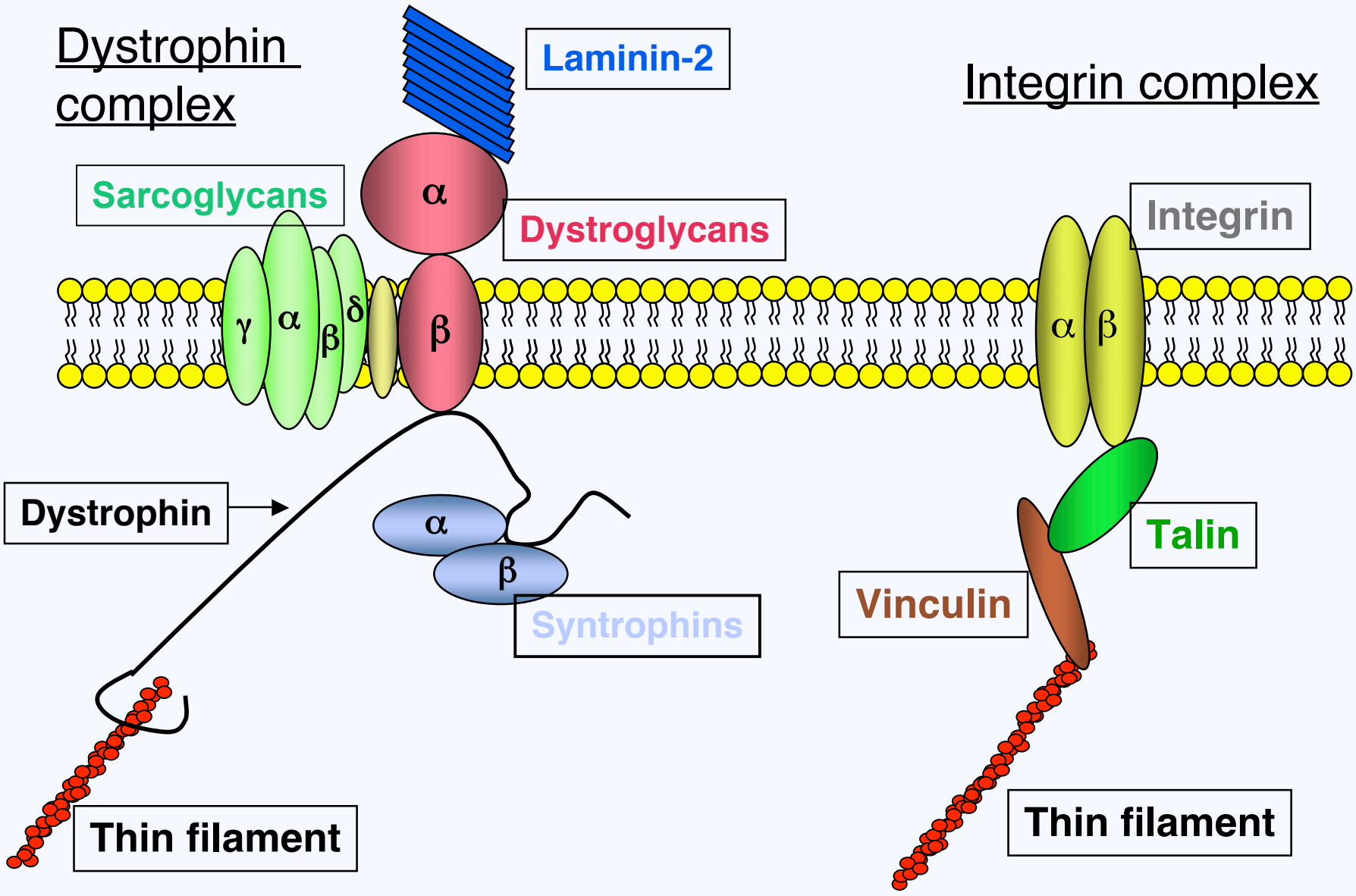
Neuromuscular Biology and Disease, Spring semester 2014.
Duchenne muscular dystrophy.

Course organizers: Denis Guttridge and Jill Rafael-Fortney

Topic: Interactions between dystrophic muscle and the immune system.

Jim Tidball
Program in Molecular, Cellular and Integrative Physiology
Department of Pathology and Laboratory Medicine
UCLA

# Mechanical signal transduction in muscle.



# Mechanical defect hypothesis

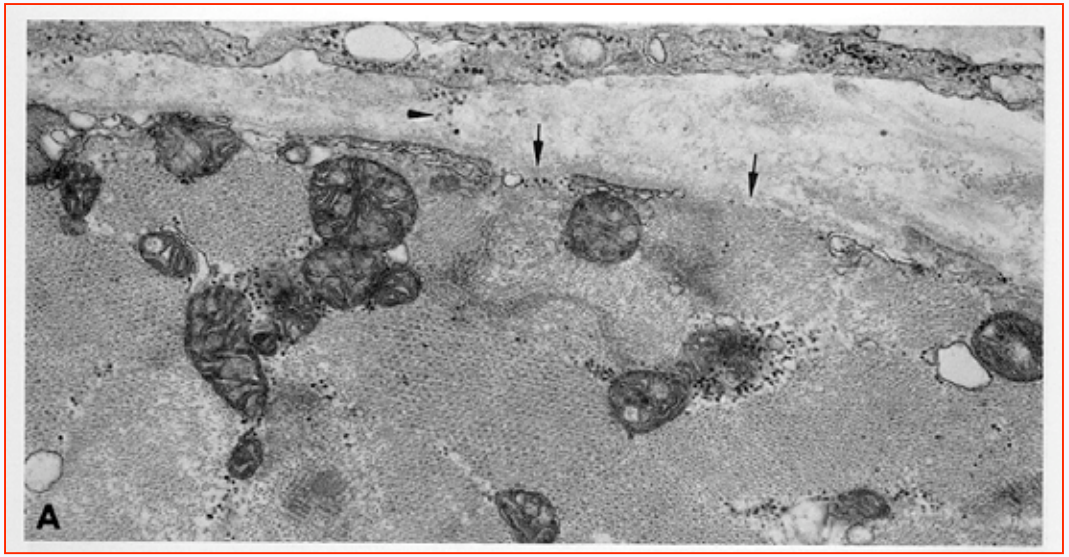
Mutation of dystrophin

Loss of normal dystrophin expression

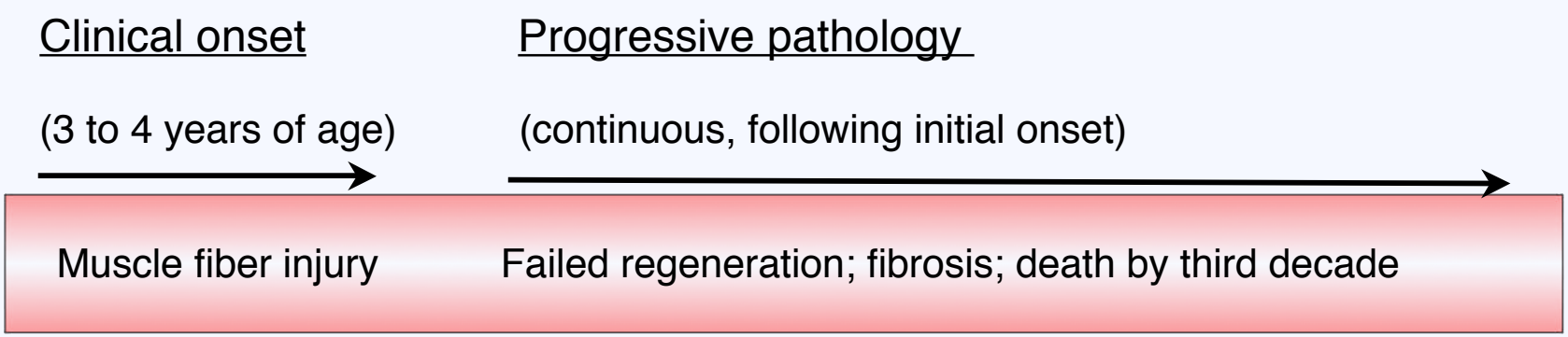
Mechanical weakening of cell membrane

Membrane lysis

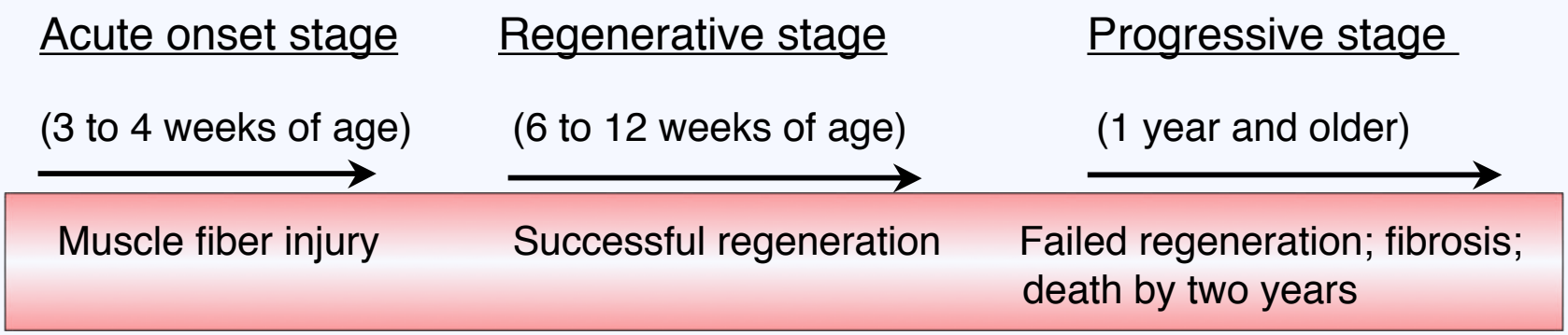
Cell death

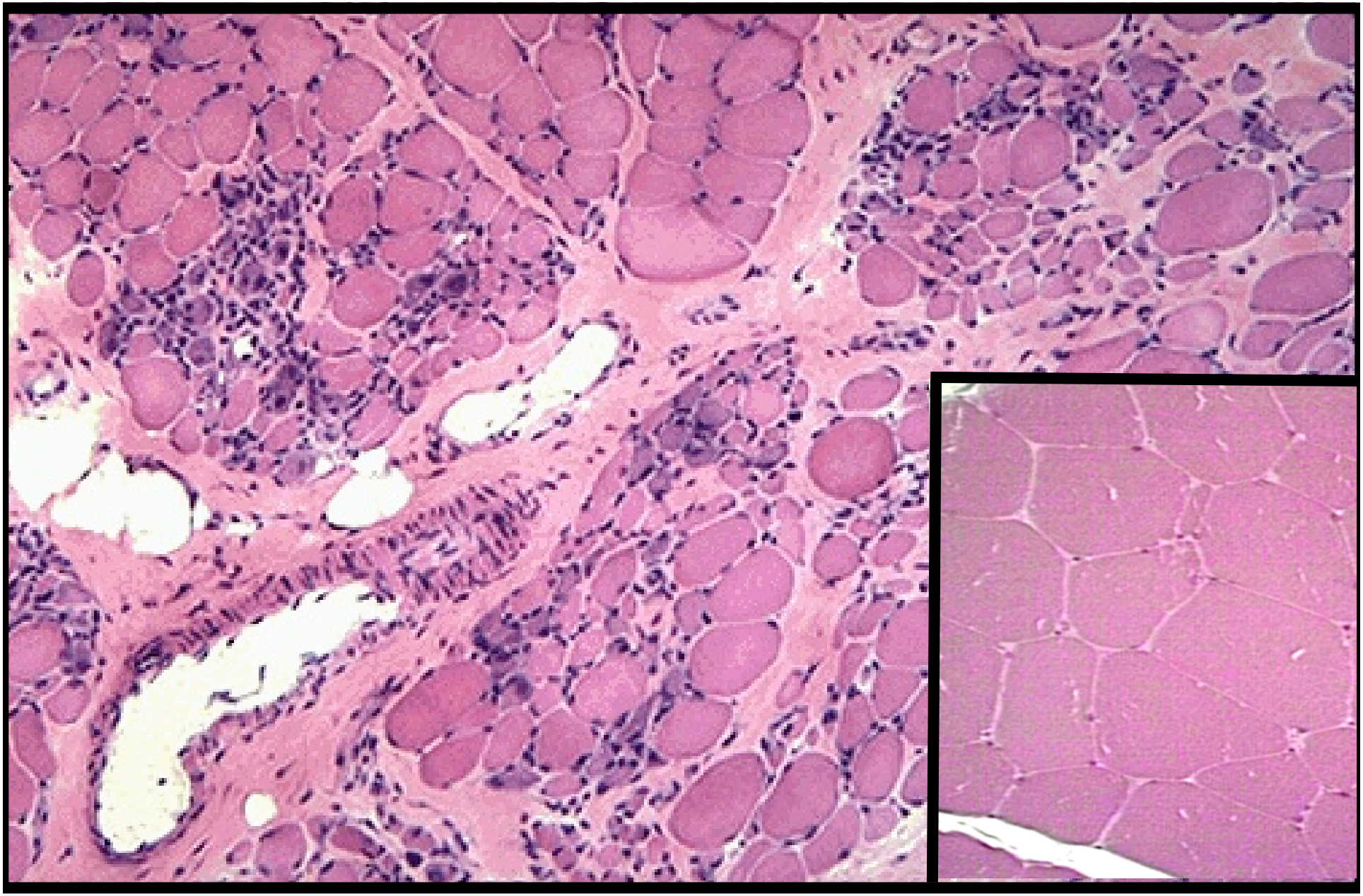


## Dystrophin-deficient DMD humans




## Dystrophin-deficient *mdx* mice





Duchenne muscular dystrophy.

