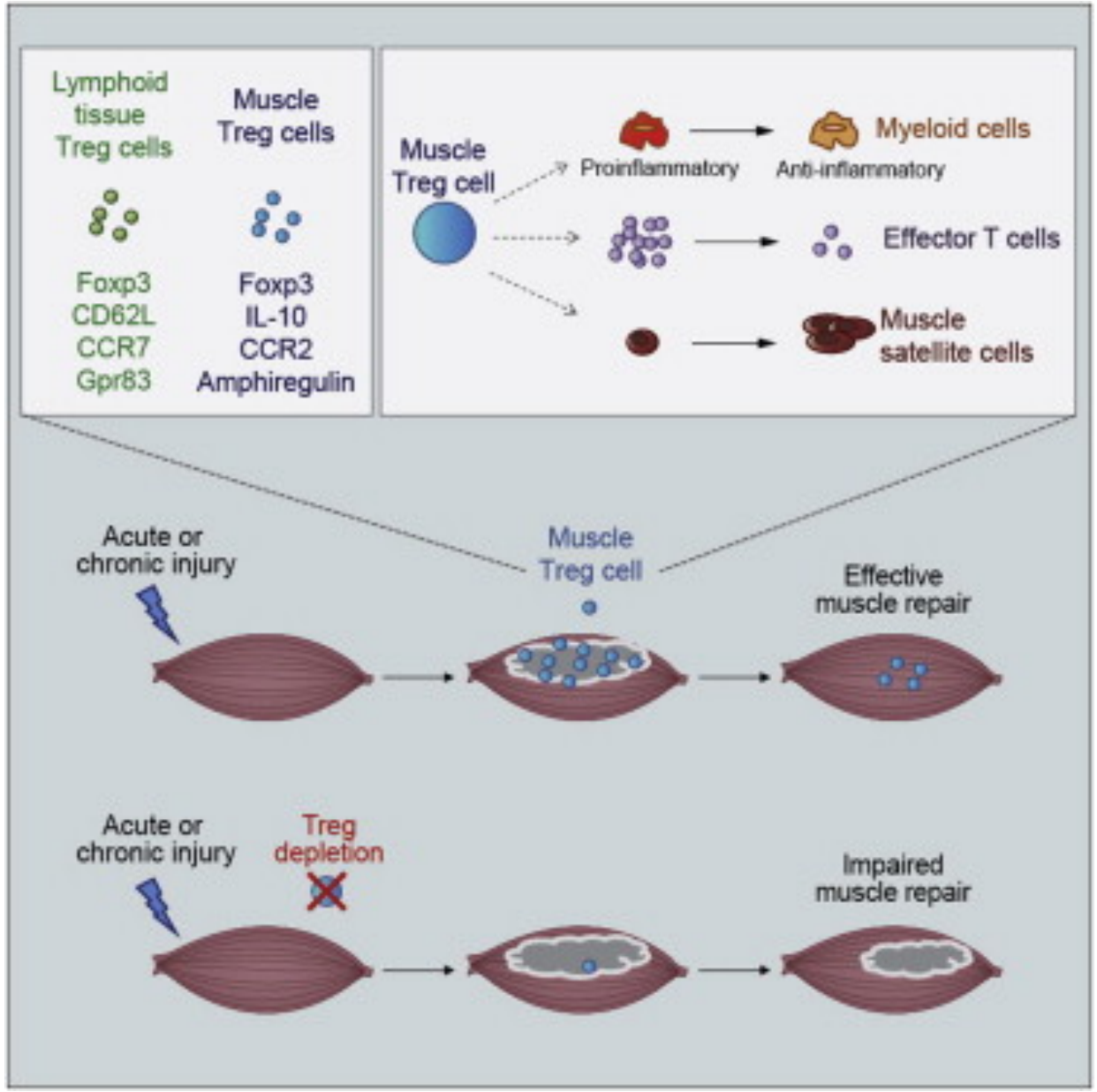
Can we intervene with drugs
-antifibrotics or immune modulators
-dystrophin replacement

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 (amphiregulain)

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...

MUSCULAR DYSTROPHY

Regulatory T cells suppress muscle inflammation and injury in muscular dystrophy

S. Armando Villalta,[1]* Wendy Rosenthal,[1] Leonel Martinez,[2] Amanjot Kaur,[1] Tim Sparwasser,[3] James G. Tidball,[4] Marta Margeta,[5] Melissa J. Spencer,[2] Jeffrey A. Bluestone[1,5,6]

Can immune modifiers limit fibrosis/promote regeneration?

Rosenburg and Woodcock, Nature Immunology

Immunomodulators in DMD

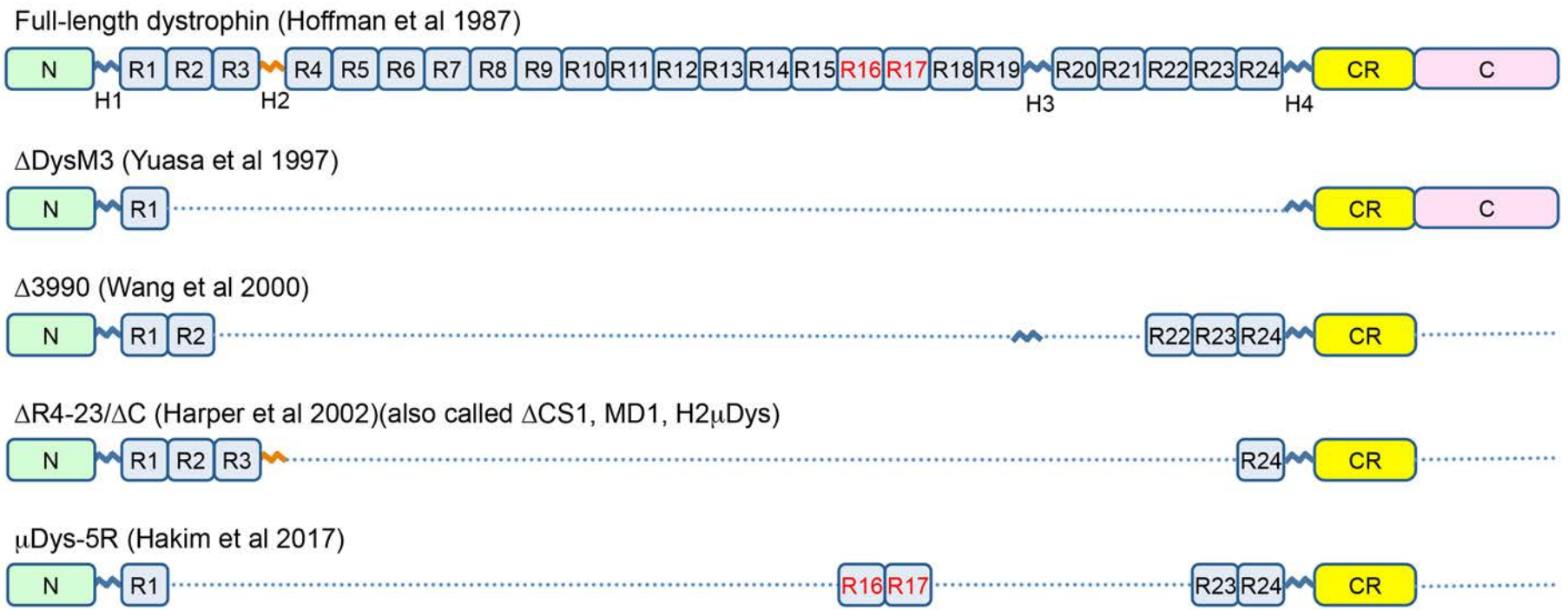
NFAT, nuclear factor of activated T cells; PDE, phosphodiesterase.

