

Supplemental Table 1.

Comparison of cell surface activation markers on TCR β and V β 8.1/8.2 positive cells.

Surface Marker	Specificity	Percent Positive Gating on TCRβ	Percent Positive Gating on Vβ8.1/8.2
CD49d	Integrin α 4	78%	66%
CD69 **	Very early activation marker	27%	41%
CD106 **	Vascular cell adhesion molecule (VCAM-1)	7%	17%
CD134 **	OX40, T cell activation marker	39%	64%
CD154 **	CD40 Ligand	37%	63%

Data shown in table are representative of one experiment performed on leukocytes isolated from skeletal muscle from four, 4 wk old mdx mice, stained for various T cell activation markers, co-stained with antibodies against TCR β or V β 8.1/8.2, and analyzed by flow cytometry. 10,000 events were collected per stain. **Markers shown to be upregulated in V β 8.1/8.2 compared to all TCR β cells.

Supplemental Table 2.

Characterization of DMM muscle immune cell infiltrate.

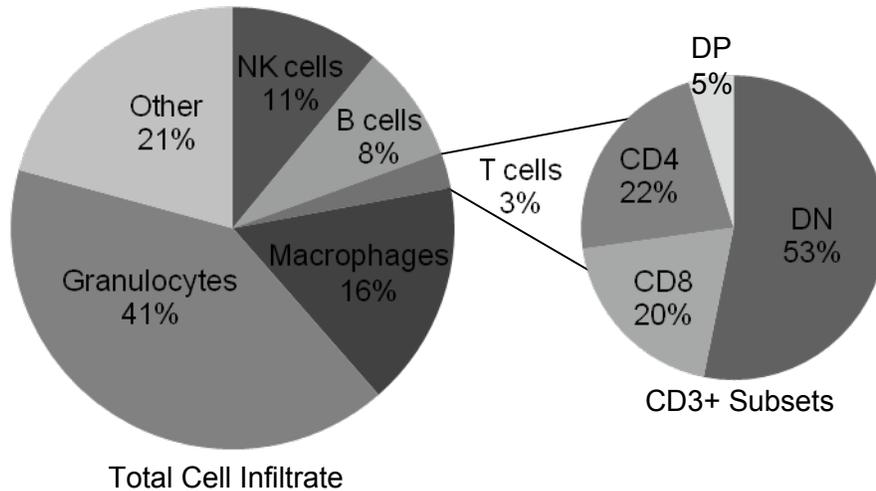
Cell Population	Cell Marker	Avg Freq		P value	Exp. (n)
		MDX	DMM		
B cells	B220	5.91	7.71	NS	3
T cells*	CD3	2.32	2.78	≤ 0.05	8
NK cells	NK1.1, DX5	7.15	6.85	NS	8
Macrophages	F4/80	15.94	16.11	NS	6
Granulocytes*	Ly6G	27.77	23.55	≤ 0.01	5
Other		40.91	57.00		

Data show cell frequencies obtained from an average of 4-6 experiments per stain. Each experiment was performed on leukocytes isolated from all of the muscles from four, 4 wk mdx or DMM mice, stained for cell surface markers and analyzed by flow cytometry. 10,000 events were collected per stain, and used to assess the % gated within the live cell population. P values were assessed by Mann-Whitney U Test.

Supplemental Table 3. Characterization of CD3+ cells in DMM muscles.

<u>Cell Marker</u>	<u>Avg. Frequency</u>		<u>P value</u>	<u>Exp. (n)</u>
	mdx	DMM		
CD3+/CD8+	17.81	22.34	NS	7
CD3+/CD4+*	19.93	31.80	≤ 0.05	7
CD3+/CD4+/CD8+ (DP)*	5.00	1.32	≤ 0.05	6
CD3+/CD4-/CD8- (DN)	57.96	44.18	NS	7
CD3+/NK1.1+*	8.58	7.00	≤ 0.05	7
CD3+/DX5+*	8.67	2.68	≤ 0.01	8
CD3+/Vβ8.1/8.2+*	10.08	6.53	≤ 0.05	8

Data in table show the CD3+ populations identified in mdx and DMM muscles. Leukocytes were isolated from four, 4 wk mdx mice, stained for cell surface markers, and analyzed by flow cytometry. Leukocytes were isolated from all body muscles. 10,000 events were collected per stain and used to assess the % gated within the live population. P values were assessed by Mann-Whitney U Test.