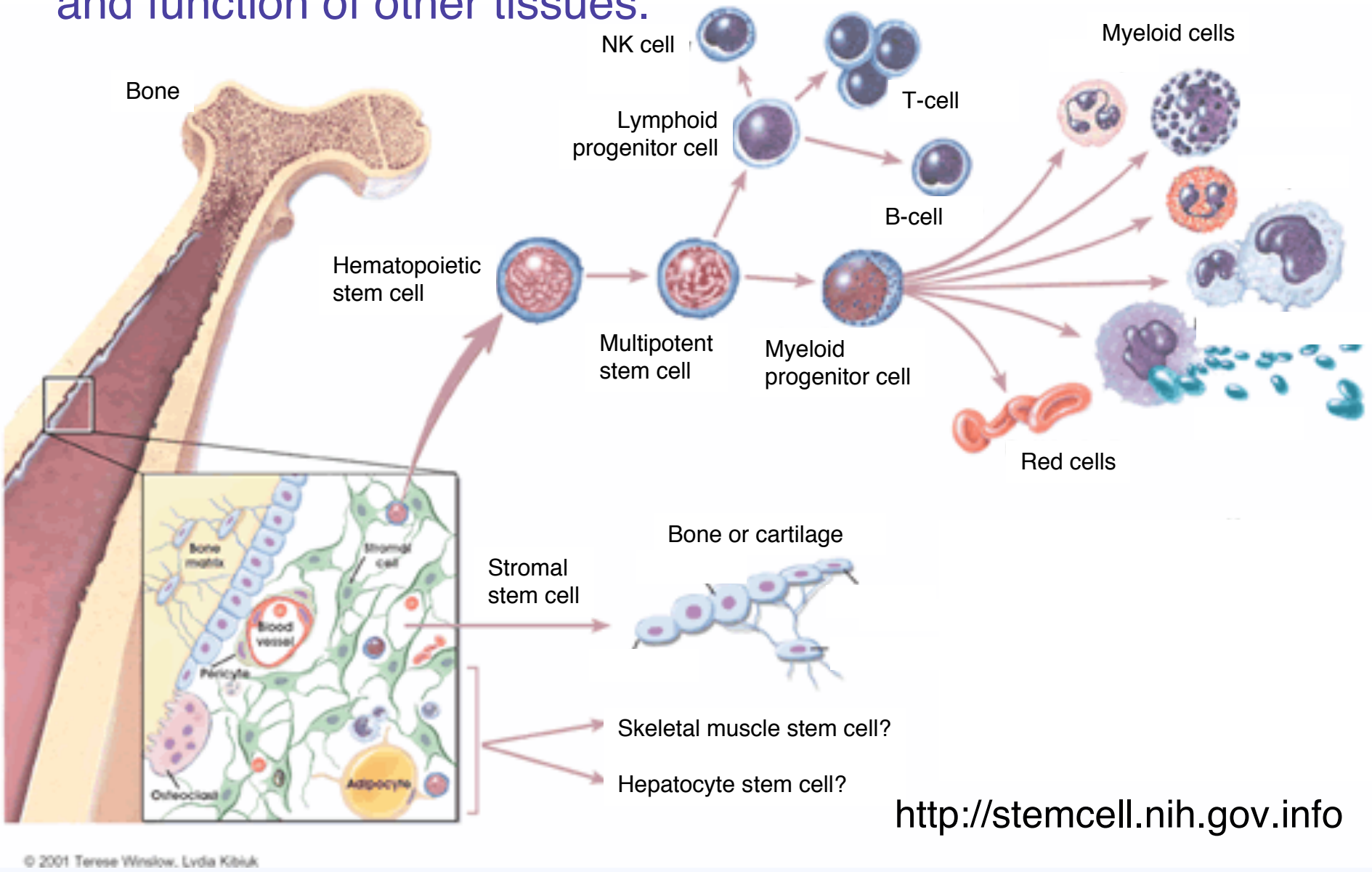
Healthy muscle.

Jorde et al. (2000) Medical Genetics.

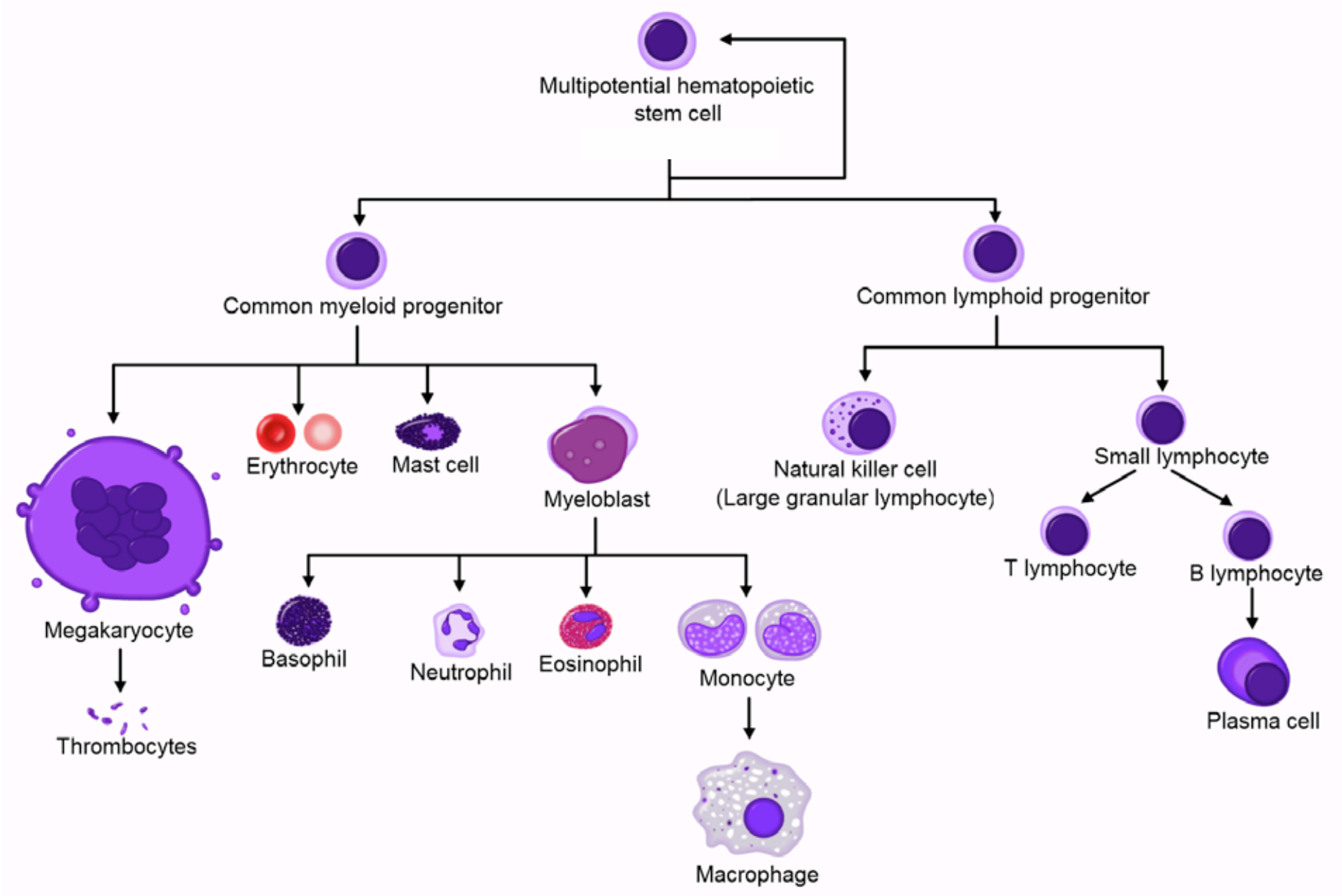
## Goals for presentation:

- learn the major populations of immune cells present in dystrophic muscle;
- learn interactions between immune cells and dystrophic muscle;
- learn mechanisms that influence inflammatory cell phenotype;
- learn relationships between inflammatory cell phenotype and stages of muscular dystrophy.

# Hematopoietic stem cells are the source of multiple cell lineages that have the potential to affect growth, regeneration and function of other tissues.



# HSCs are the source of multiple cell lineages that can affect growth, regeneration and function of other tissues.

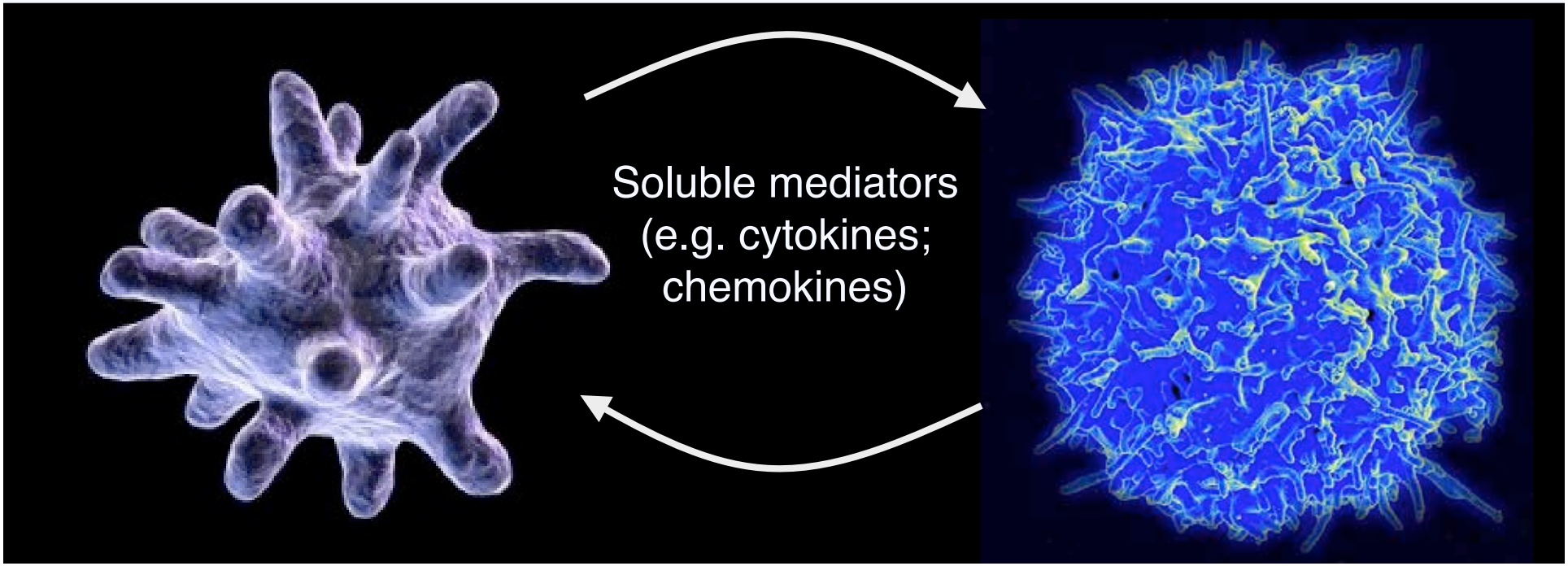


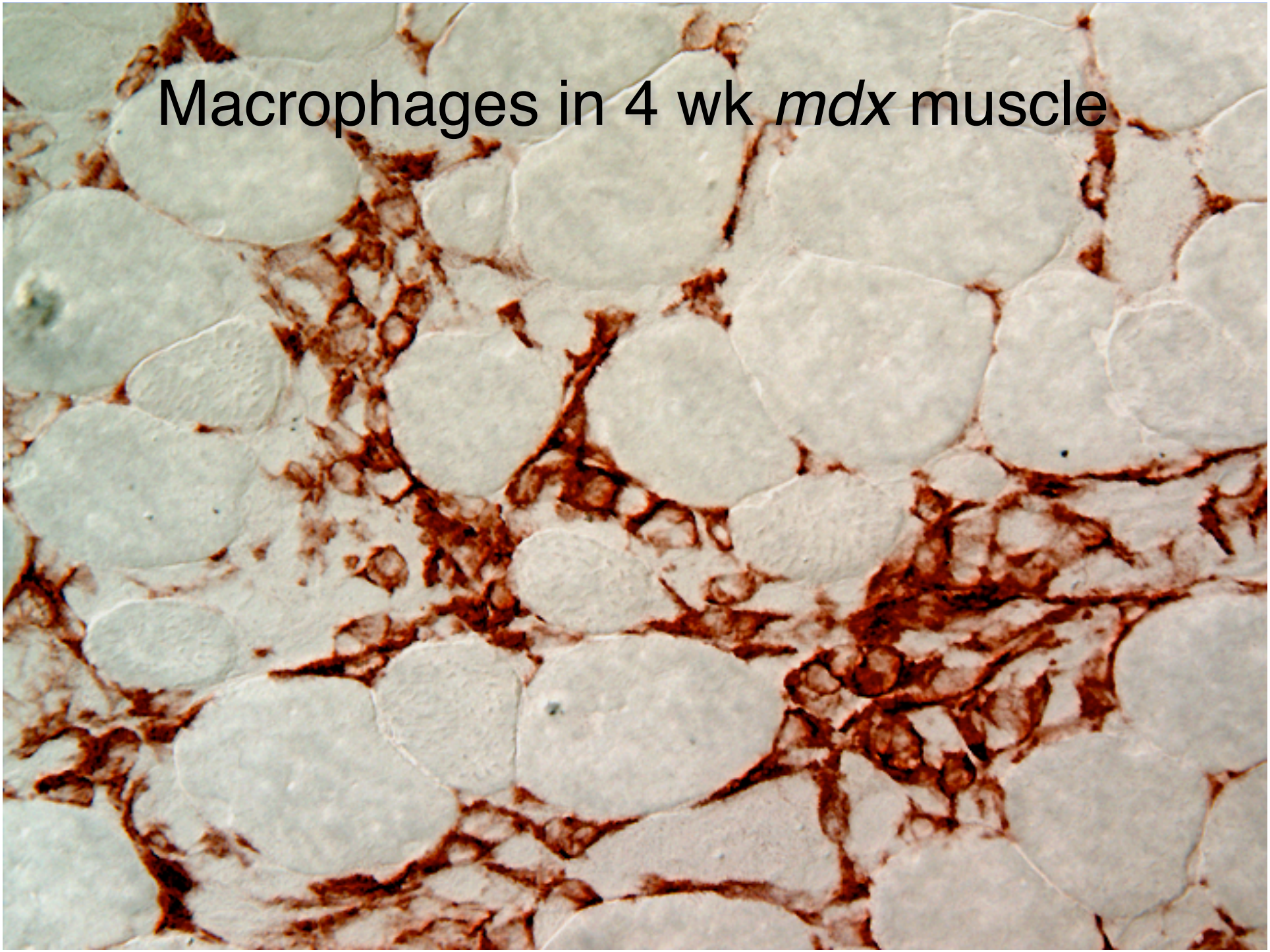
## Innate immunity:

- relatively rapid
- non-specific;
- inflammation
- humoral immunity
- (complement system)
- cellular immunity
- (mostly myeloid cells)

## Acquired immunity:

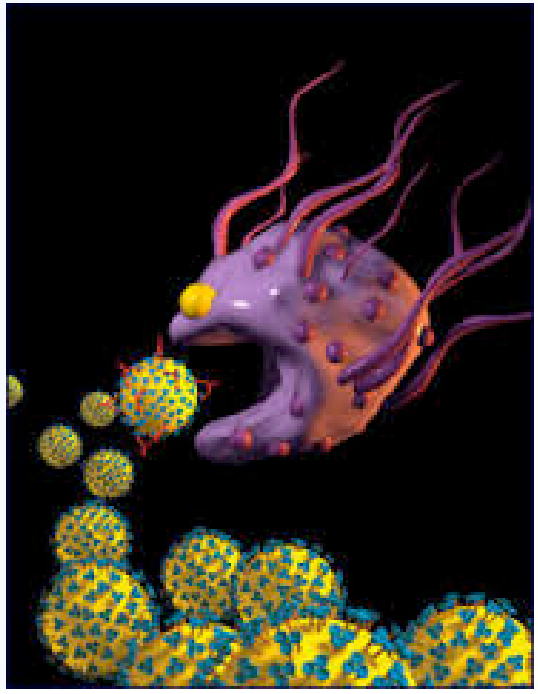
- develops over time
- pathogen specific
- immunological memory
- humoral immunity
- (antibodies);
- cellular immunity
- (mostly lymphocyte mediated)




Macrophages in 4 wk *mdx* muscle

## Macrophages:

- differentiate from monocytes;
- phagocytic;
- can lyse other cells via free radicals;
- release soluble factors that can regulate the functions of myeloid cells and lymphoid cells;
- can present antigen to lymphocytes to influence their activation.
- release factors that can influence growth and differentiation of multiple cell types.



# Macrophages isolated from 4 wk old *mdx* muscles are highly cytolytic.

(Wehling et al. (2001) J. Cell Biol.)

