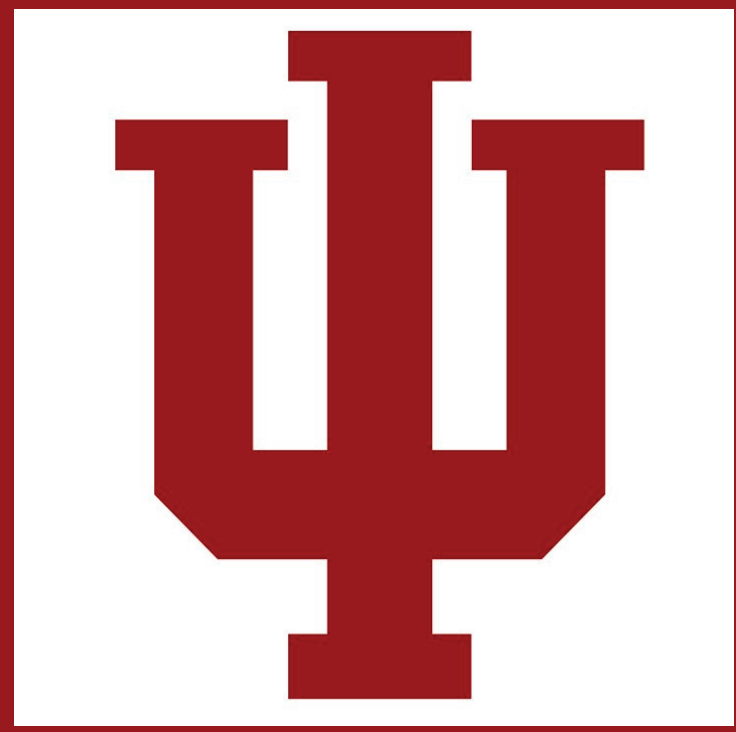


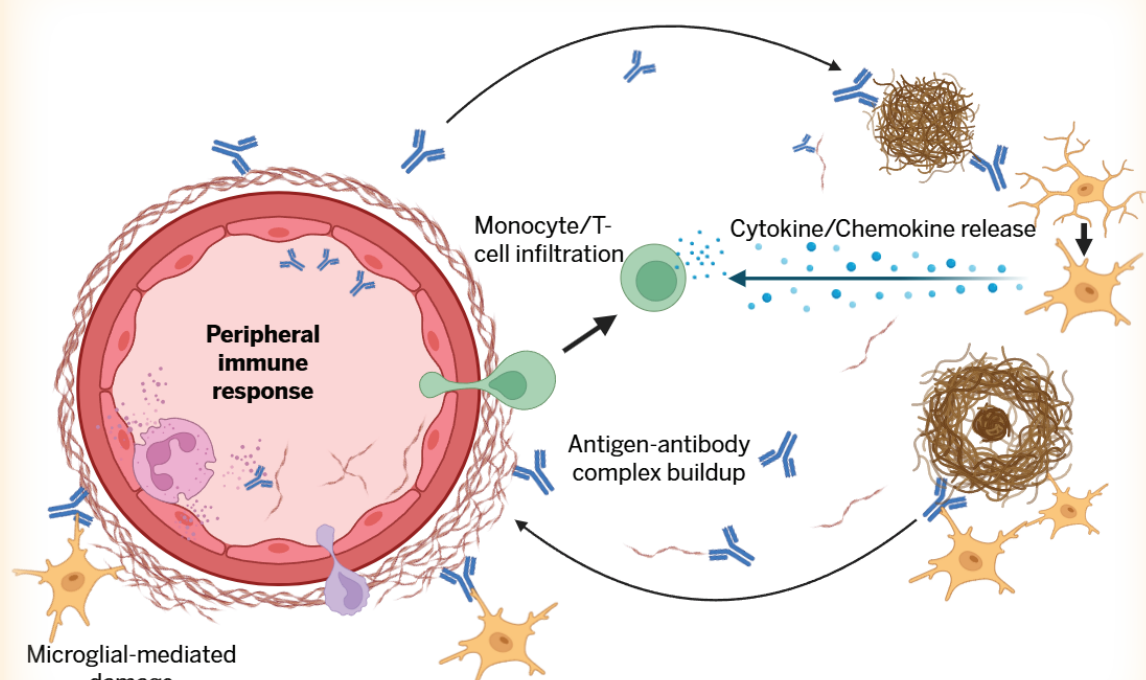


The impact of Neutrophil Inhibition on ARIA Incidence Following 3D6 Administration in Mice