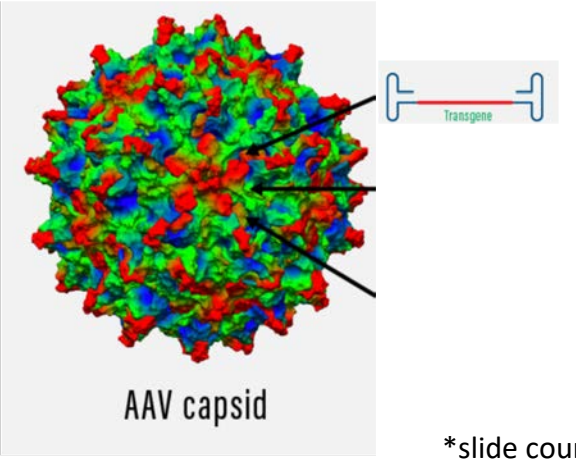
Drug/compound	Target	Pathological process	Preclinical trials	Clinical trials/use
		Current treatments		
Prednisone, deflazacort	NF-κB, others	Anti-inflammatory	Yes	Yes
VBP15	NF-κB, membrane protection	Anti-inflammatory, sarcolemma stability	Yes	Yes*
Cyclosporine	NFAT	Anti-inflammatory	Yes	Yes†
Azathioprine	Purine synthesis	Anti-inflammatory	Yes	Yes†
Poloxamer	Membrane protection	Sarcolemma stability	Yes	Yes‡
Gene therapy	Dystrophin replacement	Sarcolemma stability	Yes	Yes
Exon skipping	Dystrophin replacement	Sarcolemma stability	Yes	Yes
TLR7/8/9 antagonists	TLR7/8/9	Anti-inflammatory	Yes	No
NEMO peptide	NF-κB	Anti-inflammatory	Yes	No
Infliximab	TNF-α	Anti-inflammatory	Yes	No
IL-2/anti–IL-2 complex	T_{regs}	Anti-inflammatory	Yes	No
Pentoxifylline	PDE inhibitor	Anti-fibrotic	Yes	Yes
Pirfenidone	TGF-β signaling	Anti-fibrotic	Yes	No
Losartan	Angiotensin type 1 receptor inhibitor	Anti-fibrotic	Yes	Yes
Lisinopril	Angiotensin-converting enzyme inhibitor	Anti-fibrotic	Yes	Yes
Anti–IL-6	IL-6	Anti-inflammatory	Yes	No
Anti-myostatin antibodies	Myostatin	Anti-fibrotic, hypertrophy	Yes	Yes
Cromolyn	Mast cells	Membrane stability	Yes	No
		Future options		
Chloroquine	Lysosomal pH	Anti-inflammatory	No	No
Eculizumab	Complement C5	Anti-inflammatory	No	No
Rapamycin	T_{regs} +Akt/mTOR	Anti-inflammatory, regeneration	Yes	No
Plerixafor	CXCR4	Anti-inflammatory	No	No
IL-10	Alternatively activated macrophages	Anti-inflammatory	No	No
Anti-osteopontin antibodies	Osteopontin	Anti-inflammatory, anti-fibrotic	No	No
Candesartan	Angiotensin type 2 receptor inhibitor	Anti-fibrotic	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





Δ3990

ΔR4-23/ΔC

Sarepta

μDys

5R SOLID

*slide courtesy of Dongsheng Duan

Immune reponse to AAV Gene Therapy

Mingozzi and High Annual Review of Virology 2017

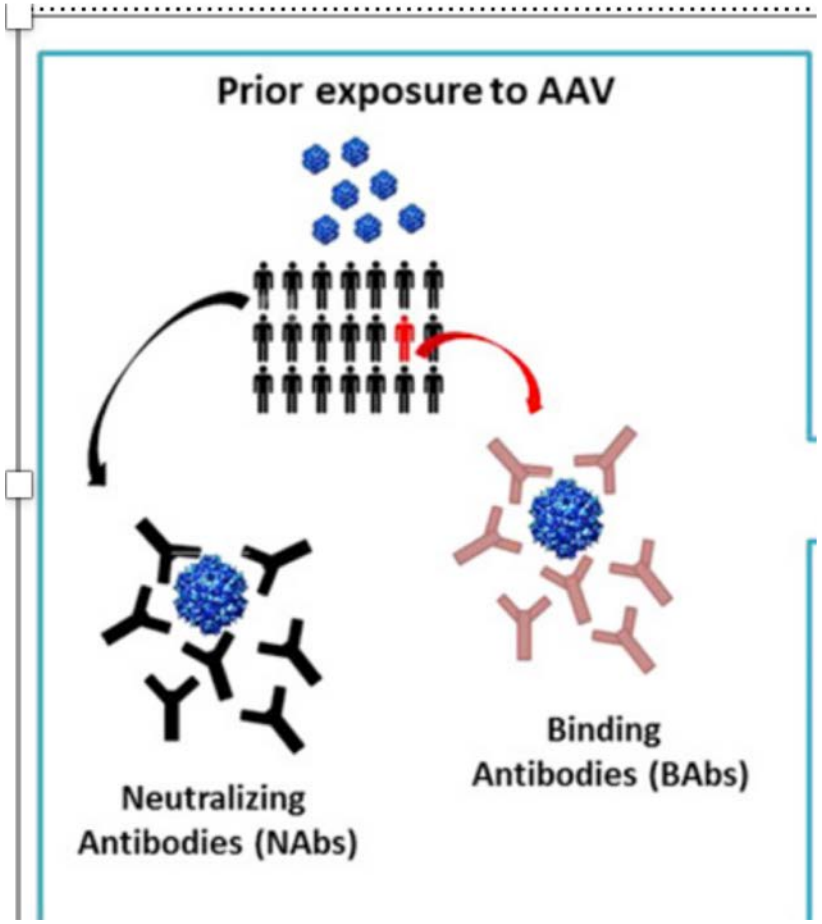
SUMMARY POINTS

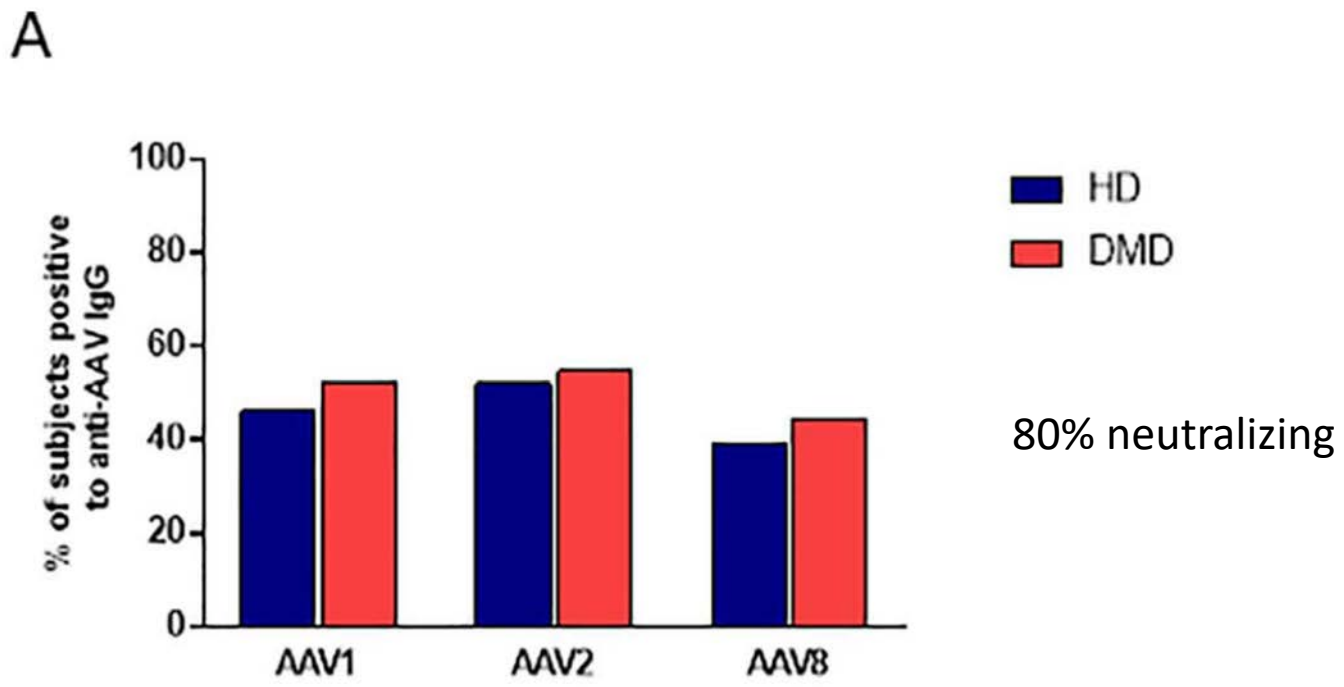
1. AAV vector–mediated gene transfer has resulted in long-term therapeutic efficacy in humans affected by a variety of diseases. However, preclinical and clinical experience indicates that components of AAV vectors can be recognized by the host immune system.
2. Thus far, no serious or permanent consequences 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 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?