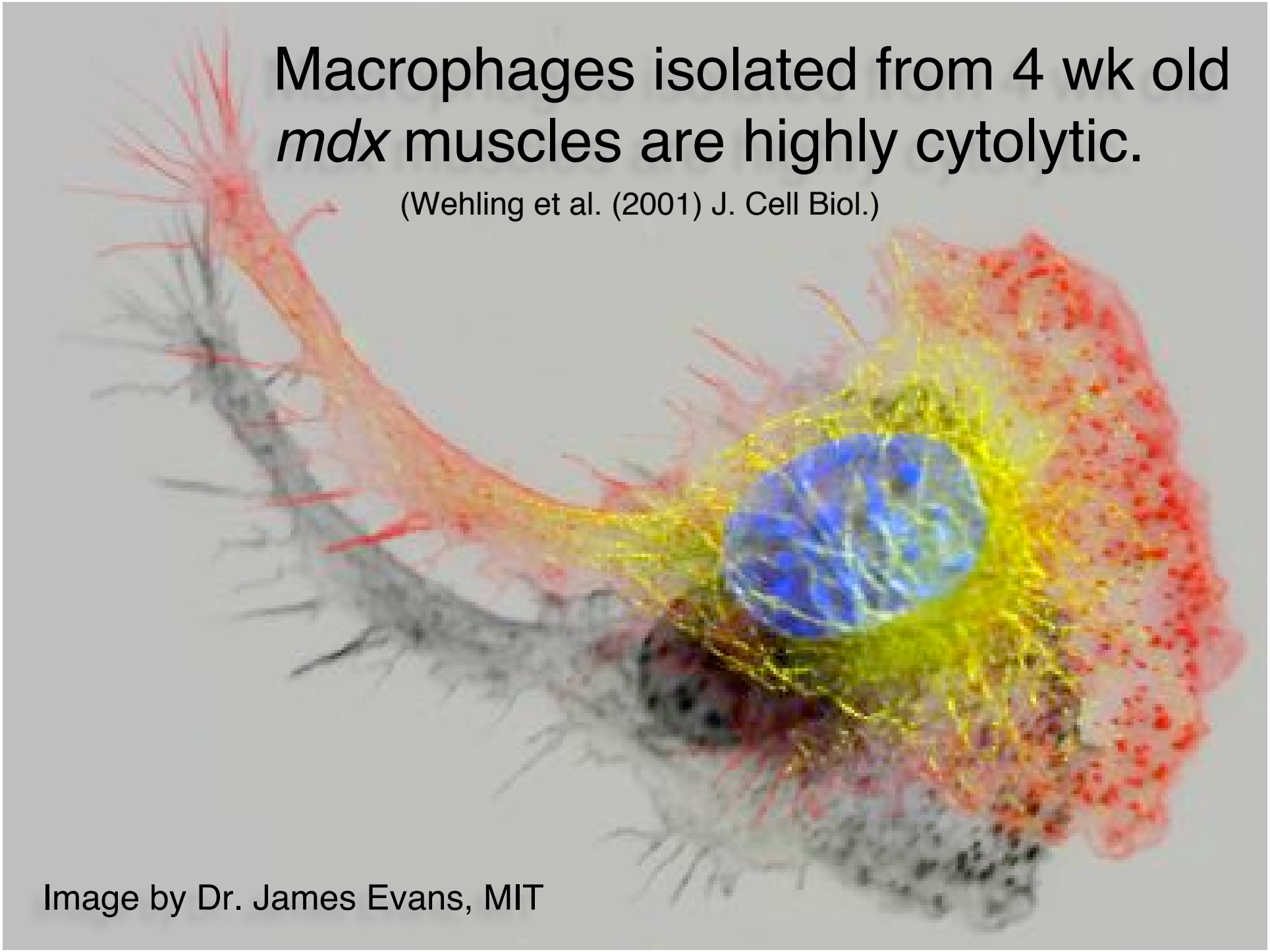
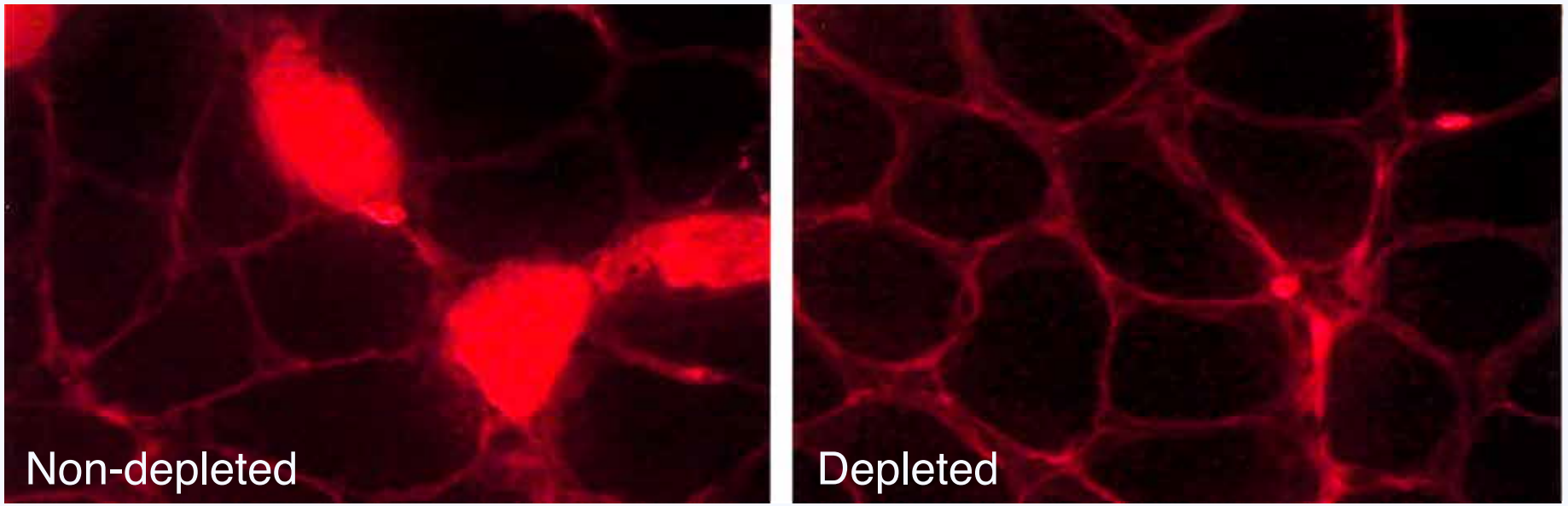


Image by Dr. James Evans, MIT

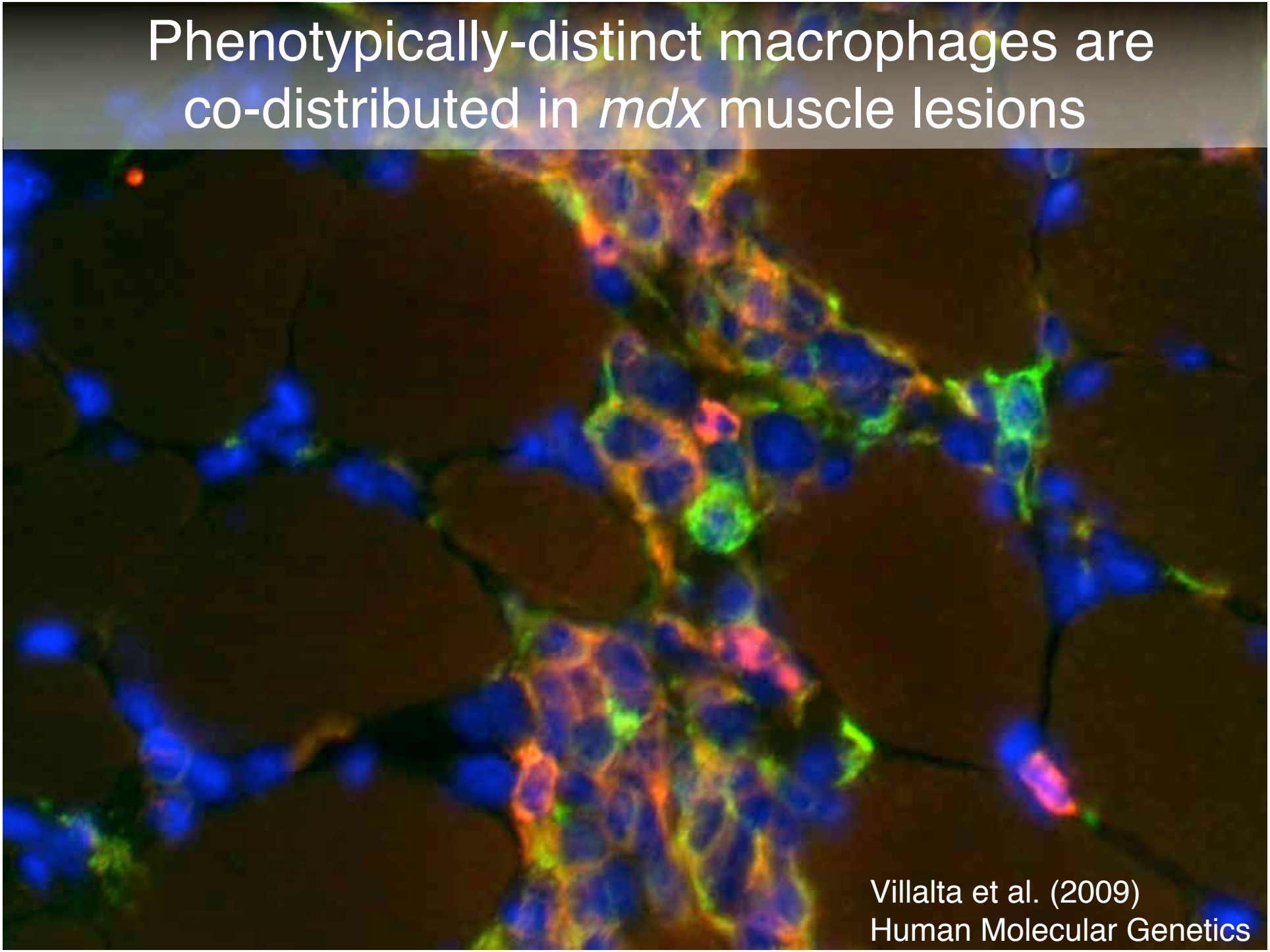
# Macrophages in 4-wk-old *mdx* muscles are highly cytolytic.




*In vivo* depletions of macrophages in *mdx* mice reduces muscle membrane lysis by more than 70%.

(Wehling et al. (2001) J. Cell Biol.)

# Phenotypically-distinct macrophages are co-distributed in *mdx* muscle lesions



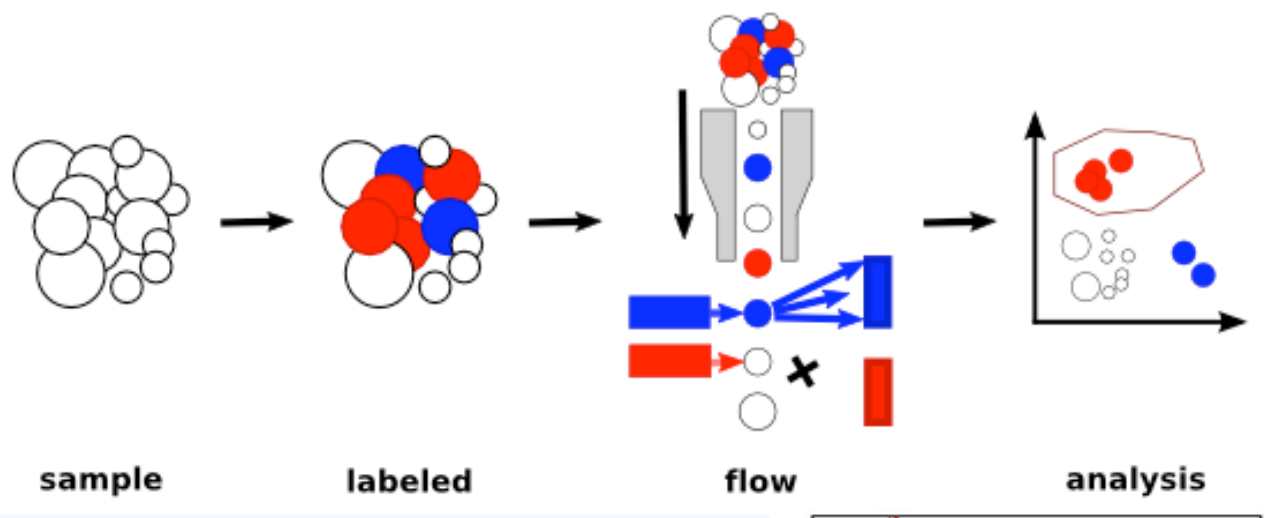
Villalta et al. (2009)
Human Molecular Genetics

# Phenotyping cell populations by flow cytometry

Assay single sample for cells expressing two or more markers

Marker 1

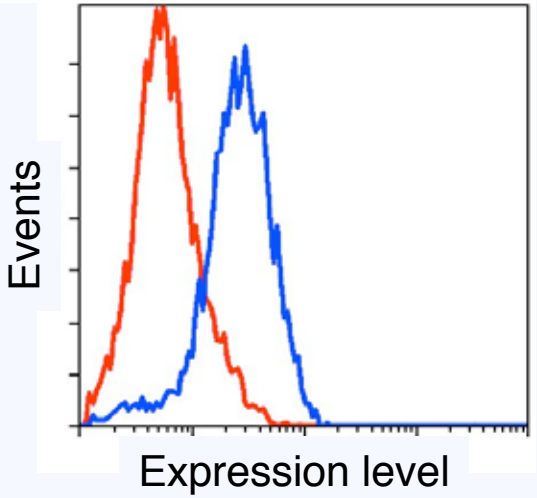
Marker 2



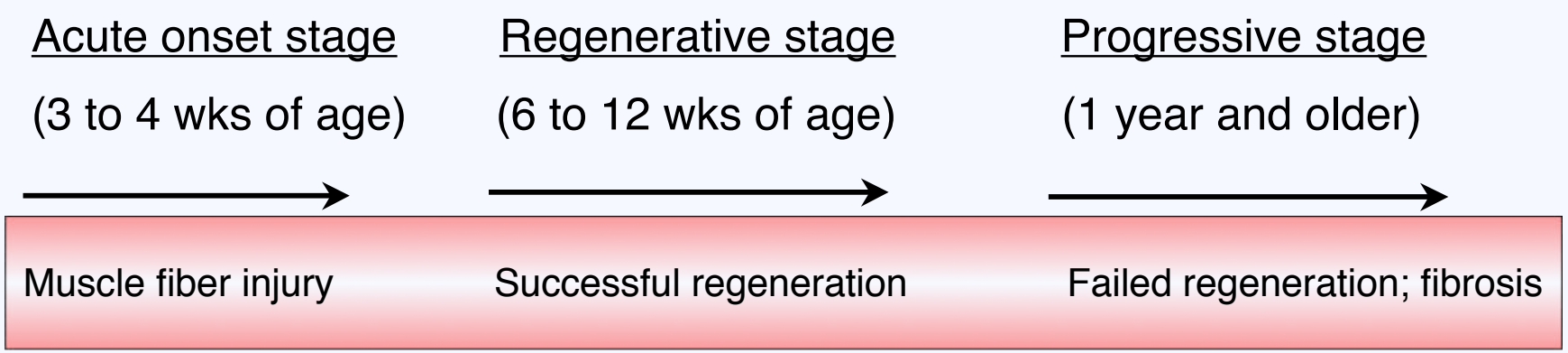

Assay two samples for expression of a single marker.