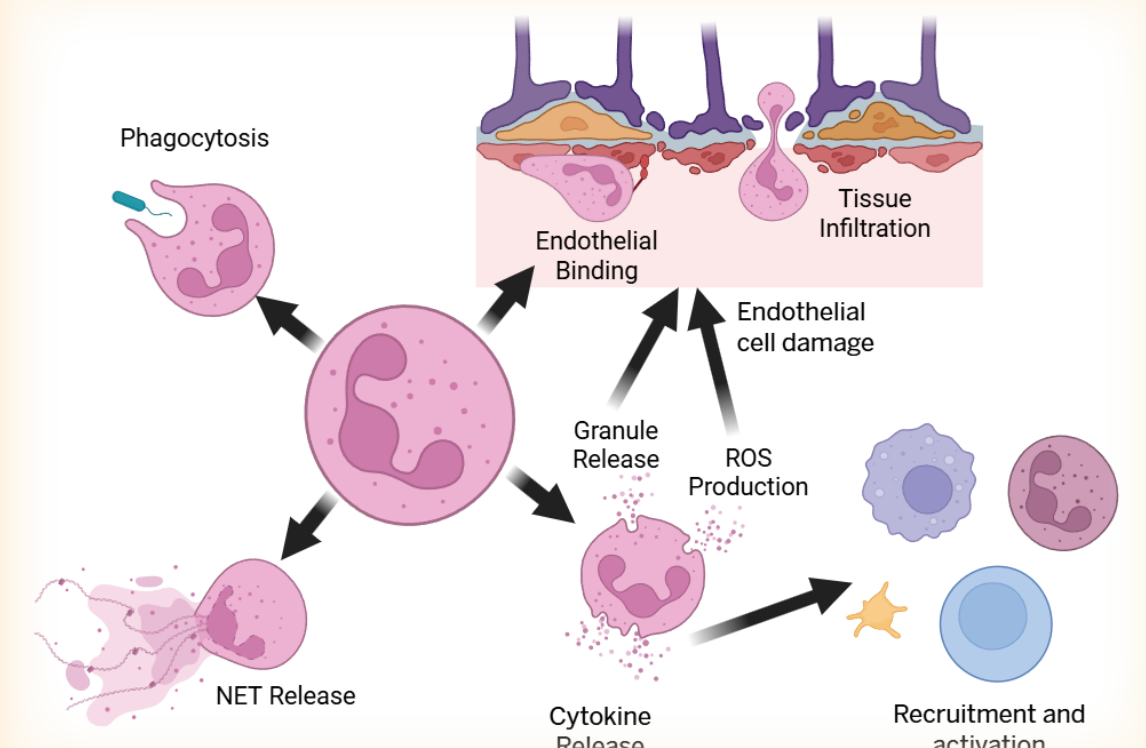
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Background: Administration of anti-amyloid antibodies to treat Alzheimer's disease has been shown to produce microhemorrhages in the brains of humans and amyloid mouse models, known as ARIA-H, and while we are becoming more aware of risk factors, an exact mechanism is not known. Some proposed mechanisms include increased amyloid load at the increasingly fragile arteriole interface, microglial recruitment of monocytes and T-cells, or direct microglial attack of the vasculature by interaction with the Fc regions of antibody bound to vascular amyloid.