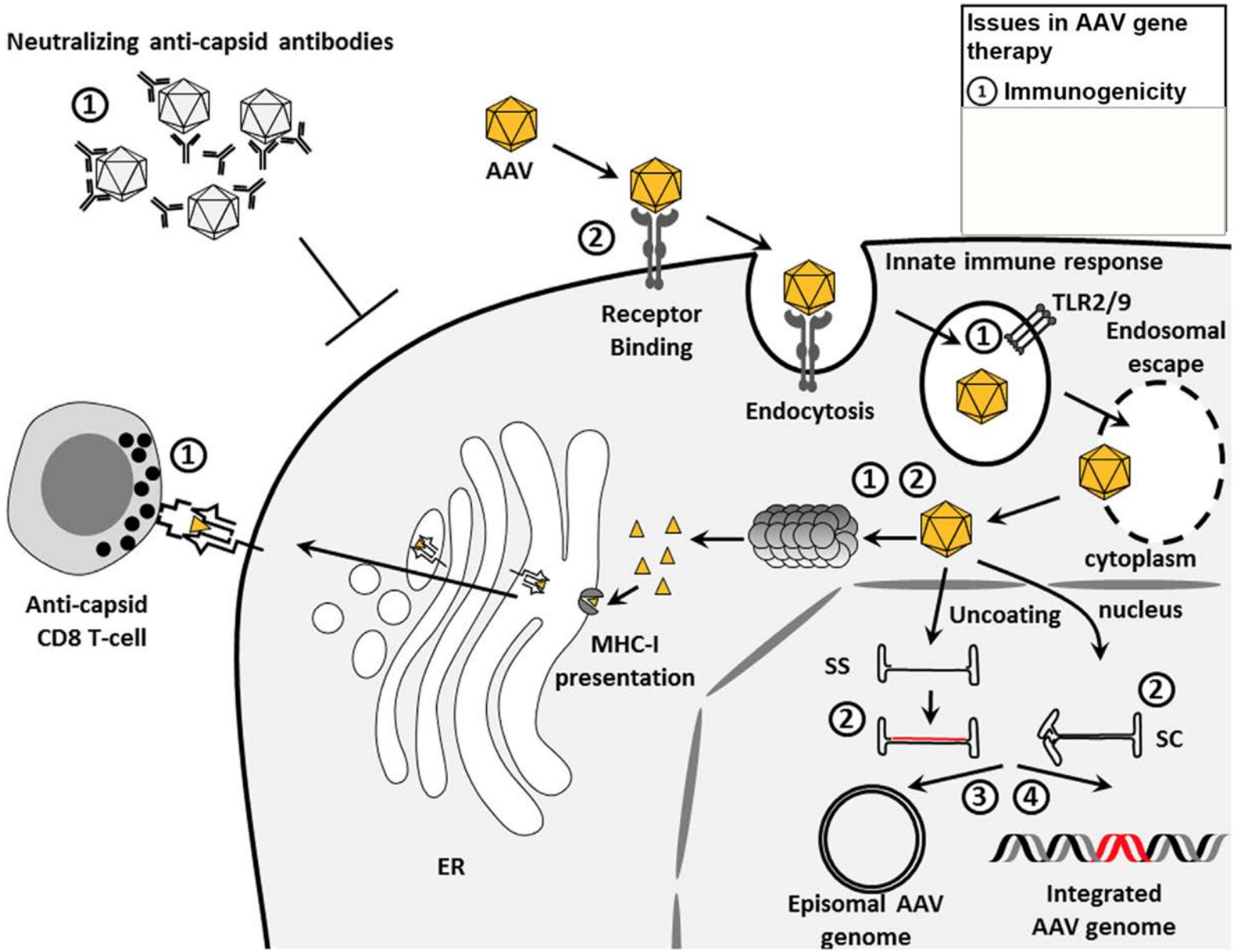
Exposure to AAV in the wild induces production of AAV specific neutralizing antibodies that can block GT delivery





80% neutralizing

Immune Response to AAV: pre-existing antibodies



Potential Solutions for Pre-formed Antibodies

Strategy	Pros	Cons	Clinical feasibility
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	■ 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?

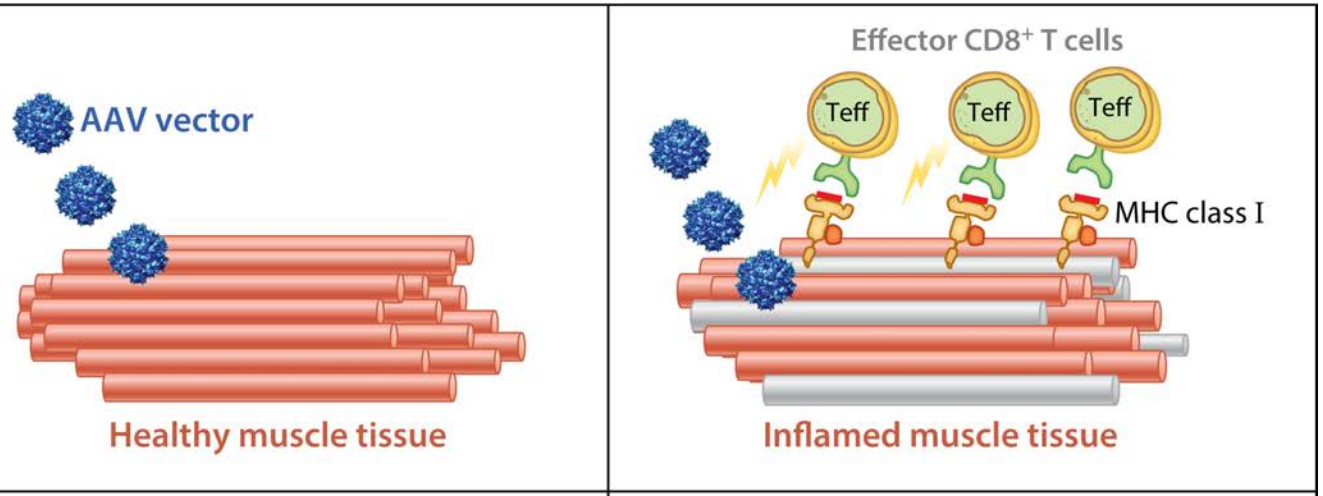
Muscle in DMD is not “normal”

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

DMD- Intra-Muscular GT Injection



Muscle environment	Normal	Inflamed
Route of delivery	Intravascular	Intramuscular
Genetic background	Presence of nonfunctional endogenous protein	Complete lack of endogenous protein
Expression cassette	Muscle specific or detargeted from antigen-presenting cells	Constitutive expression cassette
AAV vector genome	Single-stranded	Self-complementary

Mingozzi F, High KA. 2017. *Annu. Rev. Virol.* 4:511–34

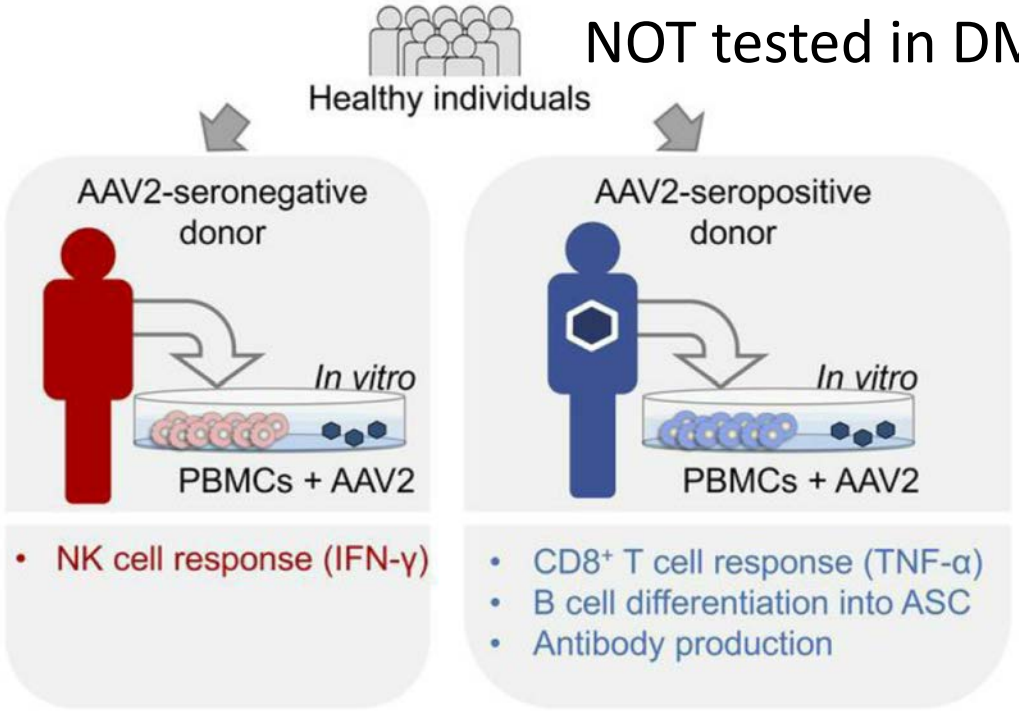
Exposure to wild-type AAV drives distinct capsid immunity profiles in humans