Sample 1

Sample 2



# Time course of muscle pathology and macrophage phenotype switching in muscular dystrophy.



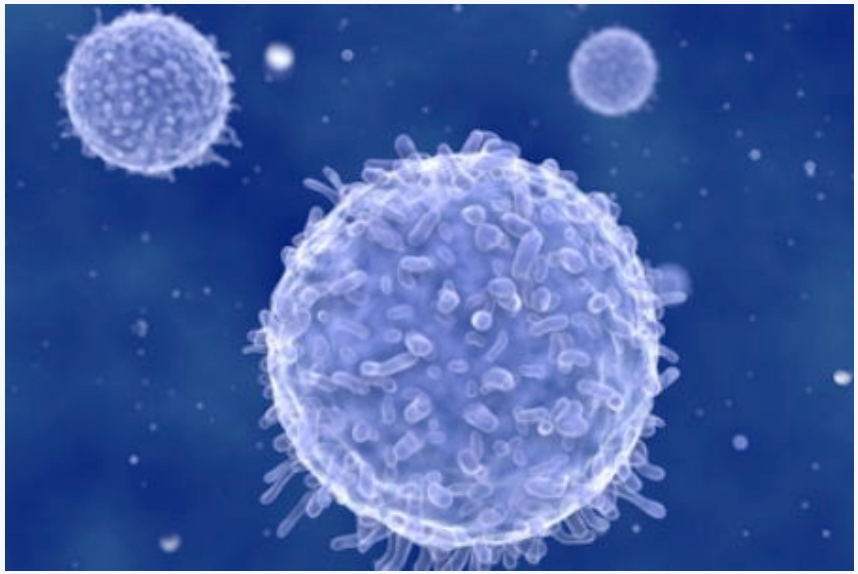

**M1 macrophages**
$F4/80^{+}/CD163^{-}/CD206^{-}$
cytolytic
pro-inflammatory
$iNOS^{high}$
$arginase^{low}$

**M2c macrophages**
$F4/80^{+}/CD163^{+}/CD206^{+}$
non-cytolytic
anti-inflammatory
$iNOS^{low}$
$arginase^{low}$

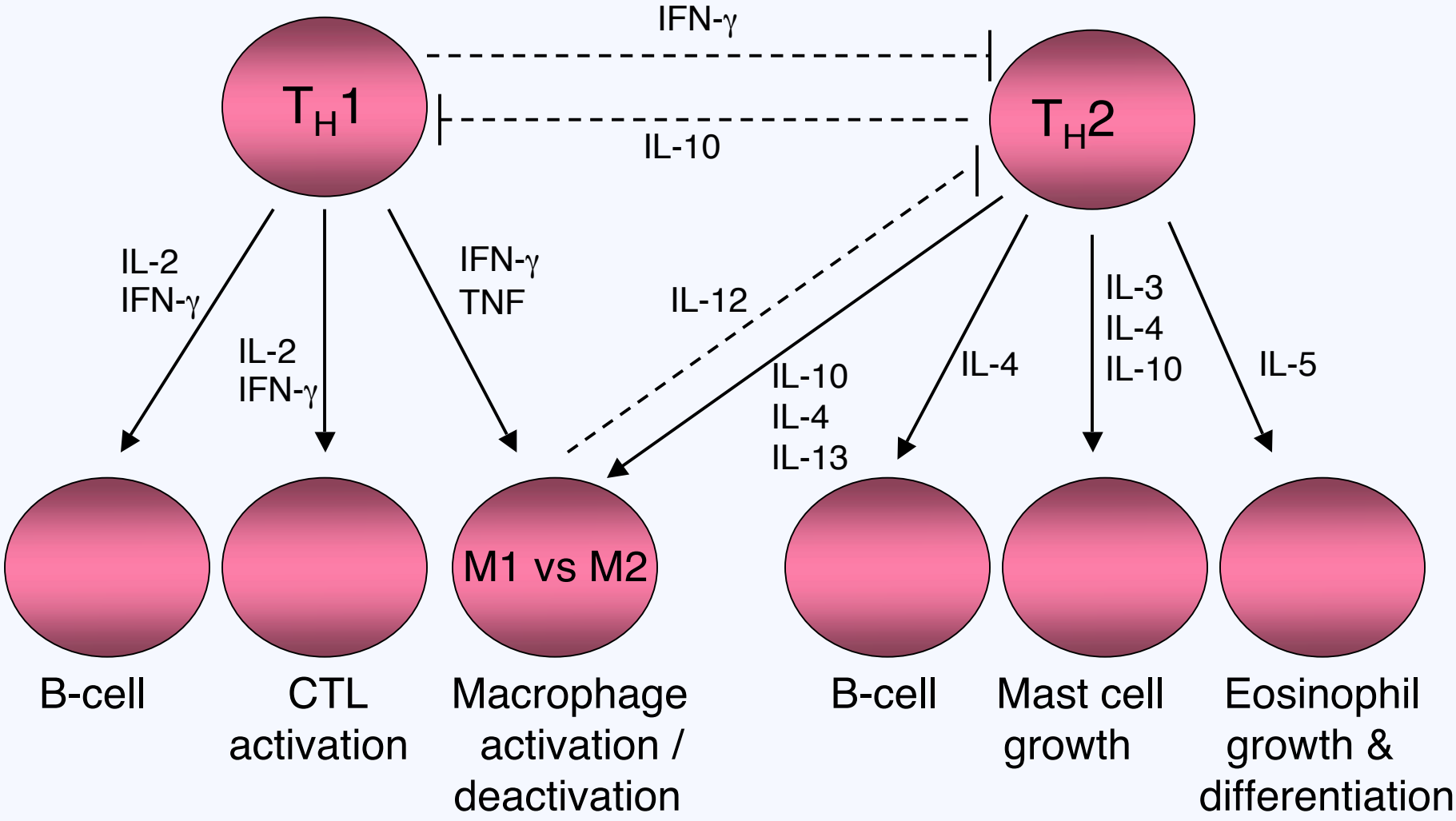
**M2a macrophages**
$F4/80^{+}/CD163^{+}/CD206^{+}$
non-cytolytic
anti-inflammatory
$iNOS^{low}$
$arginase^{high}$

## Helper T-cells ($T_h$ cells):

- differentiate in thymus;
- rich source of cytokines that influence the activities of other immune cells;
- learn to recognize and bind specific antigenic peptides that affect T-cell function and proliferation;
- can differentiate into:
    - effector T-cells (Th1 or Th2),
    - memory T-cells,
    - regulatory T-cells.



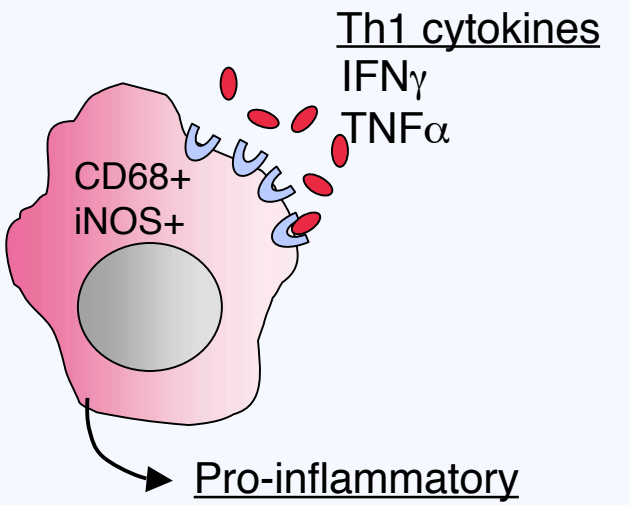
# Cytokine modulation of immune cell functions.



# Fates of M1 and M2 macrophages.

## M1 macrophages

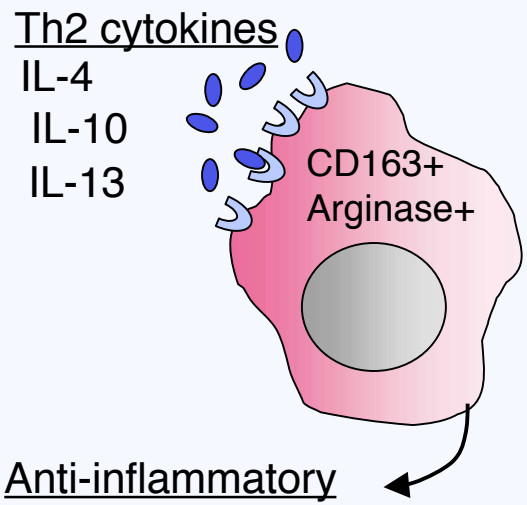
(classical activation)




Pro-inflammatory
Cytolytic (free radicals)
Phagocytic

## M2 macrophages

(alternative activation)




Anti-inflammatory
Tissue repair
Tissue fibrosis

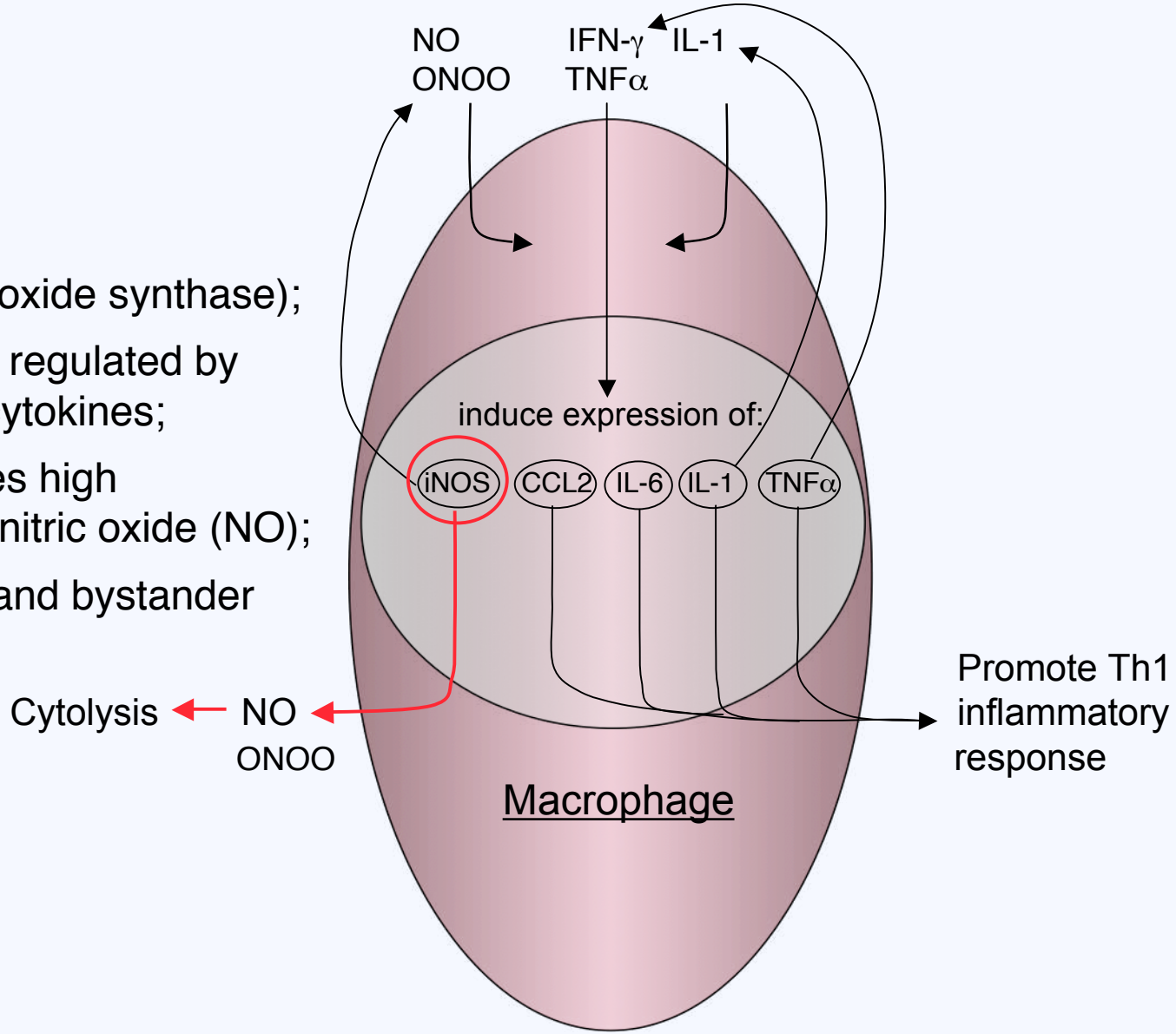
Acute peak of pathology

Regenerative and progressive stages

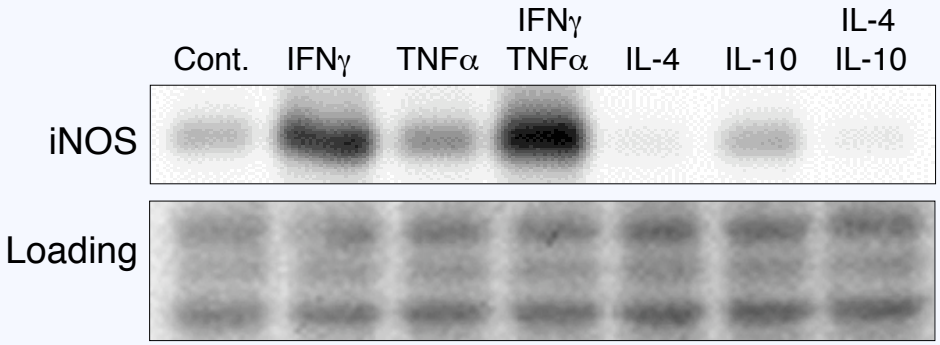
# Pro-inflammatory pathways in *mdx* macrophages

<u>iNOS</u>:

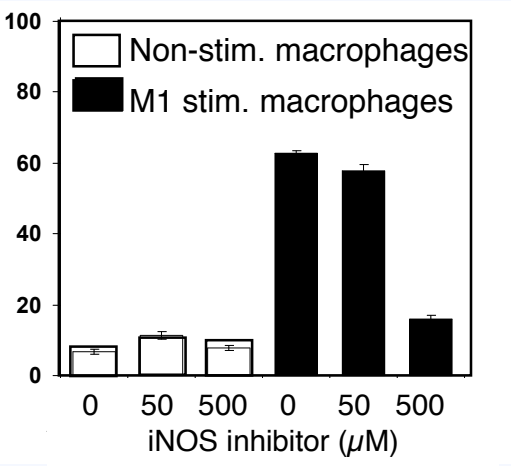
- (inducible nitric oxide synthase);
- transcriptionally regulated by proinflammatory cytokines;
- rapidly generates high concentrations of nitric oxide (NO);
- can lyse target and bystander cells.