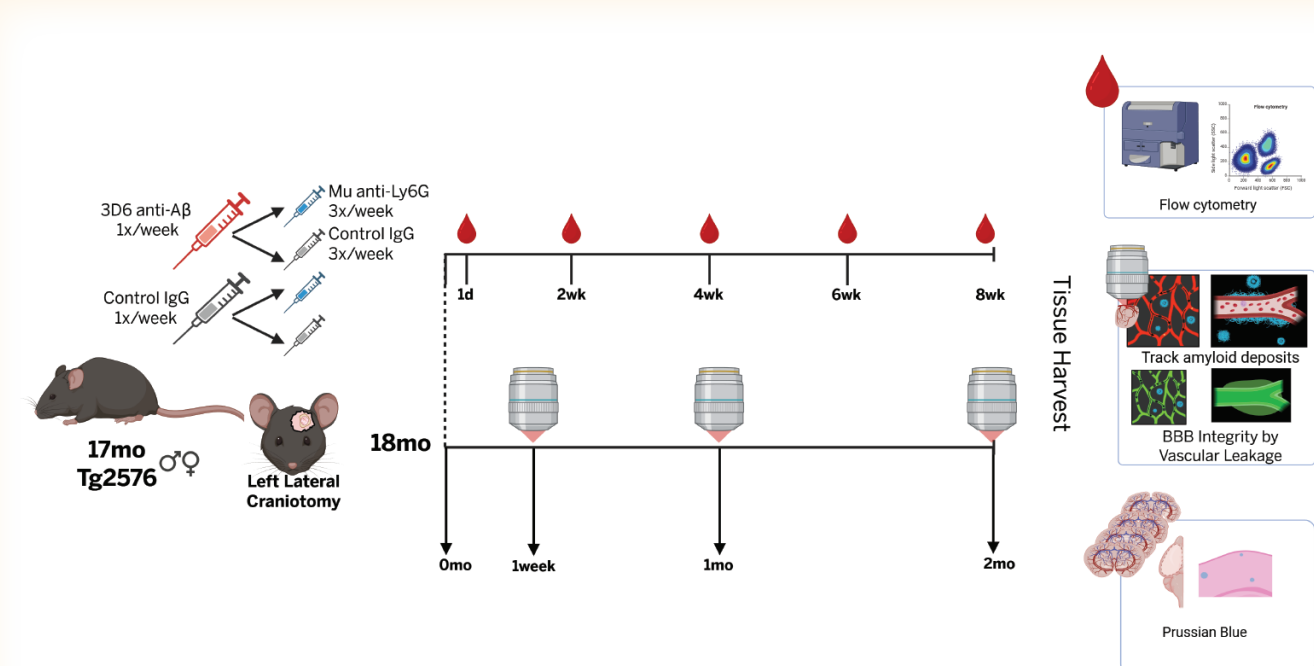


Neutrophils, the most abundant peripheral immune cell, have previously been shown to contribute to Alzheimer's disease progression. These cells also respond to the presence of Fc regions on antibodies while being equipped with tools that can cause severe endothelial damage.



Methods: 18-month old Tg2576 mice were administered weekly 3D6 injections with thrice weekly murinized anti-Ly6G antibody to chronically deplete neutrophils over 8 weeks.