Klaudia Kuranda,[1] Priscilla Jean-Alphonse,[1] Christian Leborgne,[2] Romain Hardet,[1] Fanny Collaud,[2] Solenne Marmier,[1] Helena Costa Verdera,[1] Giuseppe Ronzitti,[2,3] Philippe Veron,[2] and Federico Mingozzi[1,2,3] JCI 2018

[1]INSERM U974, Sorbonne Université, Paris, France. [2]Genethon, Evry, France. [3]INSERM S951, Université Evry, Université Paris Saclay, EPHE, Evry, France.

Deep immune profiling

NOT tested in DMD

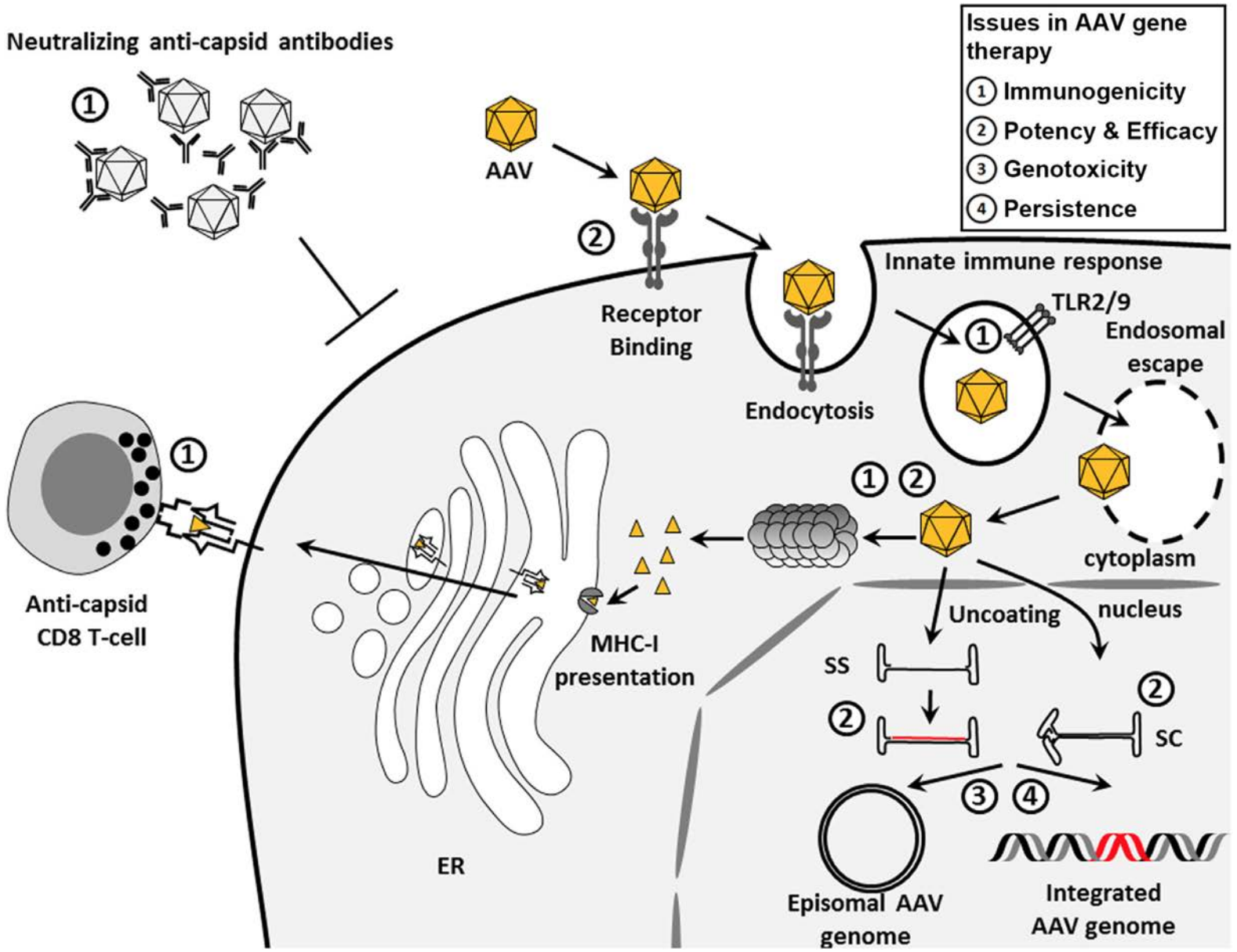


A unique moDendritic cell population identified which produces IL-6 and IL-1b ; blockingIL-1b with antibodies prevented AAV antibody production.

(AAV2 and AAV8)

Immune Response to AAV: Innate Immune Response

TRL2-AAV capsid
TRL9-AAV vector/na



Mingozzi et al

DAMPS-Danger associated molecular pattern receptors
1st line of defense; alert adaptive response
Can we identify players and modulate?