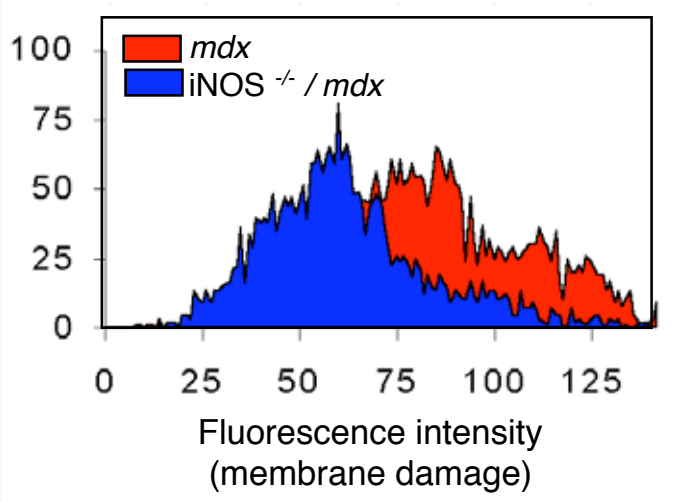
# Ablating iNOS expression reduces the pathology of *mdx* dystrophy.




Macrophage lysis of muscle cells in vitro (% total)




Number of muscle fibers

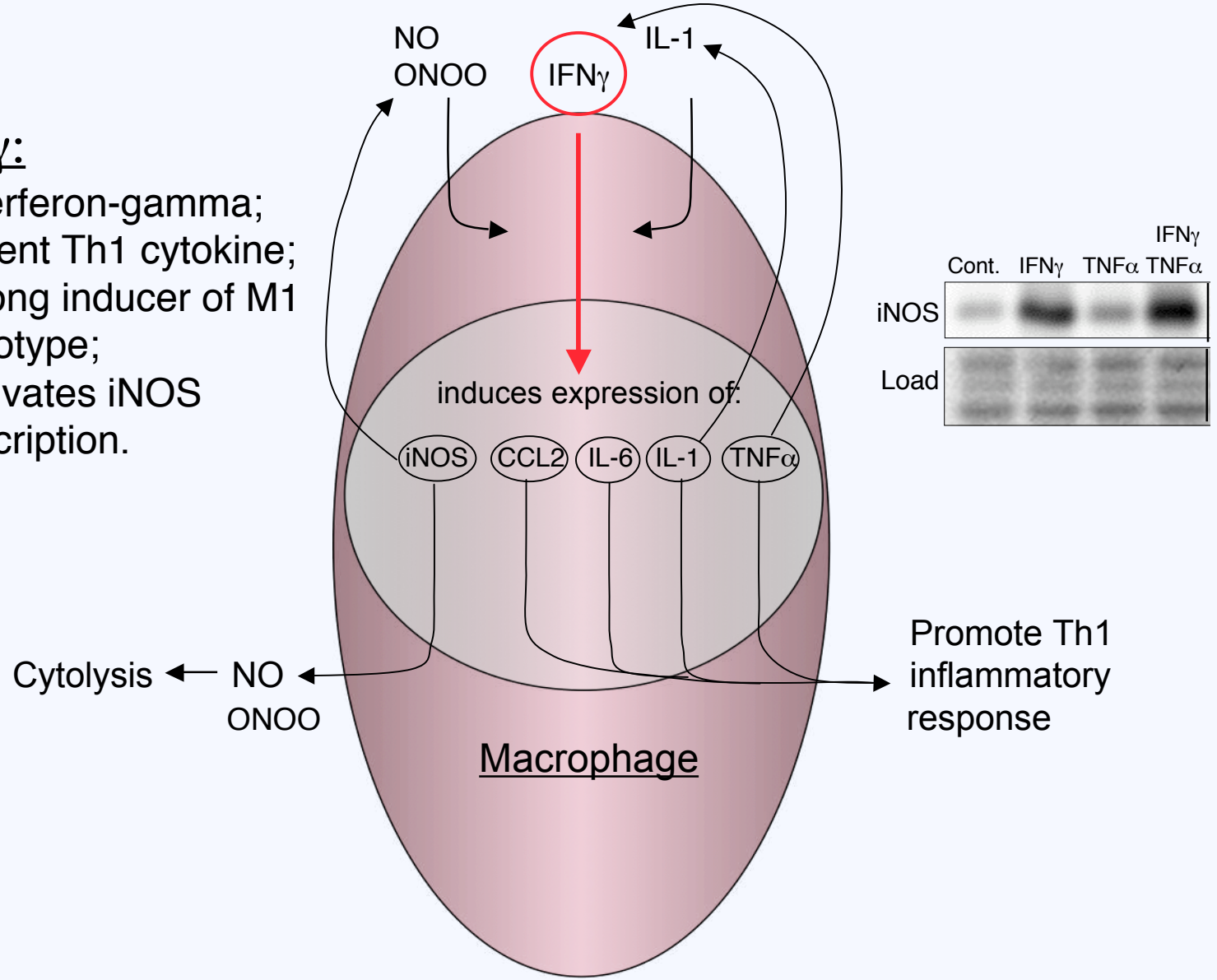



(Villalta et al. (2009) Human Molec. Genetics)

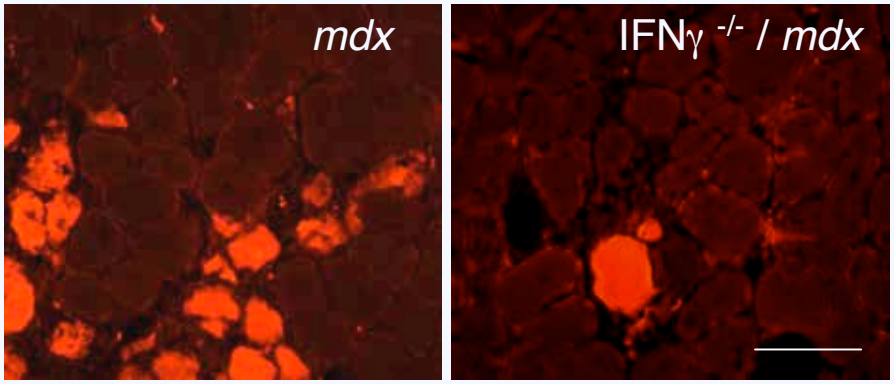
# Pro-inflammatory pathways in *mdx* macrophages

## IFNγ:

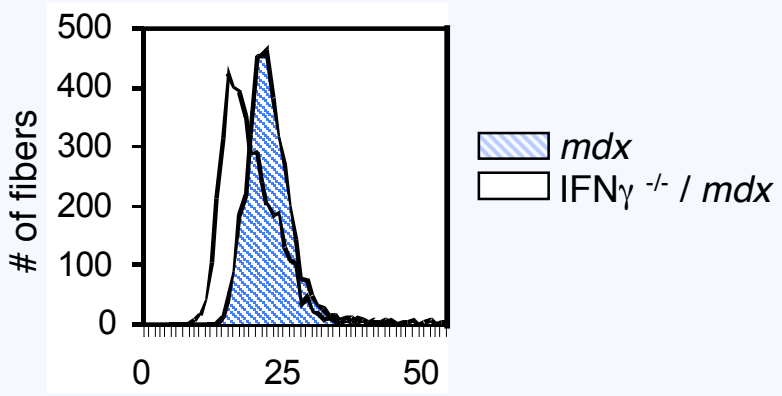
- interferon-gamma;
- potent Th1 cytokine;
- strong inducer of M1 phenotype;
- activates iNOS transcription.



# Ablating IFNγ-mediated signaling reduces the pathology of *mdx* dystrophy.

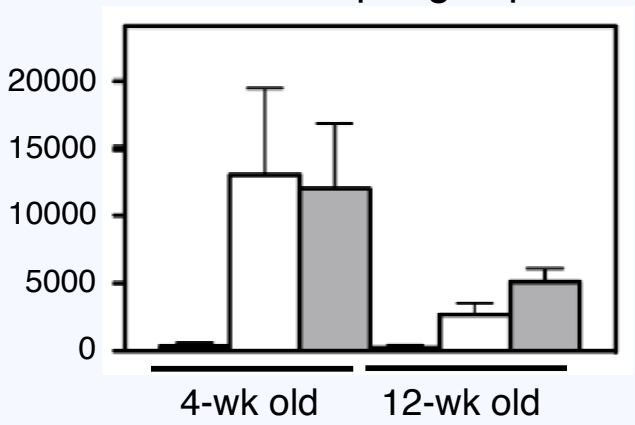



Injured fibers marked with fluorescent tracer.

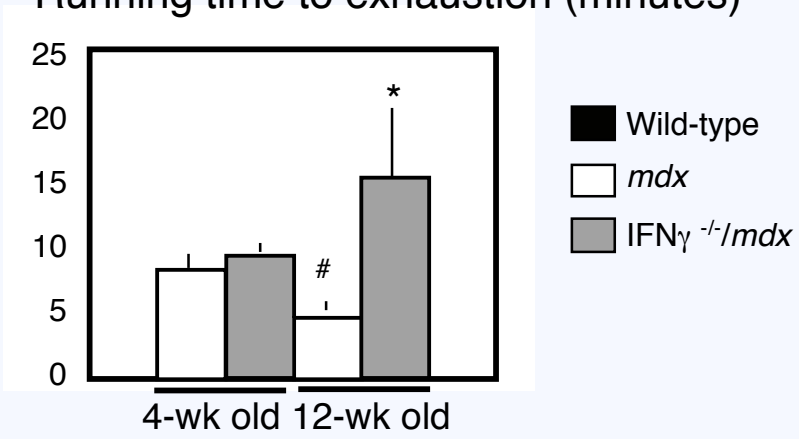



Intracellular fluorescence intensity

F4/80+ macrophages per mm$^3$




Running time to exhaustion (minutes)

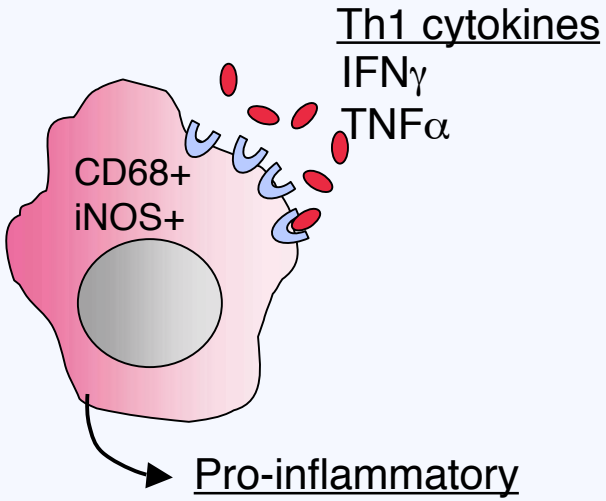



(Villalta et al. (2011) J. Immunol.)

# What regulates macrophage phenotype switch in dystrophic muscle?

## M1 macrophages

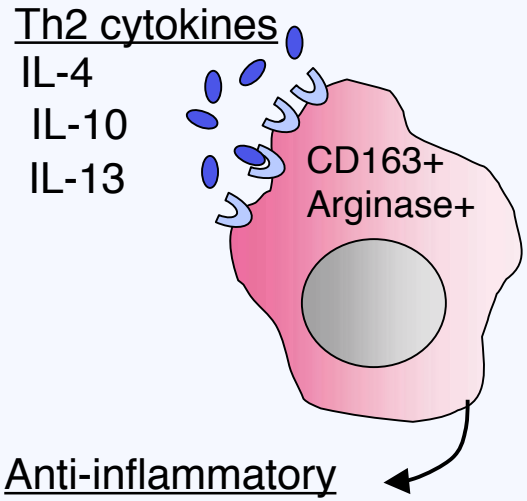
(classical activation)




Pro-inflammatory
Cytolytic (free radicals)
Phagocytic

## M2 macrophages

(alternative activation)