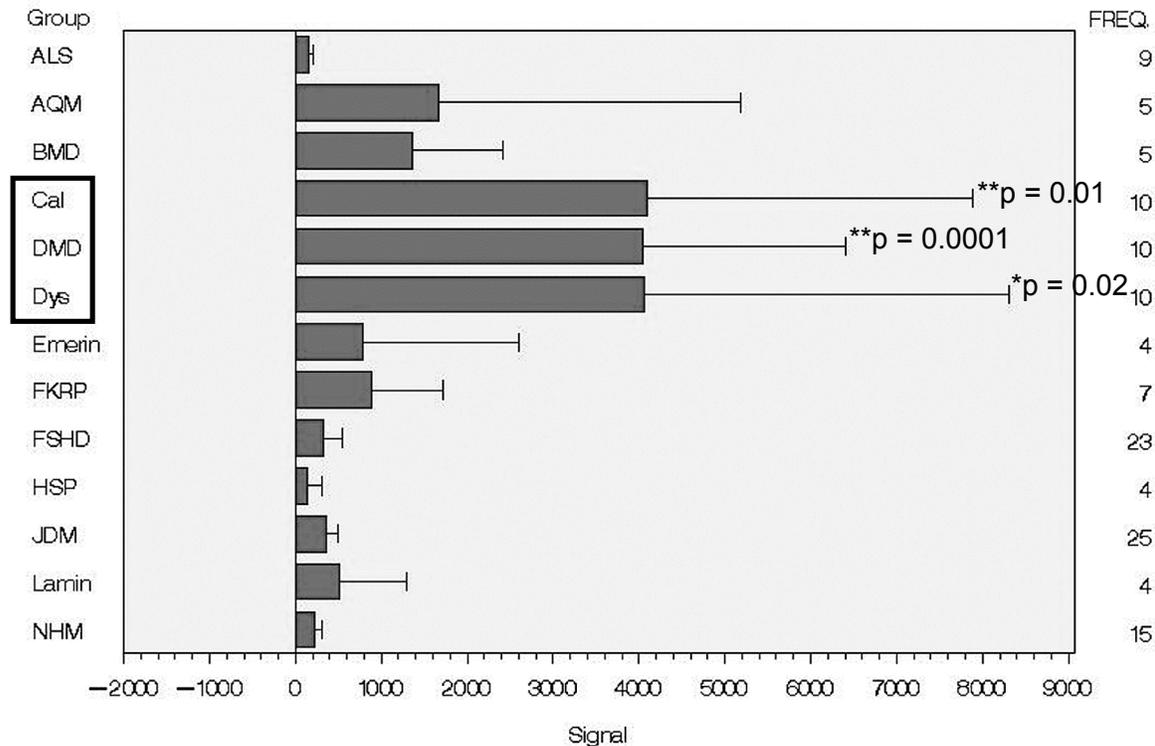
Characterization of the CD3 positive cell subsets infiltrating dystrophic skeletal muscle.

Cell Marker	% Average
CD8	19.93±4.1
CD4	22.82±9.2
DP	4.85±2.9
DN	54.02±12.3
NK1.1	5.99±3.0
DX5	8.53±5.5

Supplemental Fig. 1. Characterization of mdx muscle immune infiltrate.

Leukocytes were extracted, and purified from four, 4 wk old mdx skeletal muscle, stained for various immune cell markers and analyzed by flow cytometry. 10,000 events were collected per stain. Pie charts express the average percent of cell populations from 3 individual experiments but it should be noted that high variability between experiments was observed.

C57 mice were also used in some experiments but very few cells were isolated. Table shows the frequency of the markers present on the CD3+ gated cells. Many different types of T cells and NKT cells infiltrate dystrophic muscle.