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

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

In DMD
TRL7
ssRNA

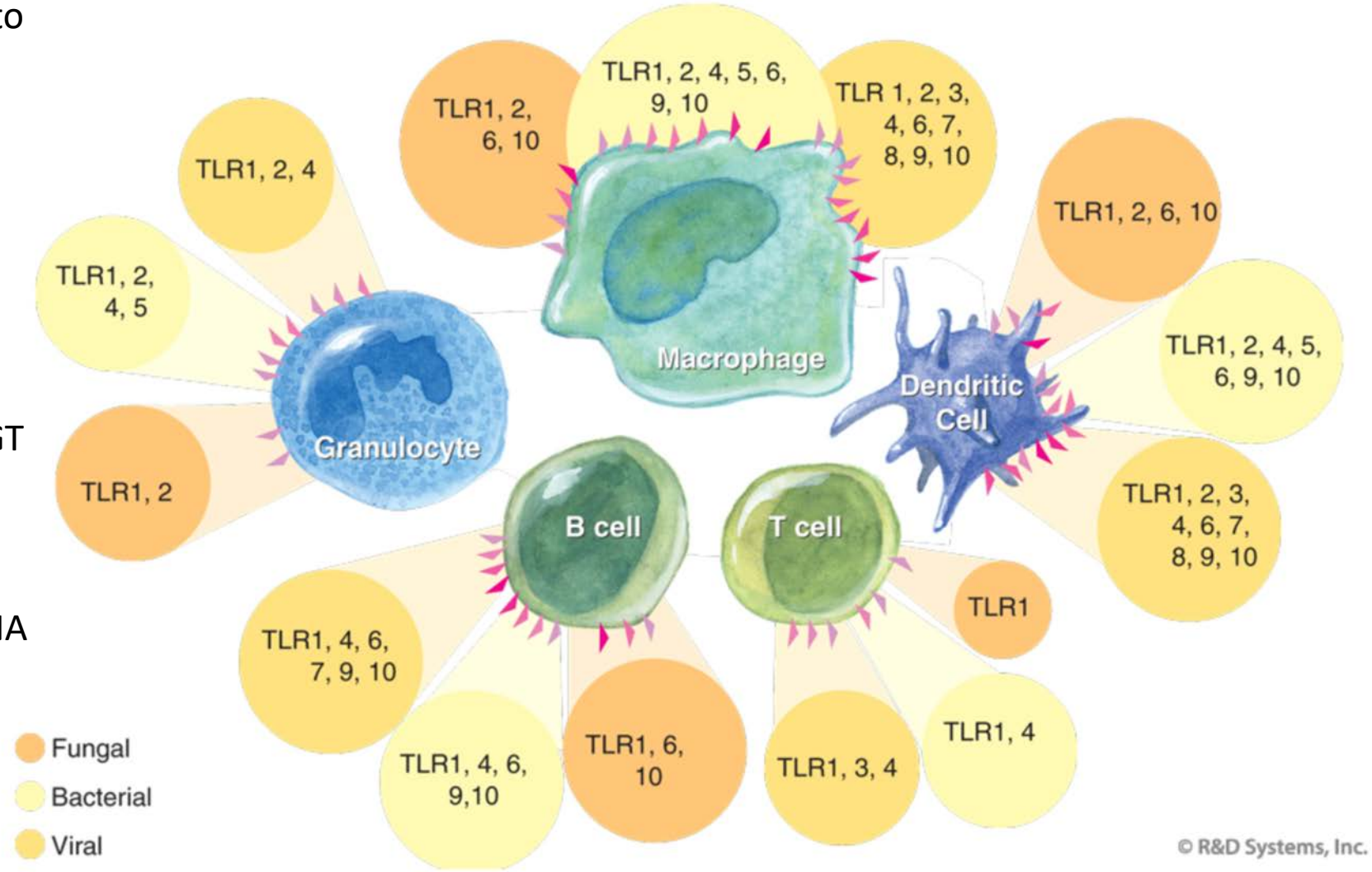


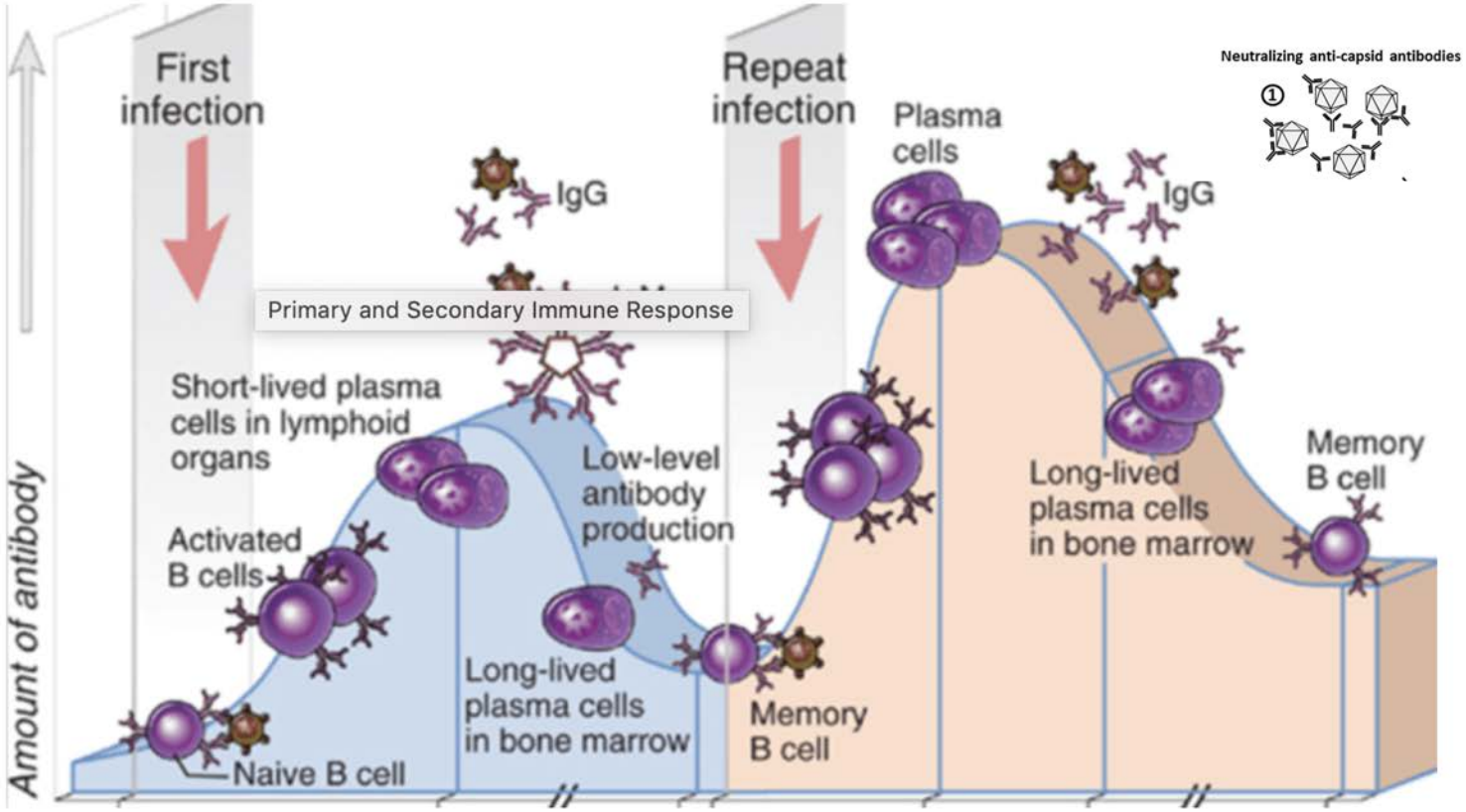
Figure 3. Toll-like receptor (TLR) leukocyte expression patterns and PAMP specificities.

Pro-inflammatory Cytokines/Chemokines IL-6 and IL-1b and others

Phagocytes
Complement Activation

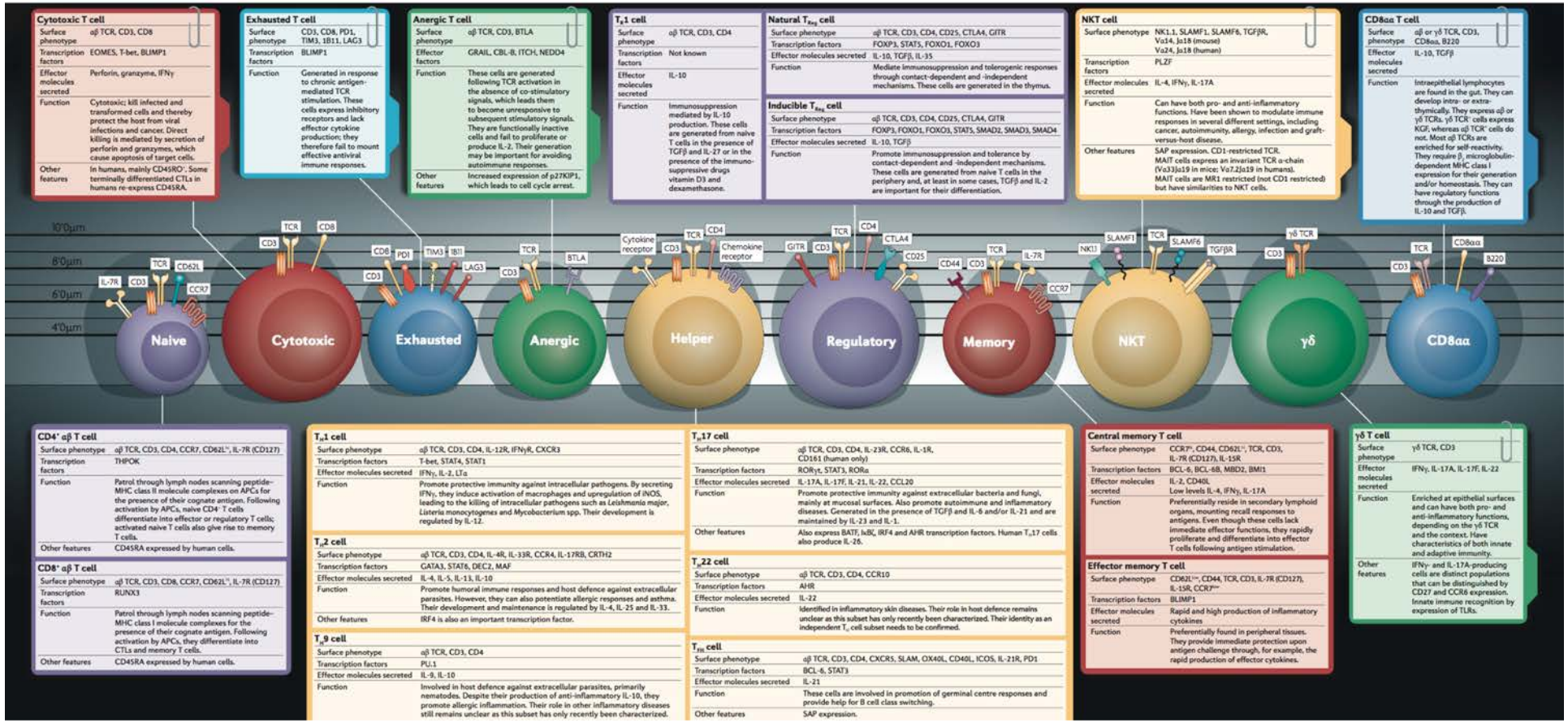
Upregulation of T cell co-stimulators on antigen presenting cells

Adaptive immunity: B cells and T cells each have surface receptors t and development of immune effectors and memory cells.