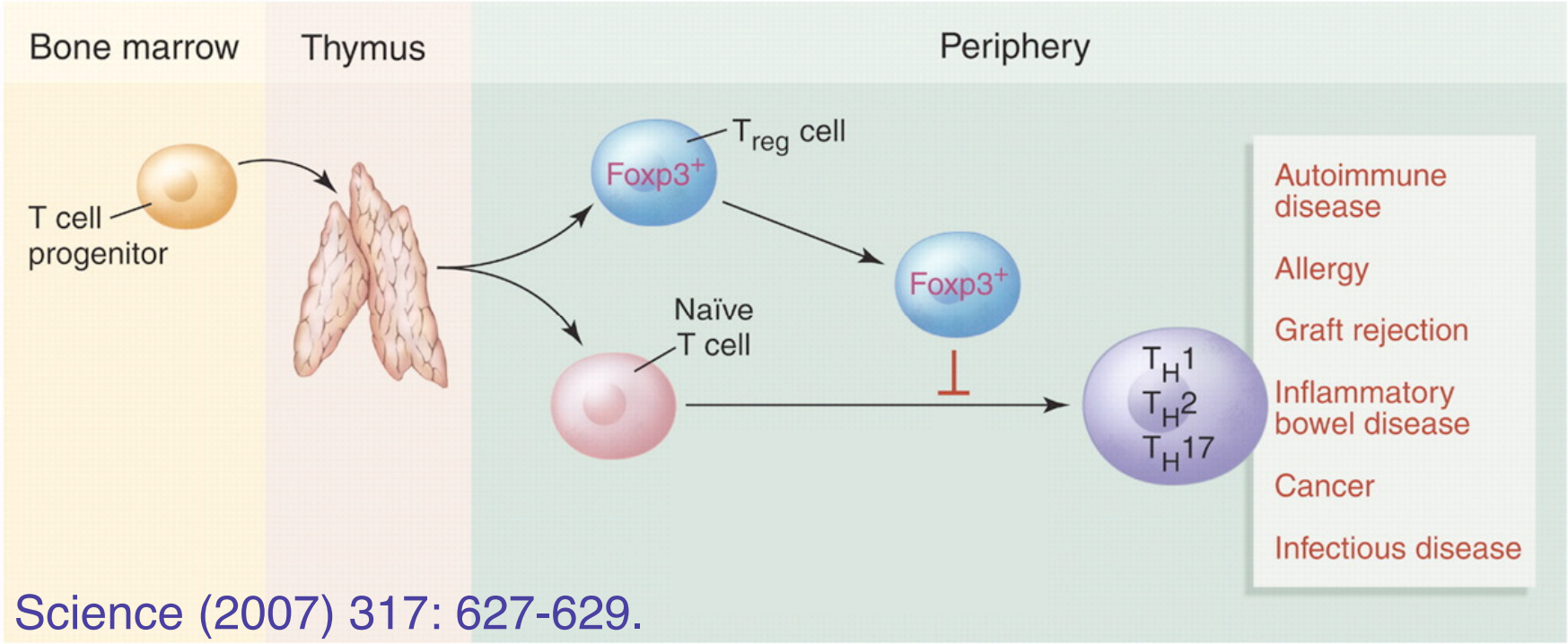

Anti-inflammatory
Tissue repair
Tissue fibrosis

Acute peak of pathology

Regenerative and progressive stages

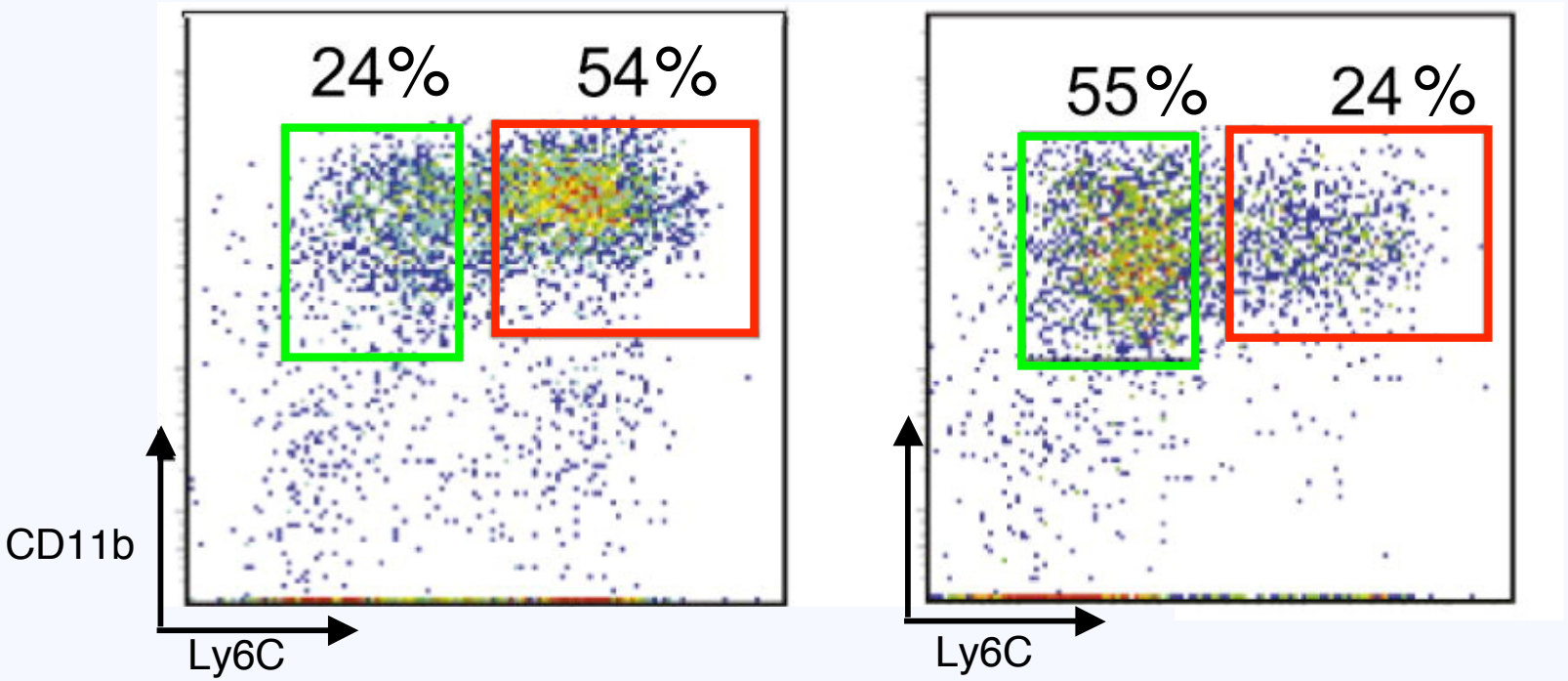
# Tregs:

- regulatory T-cells
- can suppress immune responses
- prevent pathological self-reactivity
- CD4+ / FoxP3+ / CD25+
- can inhibit interferon-γ (IFNγ) secretion
- can increase IL-10 and IL-6 secretion.




Science (2007) 317: 627-629.

# Treg depletion before acute muscle injury shifts macrophages toward an M1 phenotype.



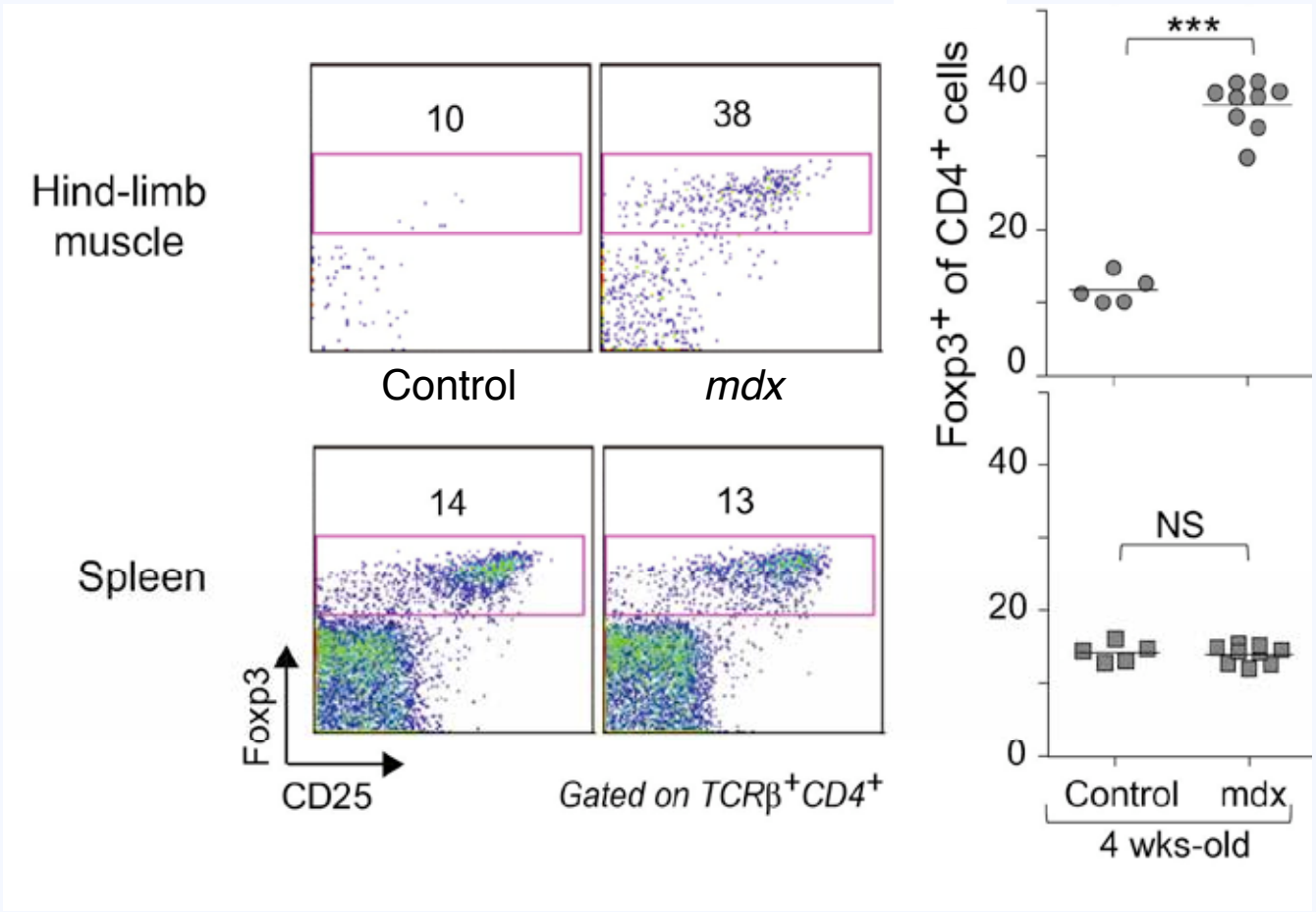

M1 macrophages

M2 macrophages

(Burzyn et al. (2013) Cell)

# Can Tregs regulate the immune response in muscular dystrophy?

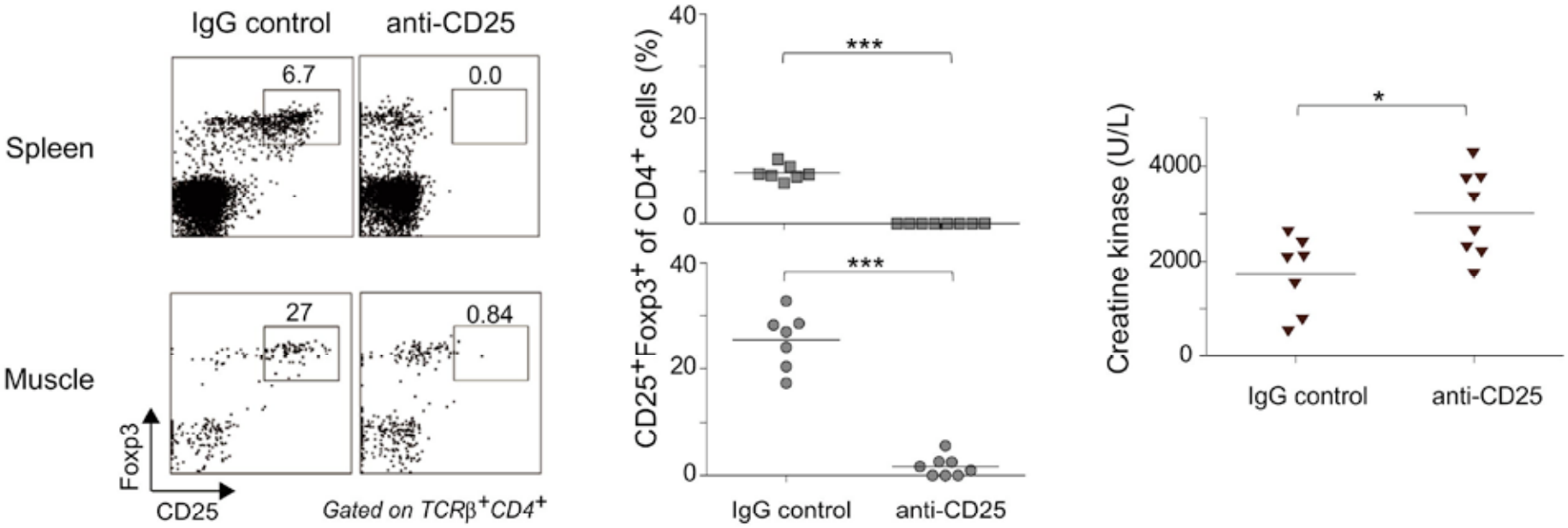
- about one-third of the T-cells in *mdx* muscle are Tregs.




(Burzyn et al. (2013) Cell)

# Can Tregs regulate the immune response in muscular dystrophy?

- Treg depletion worsens damage of dystrophic muscle.

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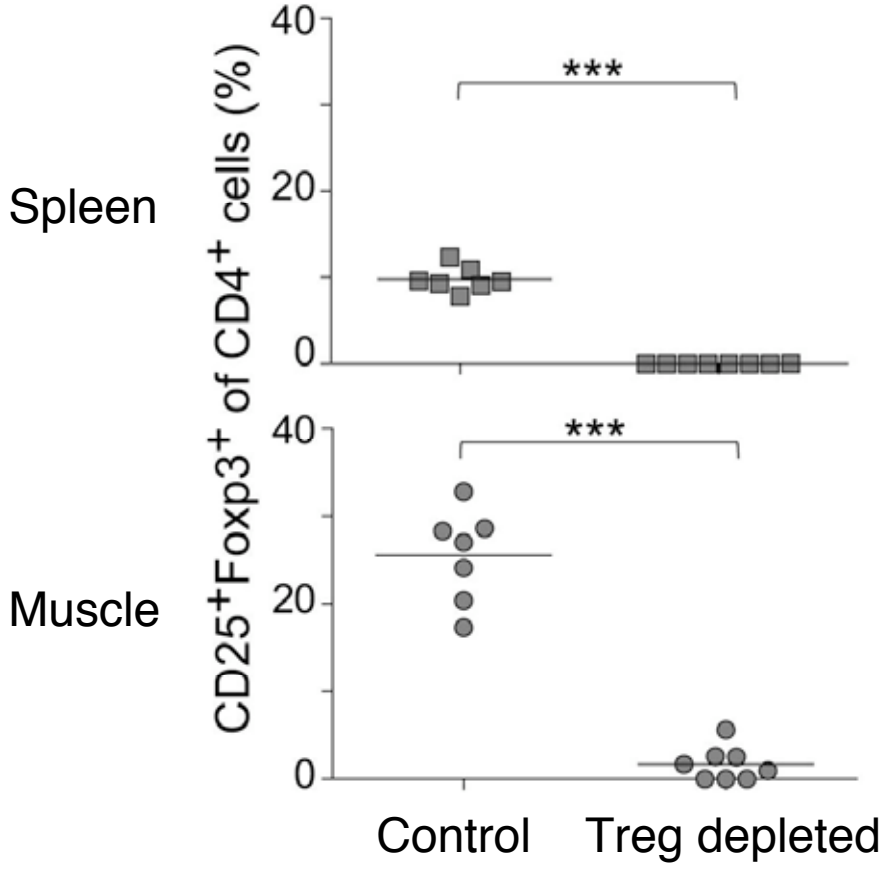
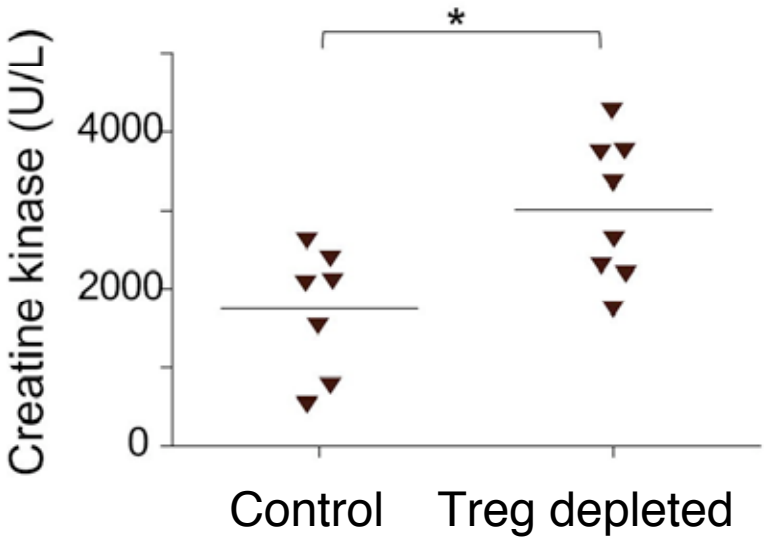
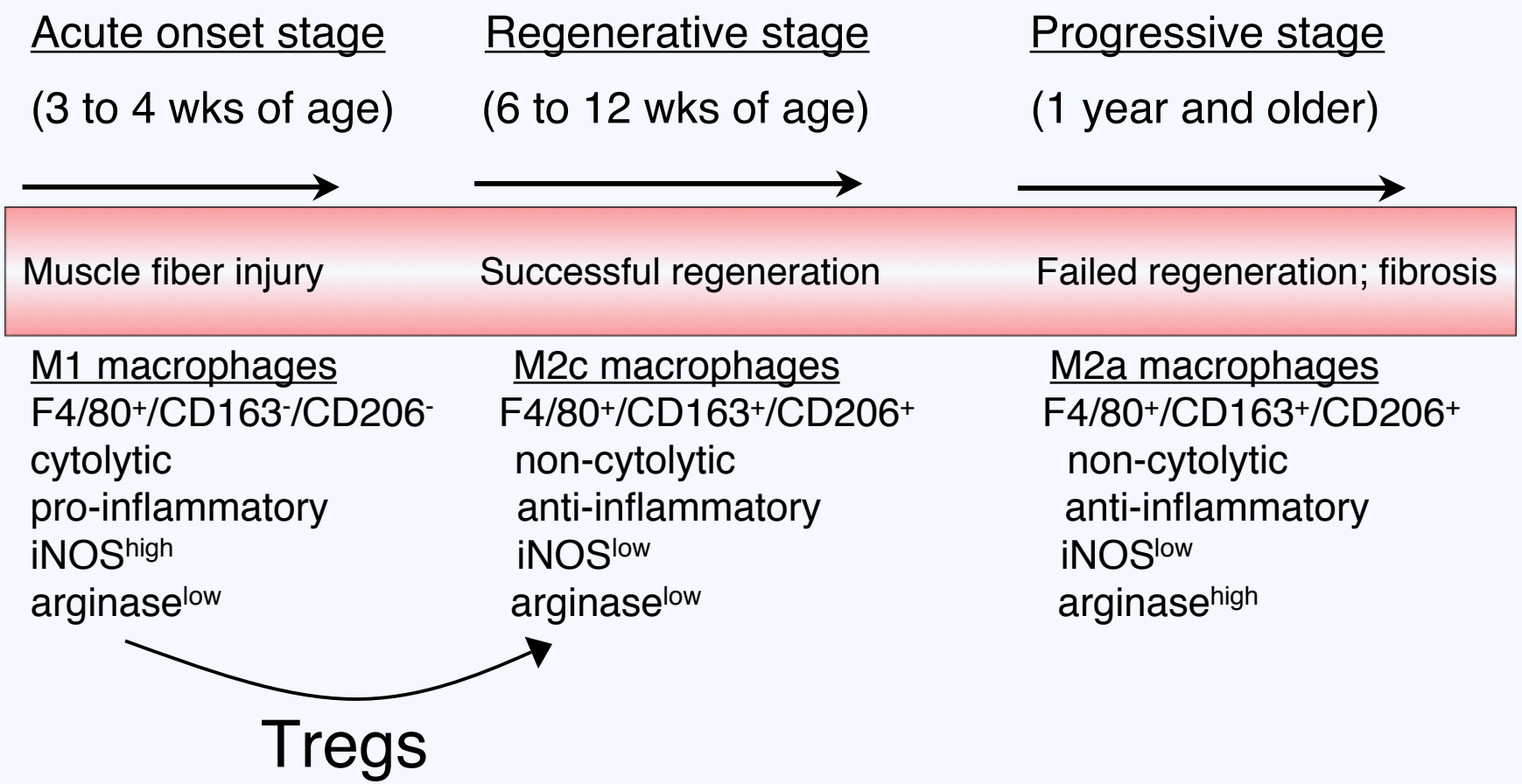