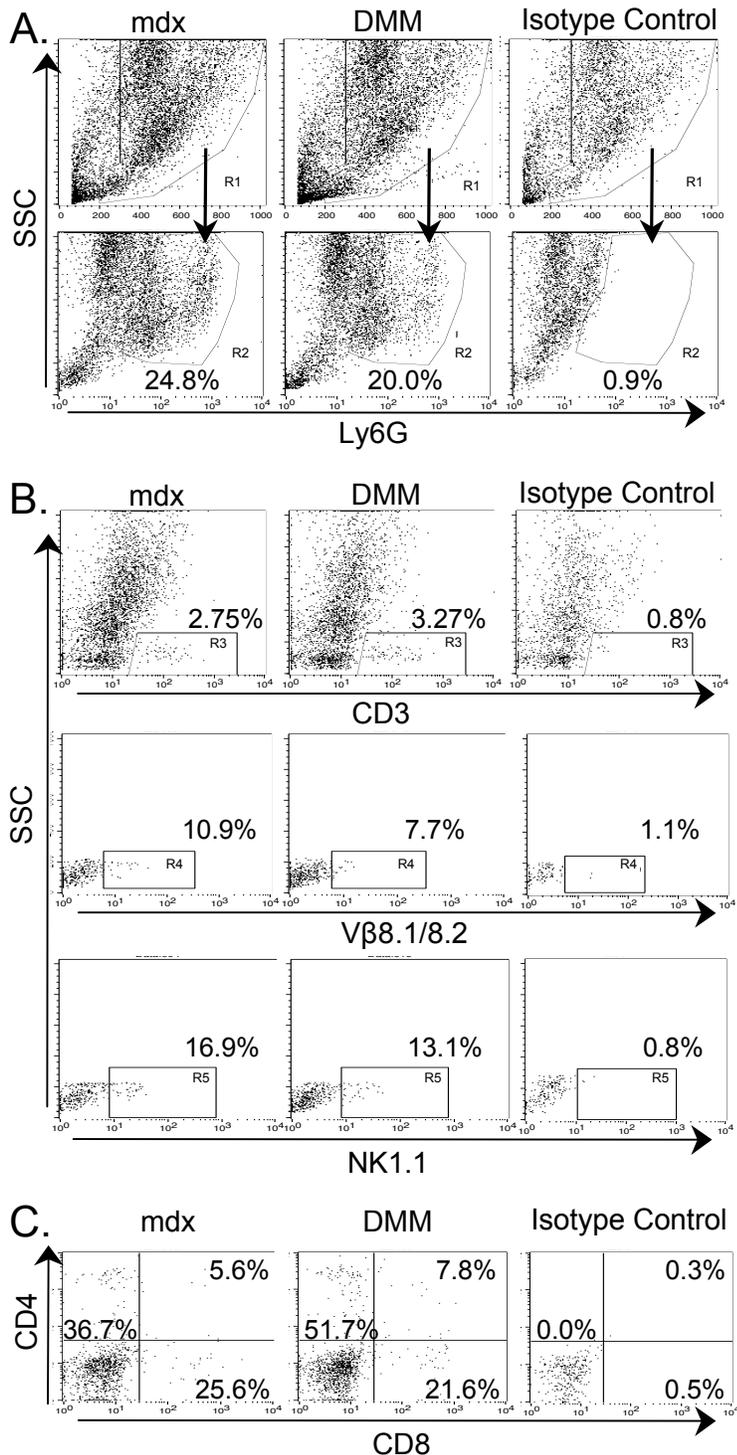


Supplemental Figure 2. OPN mRNA is highly expressed in DMD biopsies as assessed by microarrays.

Profiling of various human biopsies for OPN mRNA expression. This data set of various muscle disorders was queried with a publicly accessible SAS server with box plot visualizations (<http://sas.cnmcresearch.org>).

These data include 13 diagnostic groups, with a total of 125 muscle biopsies tested on both Affymetrix U133A and U133B microarrays (250 microarrays total). Shown are microarray results of OPN expression from

Affymetrix gene chips. Biopsies shown are the following : ALS: amyotrophic lateral sclerosis, AQM: acute quadriplegic myopathy, BMD: Becker Muscular Dystrophy, Cal: dystrophy due to Calpain-3 mutations, DMD: Duchenne Muscular Dystrophy, Dys: dysferlin, Emerin: Emery-Dreifuss muscular dystrophy, FKRP: dystrophy due to fukutin related protein mutations, FSHD: fascial-scapular-humeral dystrophy, HSP: human spastic paraplegia, JDM: juvenile dermatomyositis, LaminA/C: Another type of Emery-Dreifuss muscular dystrophy, NHM: Normal Healthy Muscle.



Supplemental Figure 3 FACS analysis of leukocytes infiltrating mdx and DMM muscles.

Muscle infiltrating leukocytes were isolated from 4 wk old mdx and DMM mice, stained for cell surface markers, and analyzed by flow cytometry. Each experiment contained cells pooled from 4 mice per group. 10,000 events were collected per stain. Data in charts are representative of 1 experiment but a total of 8 experiments were performed. (A) Charts of the forward and side scatter used to gate on the live cell population (gate R1), this population was used to assess the Ly6G (neutrophil) positive population (gate R2). (B) The CD3 positive population (gate R3) assessed from the live gate, was used to assess the co-expression of V β 8.1/8.2 (gate R4) and NK1.1 (gate R5) subsets within the CD3 positive population. (C) The CD3 positive population was assessed for the co-expression of T cell markers, CD4 and CD8.