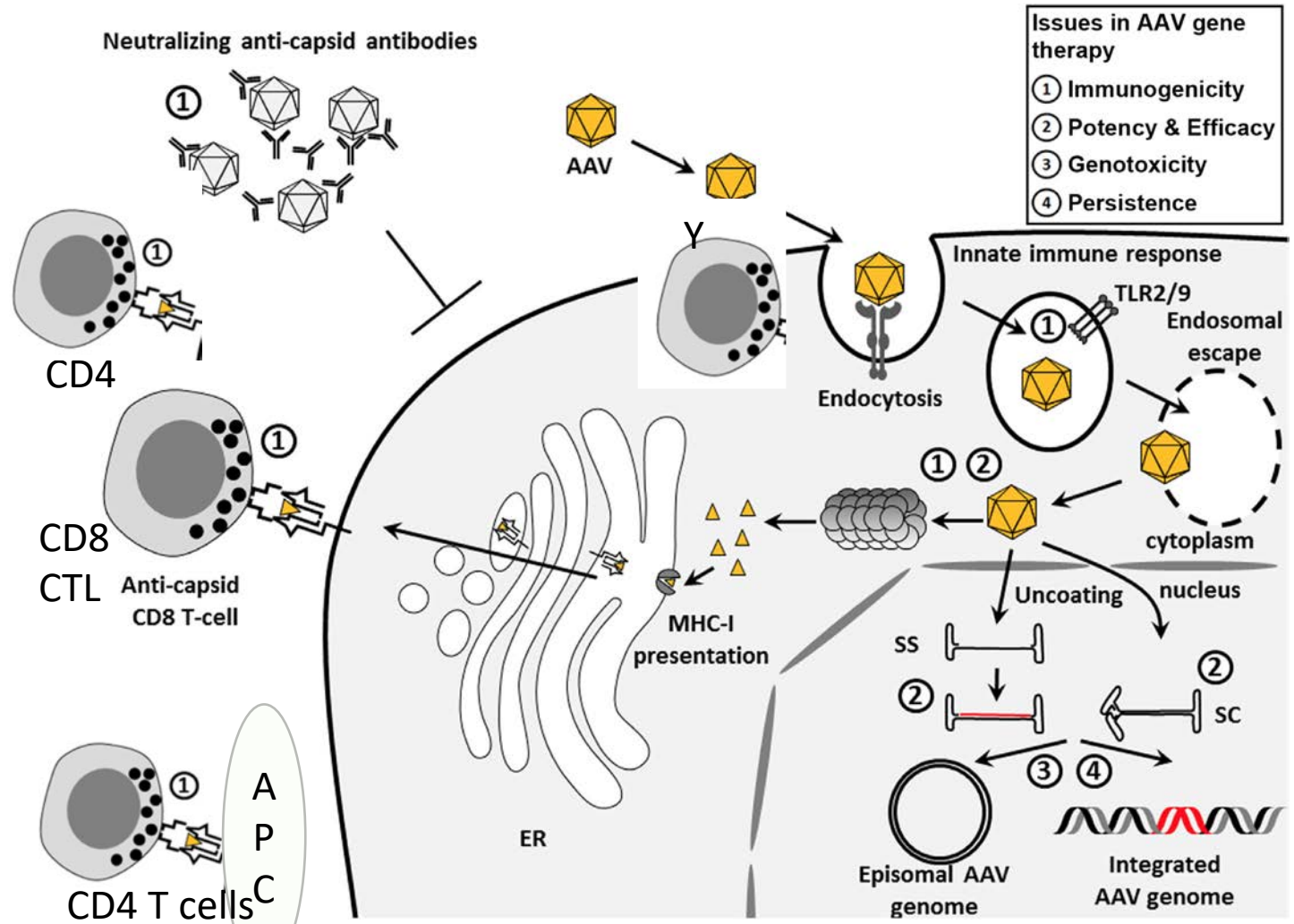
Differences in the Primary and Secondary Immune Response Image source: Abbas et. al: Cellular and Molecular Immunology

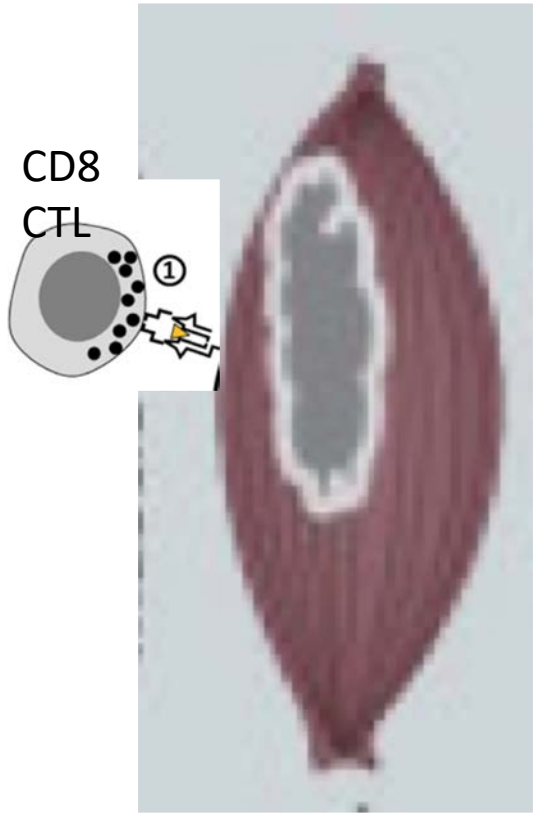
Diverse CD4 and CD8 T cells subsets regulate immune activation and self tolerance



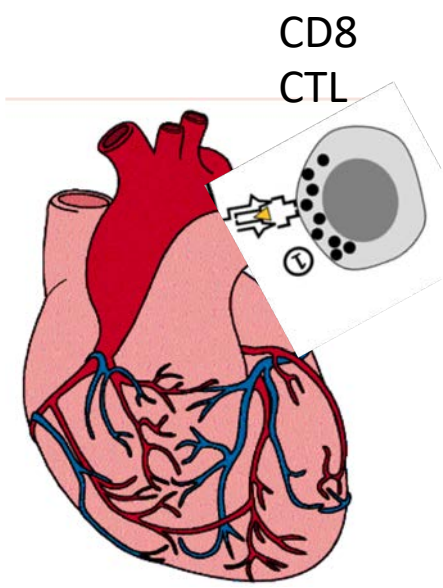
Subset distinguished by co-expression of surface antigens and functional output; plasticity and intermediates observed

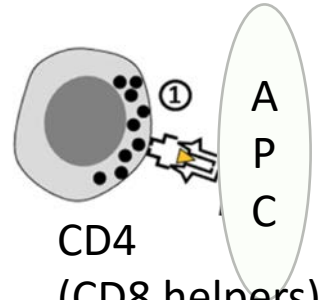
Immune Response to AAV: Adaptive Immunity Specific for AAV-vector or for dystrophin transgene?)