(Burzyn et al. (2013) Cell)

# Time course of muscle pathology and macrophage phenotype switching in muscular dystrophy.



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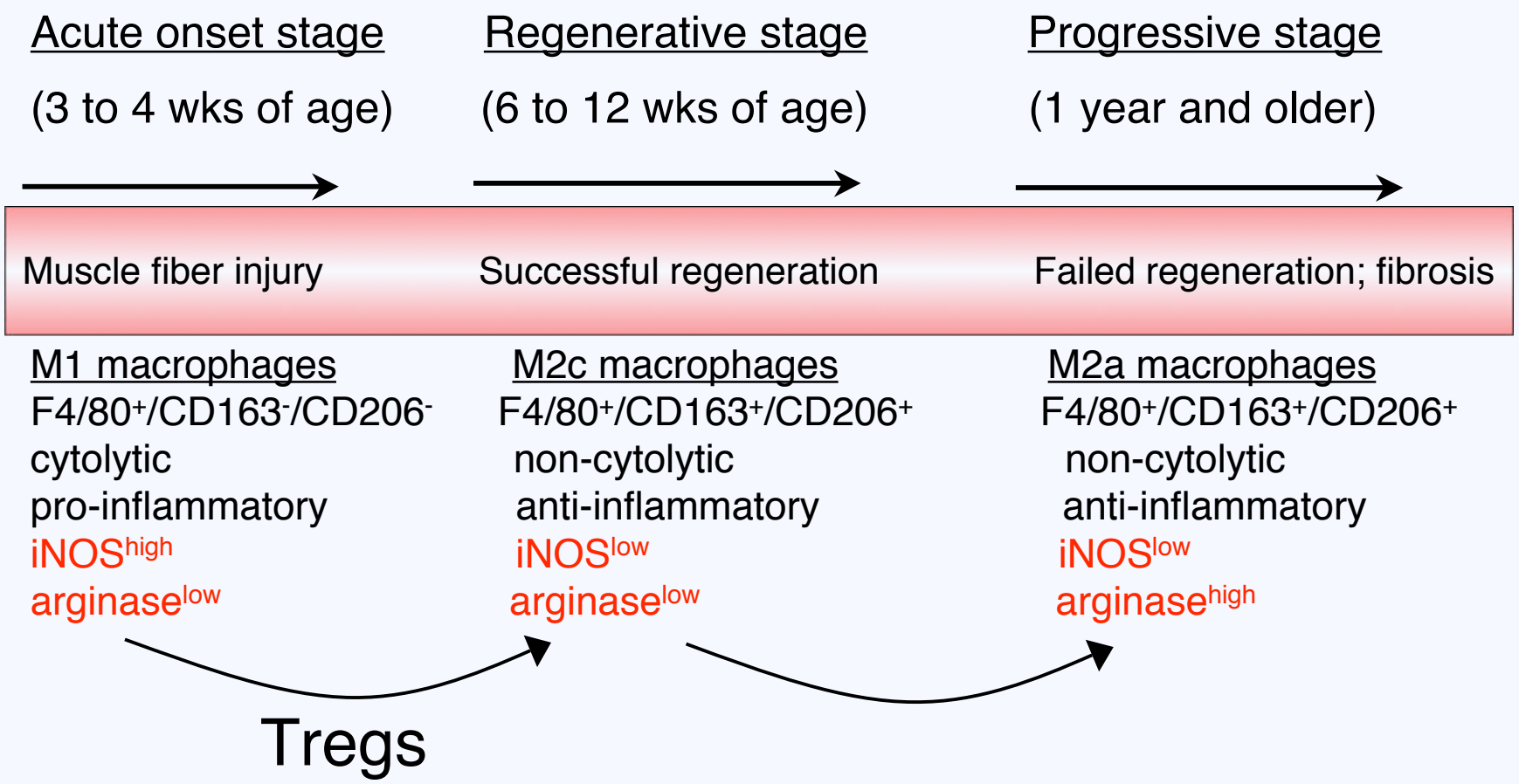
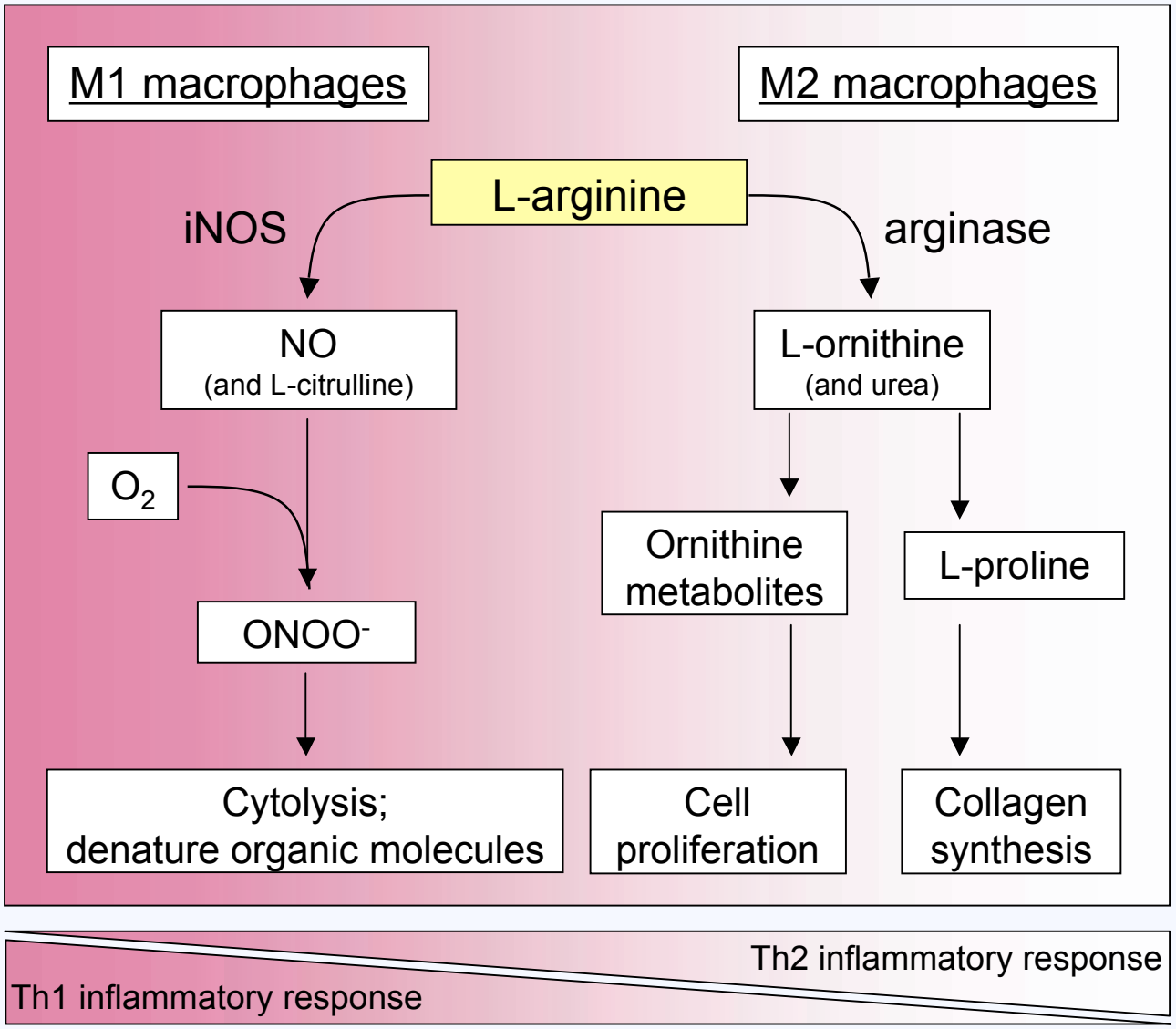
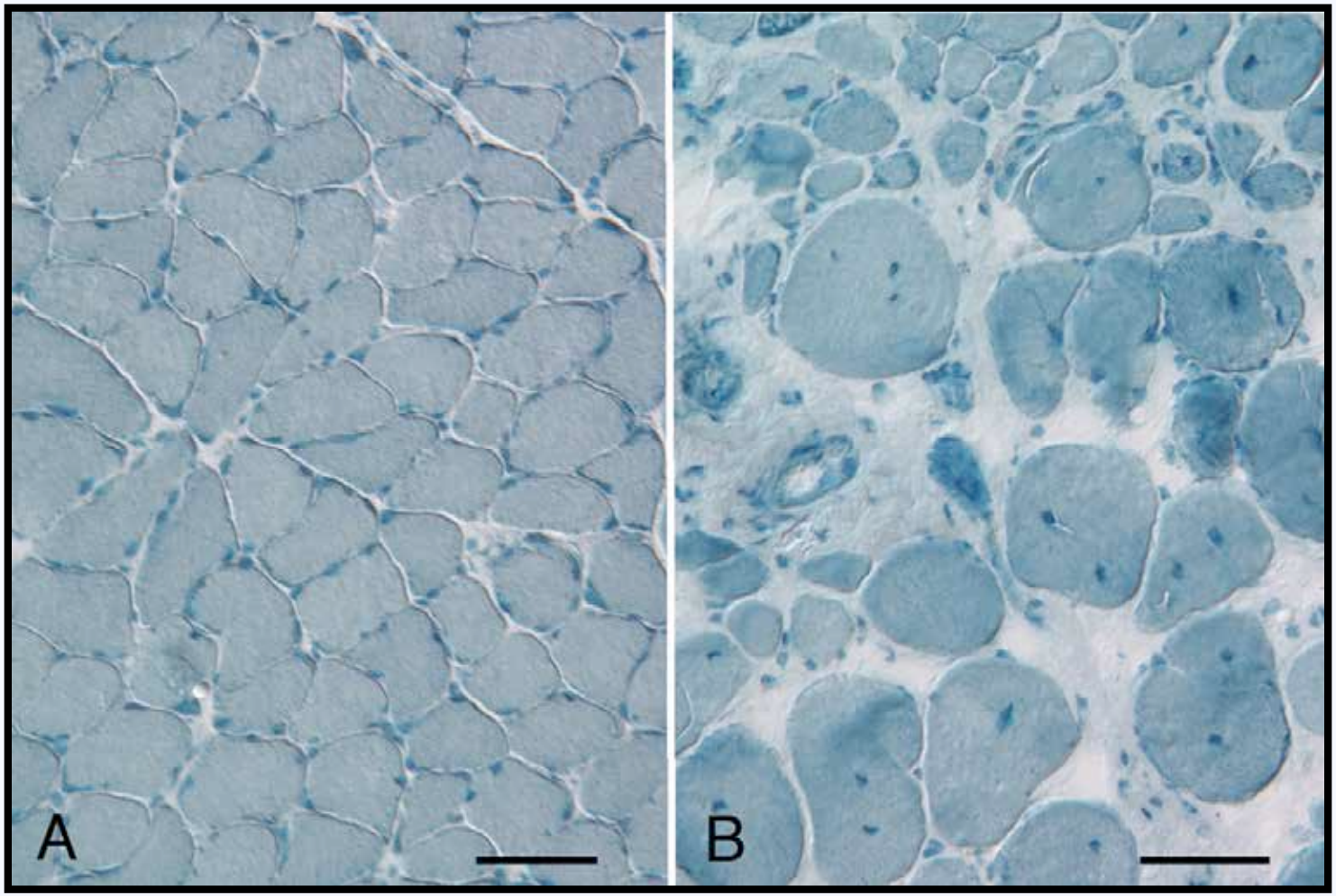


# Arginine metabolism by macrophages




Villalta et al., 2009. Human Molecular Genetics.
Wehling-Henricks et al. 2010. PLoS One.