Micro-dystrophin or AAV capsid





CD4
(CD8 helpers)
(Tregs and other

Potential Problems and Solutions

AAV Immune Response

Dystrophin Immune Response

Table 1. Immune Responses to AAV Gene Therapy and Possible Solutions

Immune Responses in the Human Host	Possible Solutions[a]
Anti-capsid Immunity	
Pre-existing neutralizing antibodies (NAbs) toward the capsid proteins[3,61,81]	selection of patients with low or no neutralizing antibodies[81]
	plasmapheresis[196,197]
	use of less seroprevalent capsids[61] capsid serotype switching[191–193] not-cross-reactive engineered capsids[25] exo-AAV[129] capsid decoy[67]
	prevention of NAb induction by using immunosuppressive drugs to allow AAV re-administration (if required)[195,198]
CD8$^+$ T cell-mediated cytotoxic immune response toward transduced cells presenting AAV capsid antigens	reduction of AAV capsid antigen load by decreasing therapeutic doses[149] 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)[29,48,49]
Anti-transgene Immunity	
Development of antibodies toward the transgene product[b]	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]
CD8$^+$ T cell-mediated cytotoxicity toward the transgene-expressing cells[200,201 c]	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).

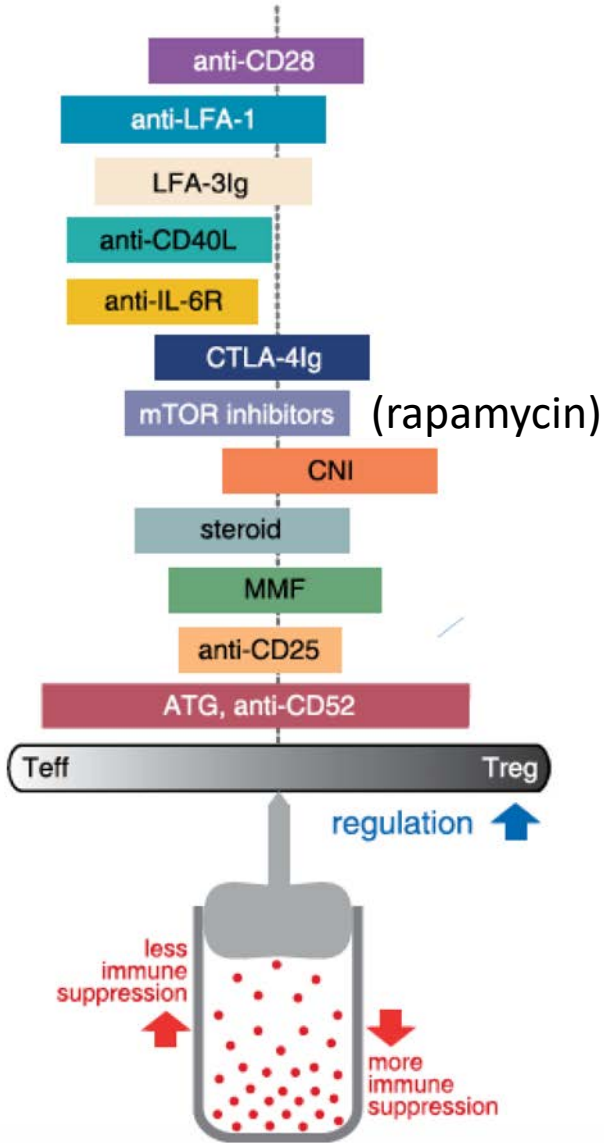
Impact of Immune-Modulatory Drugs on Regulatory T Cell
Furukawa, Wisel, MD, and Tang,
Transplantation 2016;100: 2288–2300)

All T/B cell responses

Vs novel mechanism/drugs for inducing antigen specific tolerance.

Barry B

Tacromilus /rituximab (rapamycin)

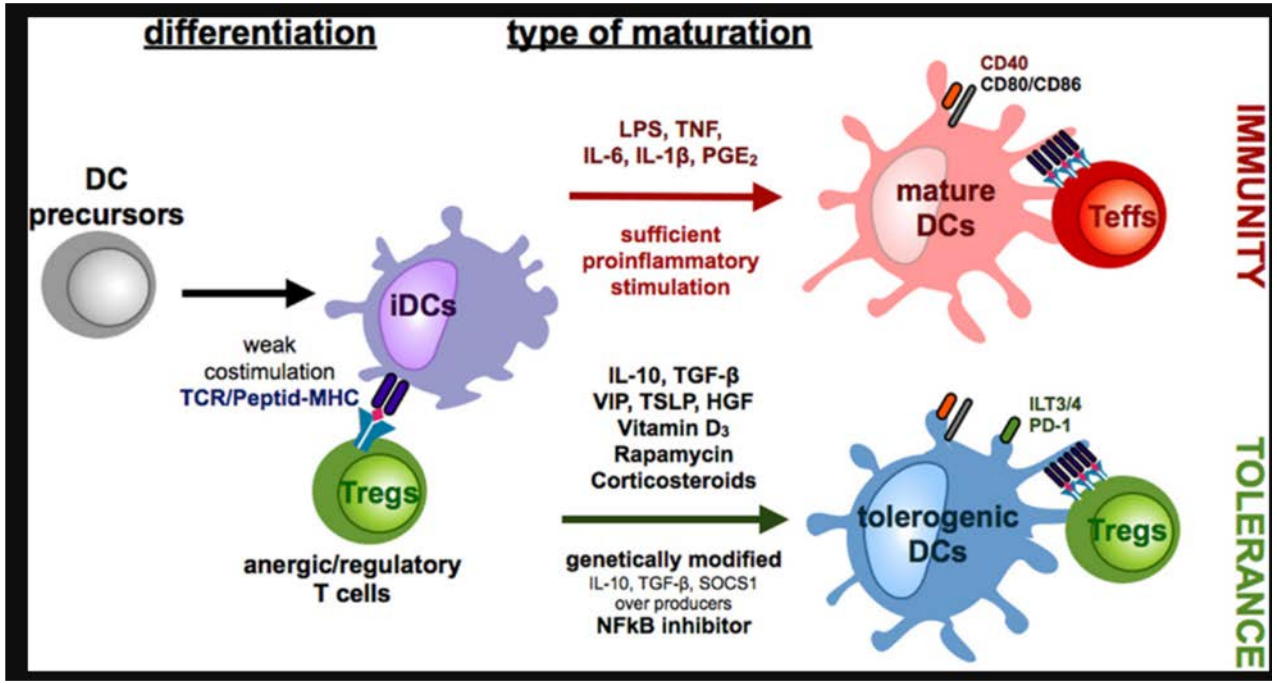


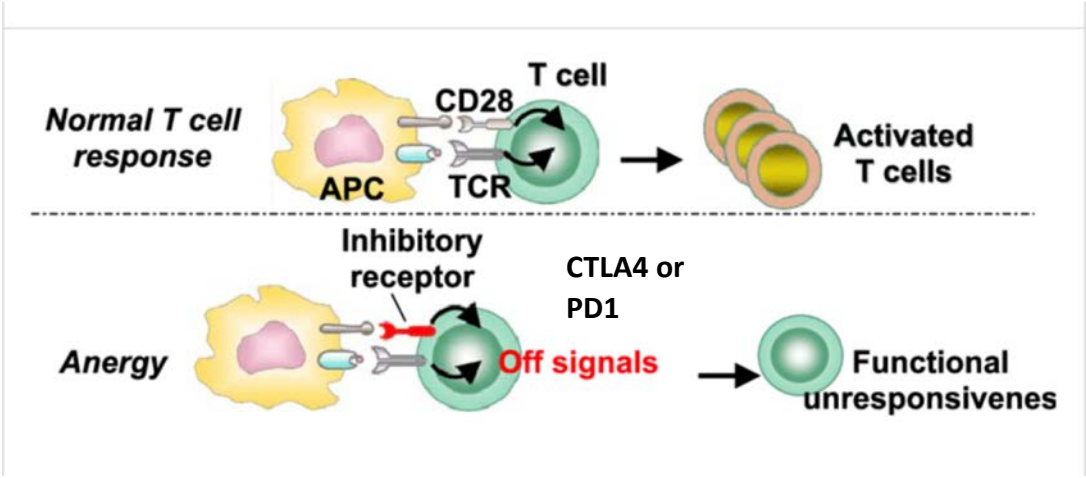
Can we borrow from fields of transplantation or autoimmunity where the goal is also to dampen inflammation and tolerize?

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

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

J Exp Med
16 January, 2019 http://doi.org/10.1084/jem.20182287

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?