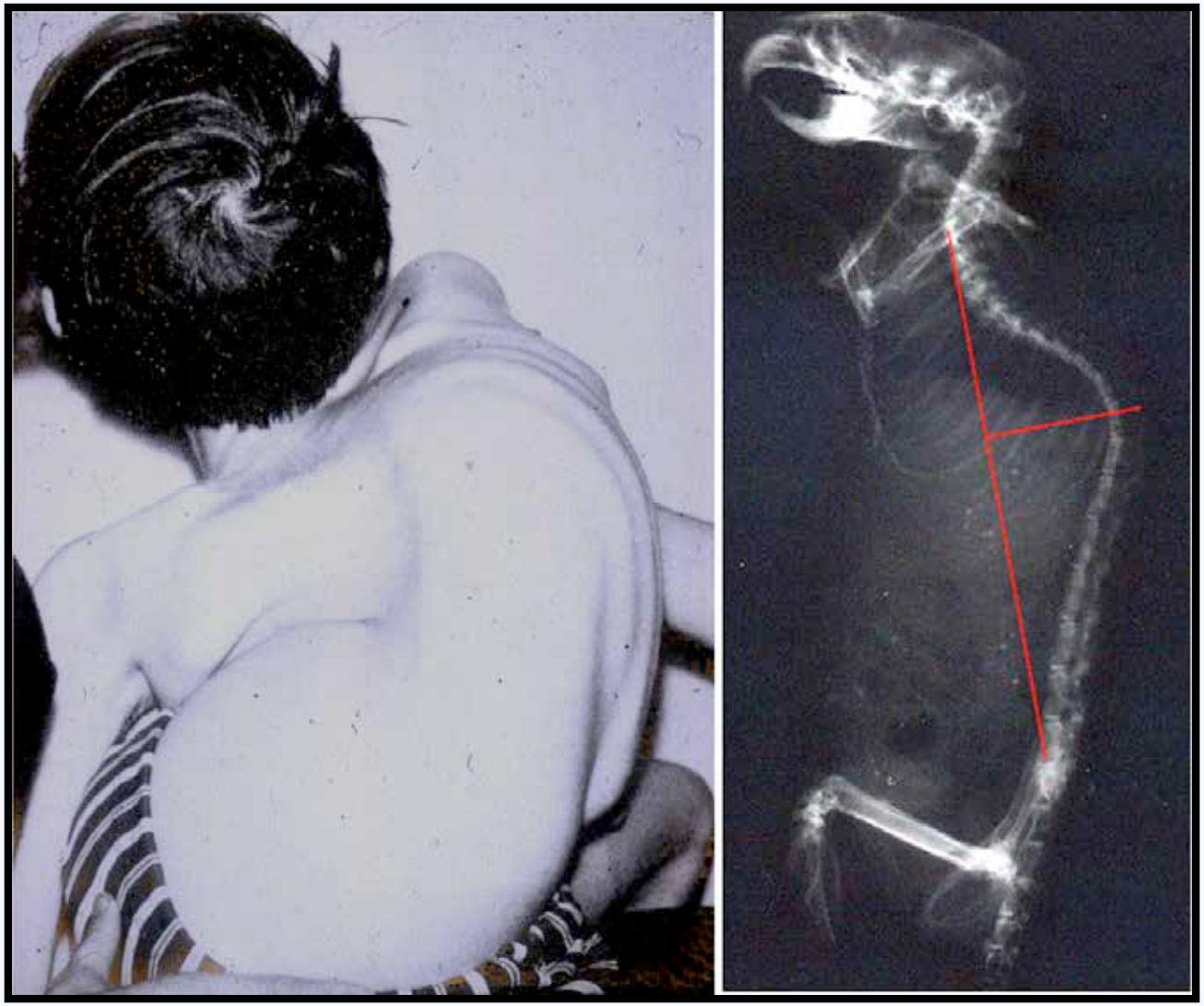
# Quadriceps of 24 month old mice.



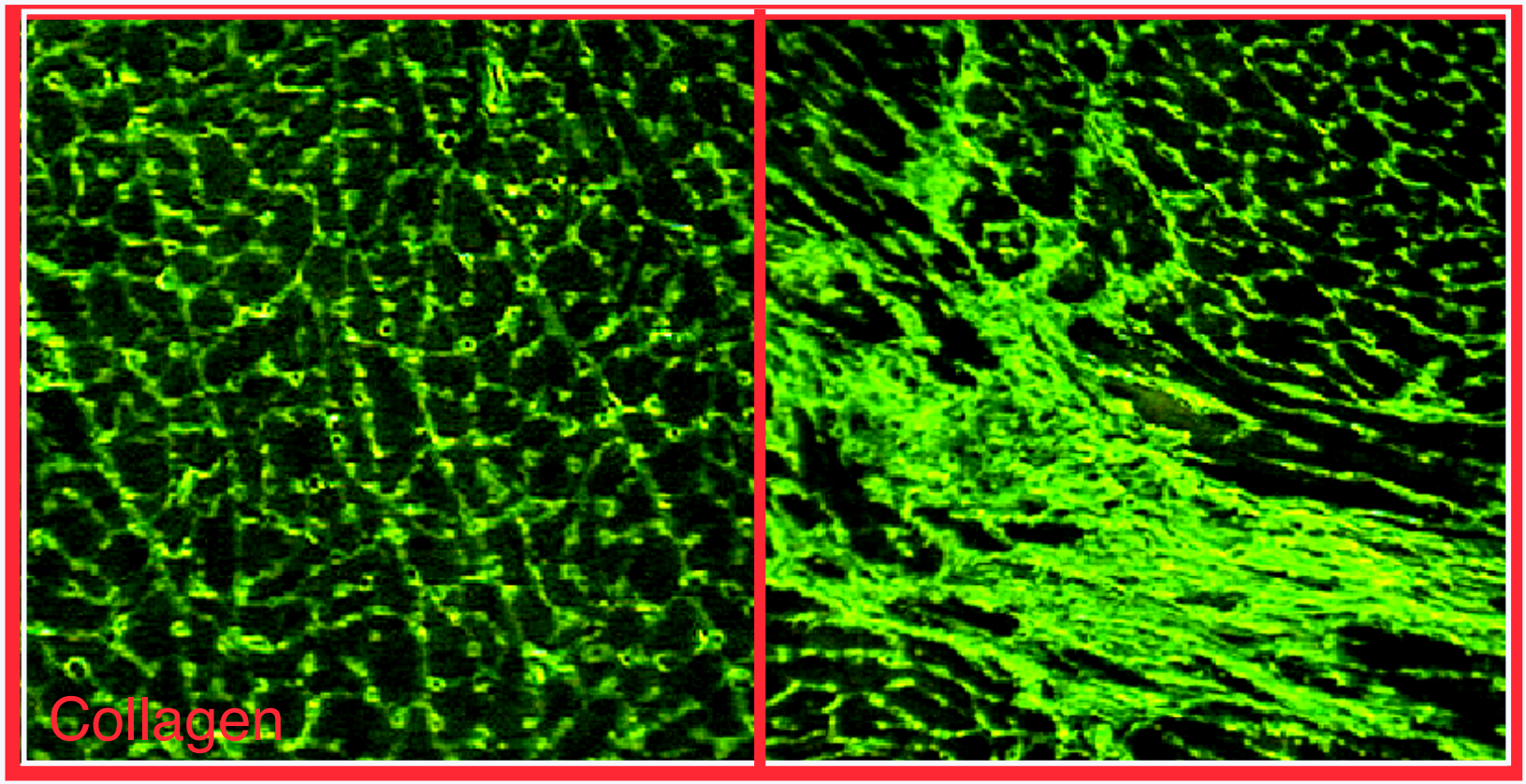

C57 *mdx*

Tidball and Wehling-Henricks. Pediatric Res. (2004)

Kyphoscoliosis is a prominent feature of DMD and *mdx* pathologies.



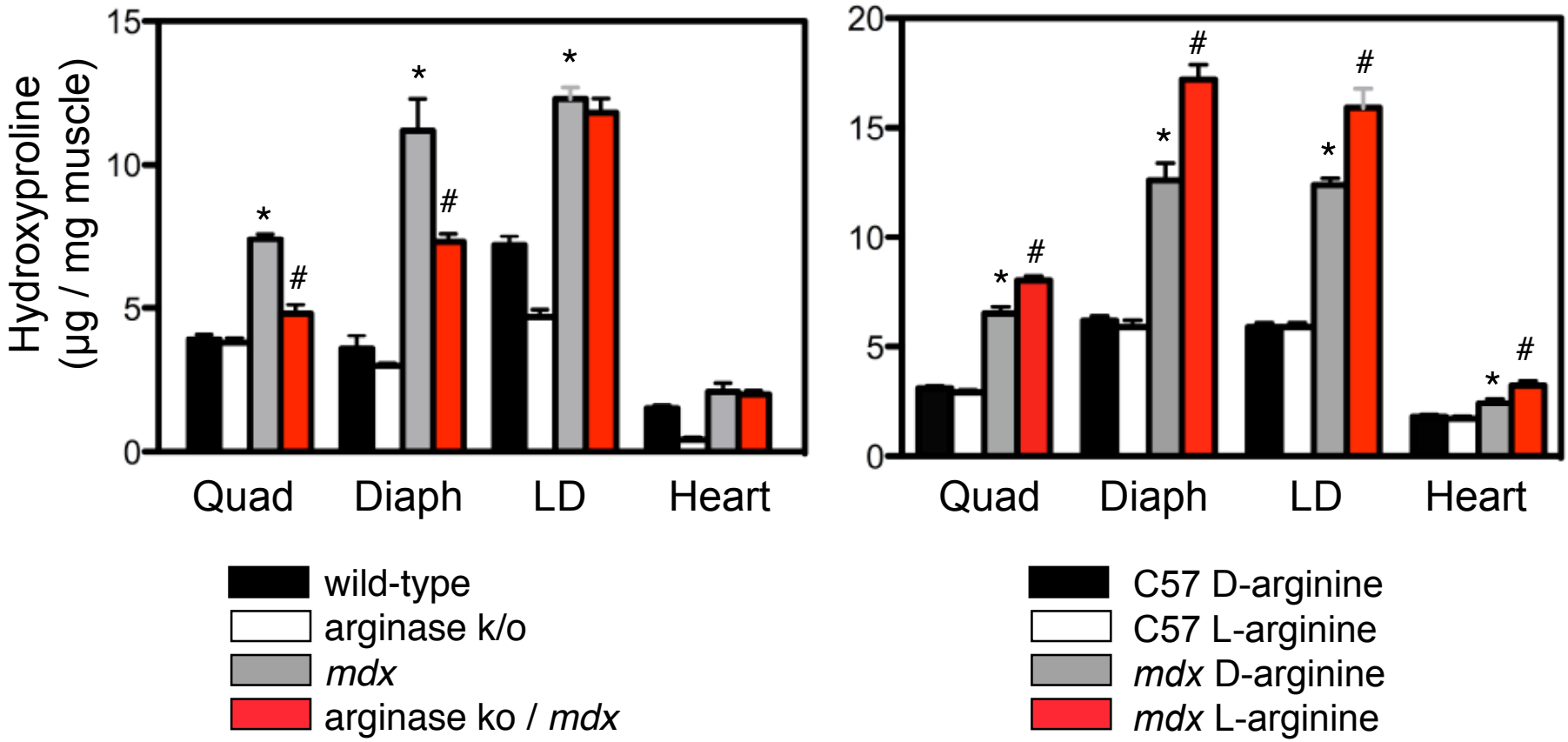
# Dystrophin-deficient hearts display fibrotic lesions.




Wild-type Dystrophin-deficient

Wehling-Henricks et al. Human Molec. Genetics (2005)

# Arginine metabolism by M2 macrophages increases fibrosis of dystrophin-deficient muscles and hearts.




Wehling-Henricks et al *PLoS One (2010)*

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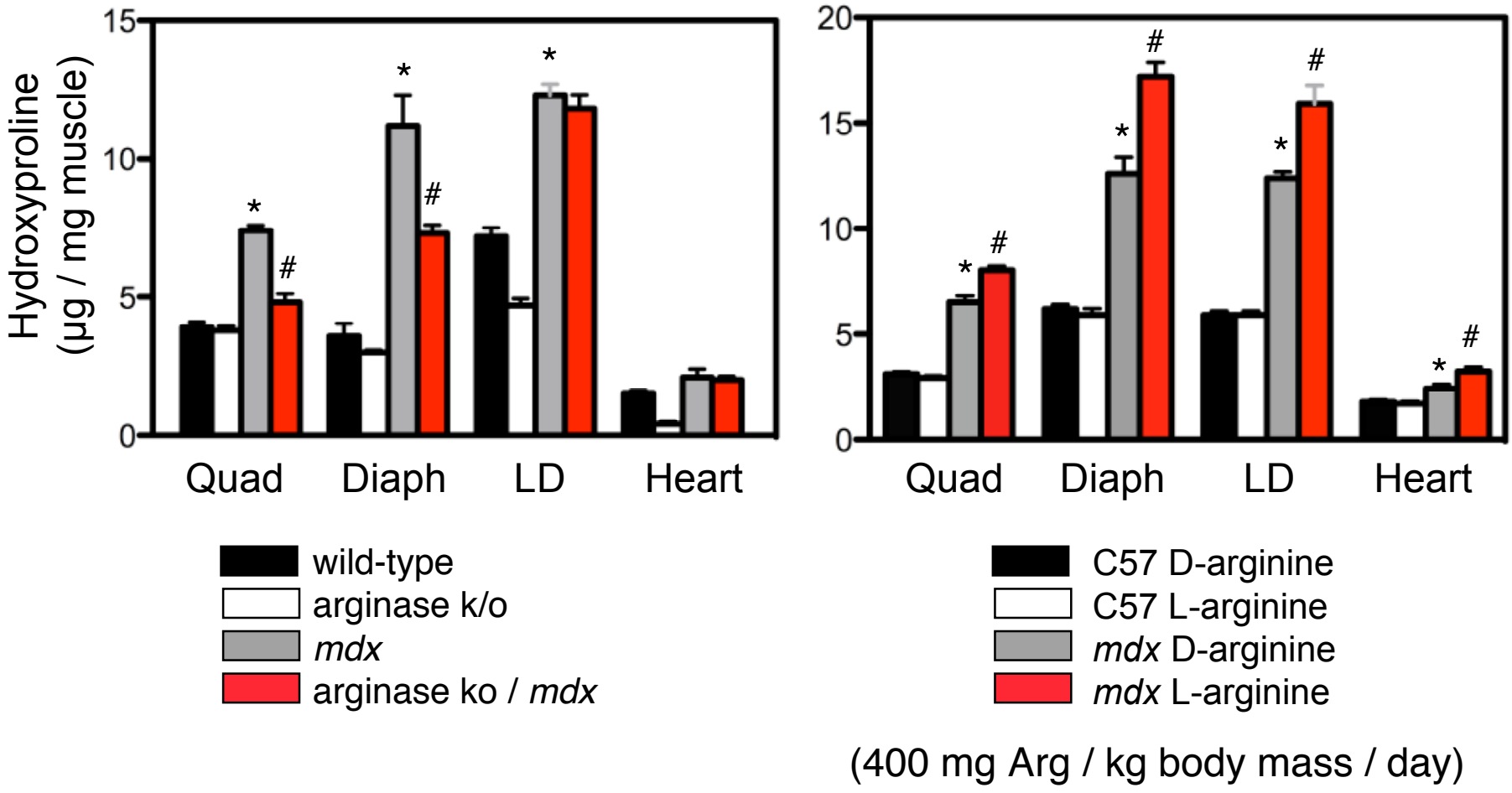


Wehling-Henricks et al *PLoS One* (2010)

# Conclusions:

| Muscle fiber injury | Successful regeneration | Failed regeneration; fibrosis |
|---|---|---|
| M1 macrophages | M2c macrophages | M2a macrophages |

- the immune system plays a significant role in regulating the pathology of muscular dystrophy;
- specific subpopulations of macrophages serve distinct and potentially competitive functions in muscular dystrophy;
- manipulation of macrophage activation, numbers or phenotype can affect the course of muscle disease;
- short-term gains achieved by therapeutic interventions into muscle disease may lead to unpredictable and detrimental long-term effects.

# Dystrophin-deficient DMD humans

Clinical onset

(3 to 4 years of age)

Progressive pathology

(continuous, following initial onset)

Muscle fiber injury — Failed regeneration; fibrosis; death by third decade

*Can manipulation of the immune response to DMD improve the regenerative capacity of muscular dystrophy patients?*

Muscle fiber injury — Successful regeneration — Failed regeneration; fibrosis; death by two years

Acute onset stage

(3 to 4 weeks of age)

Regenerative stage

(6 to 12 weeks of age)

Progressive stage

(1 year and older)

# Dystrophin-deficient *mdx* mice