ARTICLE

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

Nature Communications 2018

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

Amine Meliani[1,2], Florence Boisgerault[2], Romain Hardet[1], Solenne Marmier[1], Fanny Collaud[2], Giuseppe Ronzitti[2], Christian Leborgne[2], Helena Costa Verdera[1,2], Marcelo Simon Sola[1,2], Severine Charles[2], Alban Vignaud[2], Laetitia van Wittenberghe[2], Giorgia Manni[3], Olivier Christophe[4], Francesca Fallarino[3], Christopher Roy[5], Alicia Michaud[5], Petr Ilyinskii[5], Takashi Kei Kishimoto[5] & Federico Mingozzi[1,2]

- 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 Tregulatory 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

Carsten T. Charlesworth, Priyanka S. Deshpande, Daniel P. Dever, Joab Camarena, Viktor T. Lemgart, M. Kyle Cromer, Christopher A. Vakulskas, Michael A. Collingwood, Liyang Zhang, Nicole M. Bode, Mark A. Behlke, Beruh Dejene, Brandon Cieniewicz, Rosa Romano, Benjamin J. Lesch, Natalia Gomez-Ospina, Sruthi Mantri, Mara Pavel-Dinu, Kenneth I. Weinberg & Matthew H. Porteus

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

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

Letter | Published: 29 October 2018

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

Dimitrios L. Wagner, Leila Amini, Desiree J. Wendering, Lisa-Marie Burkhardt, Levent Akyüz, Petra Reinke, Hans-Dieter Volk & Michael Schmueck-Henneresse

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

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

B cells

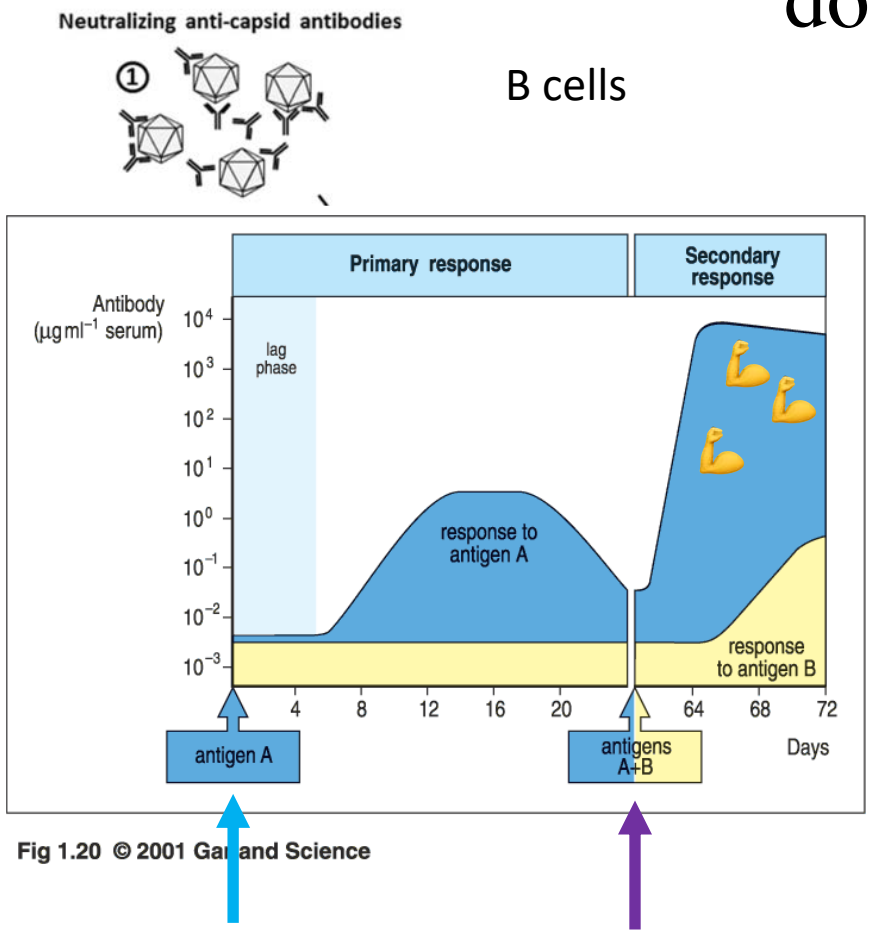
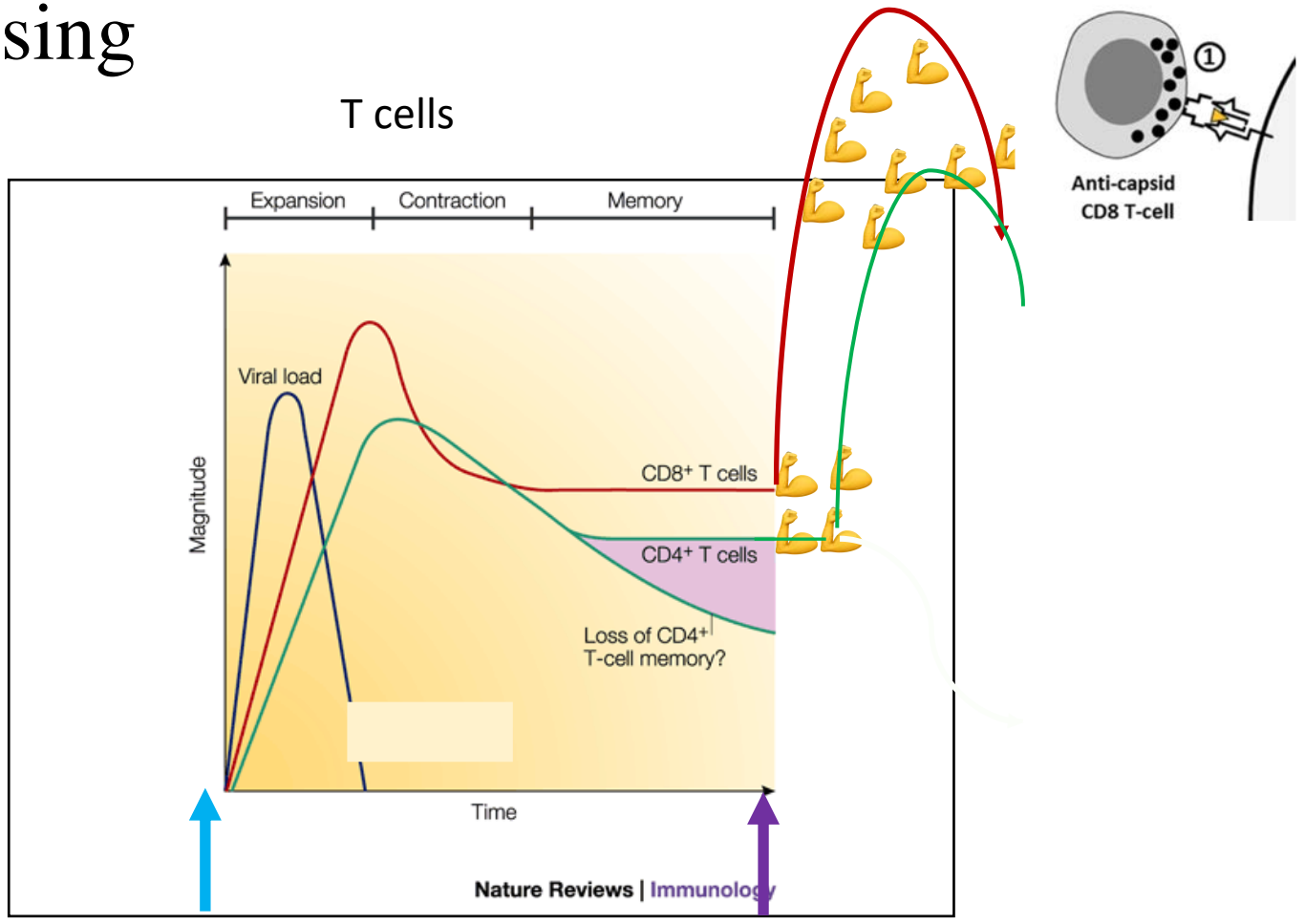


Fig 1.20 © 2001 Gar and Science

T cells



Natural AAV infection

AAV-GT infection

AAV-GT infection

AAV-GT re-infection

Natural AAV infection

AAV-GT infection (High dose)

AAV-GT infection

AAV-GT re-infection (high dose)