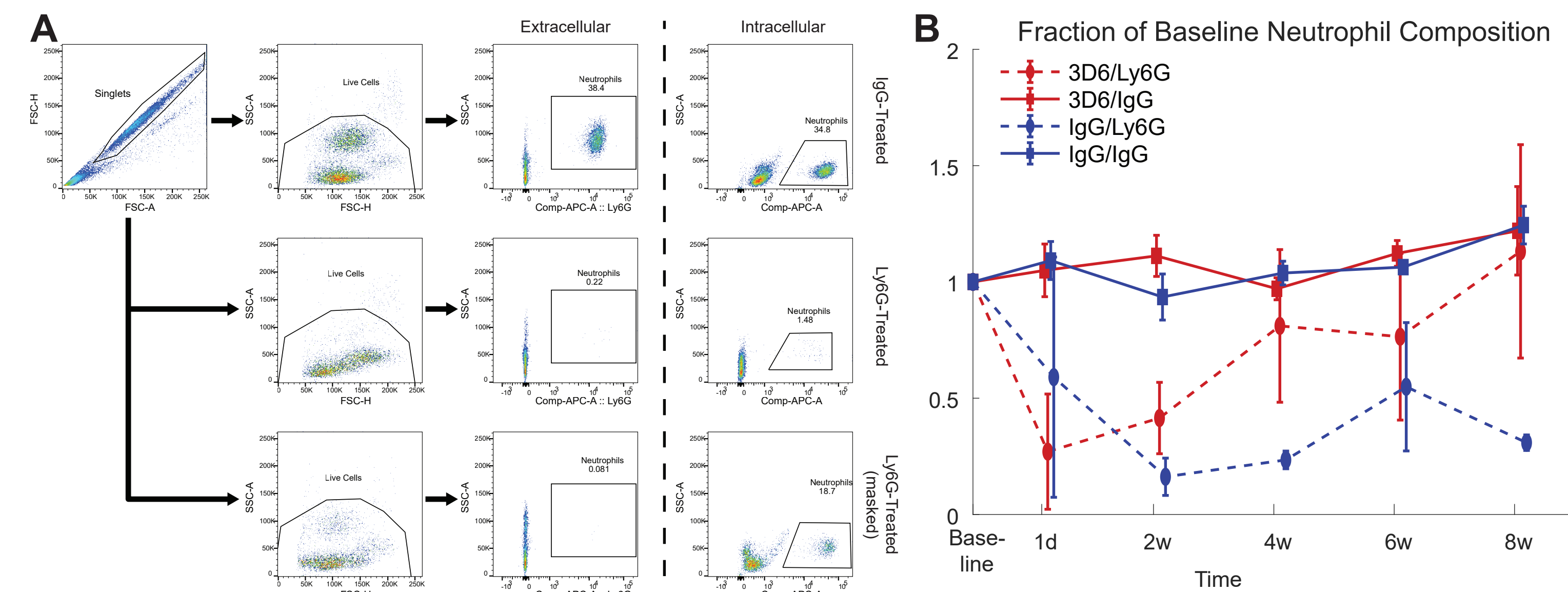
Depletion efficacy was tracked using Flow Cytometry, *in vivo* tracking of amyloid deposition and vascular leakage was performed monthly using multiphoton microscopy, and microhemorrhages were assessed after 8 weeks of treatment using Prussian Blue staining.



Results: Chronic depletion of neutrophils did not impact the rate of amyloid deposition or removal, however, vascular leakage was attenuated in mice treated with 3D6 and the rate of microhemorrhages was greatly reduced to near-IgG treated controls.

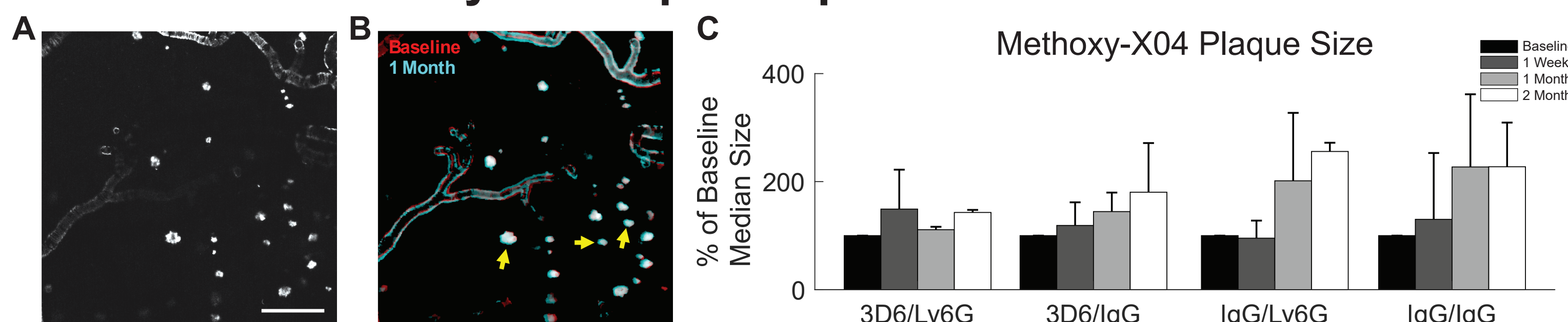
Conclusions: While still preliminary, these results support the hypothesis that neutrophils are involved in vascular damage from anti-amyloid immunotherapies.

Murinized anti-Ly6G antibody reduced peripheral neutrophils with decreased efficacy by 2 months



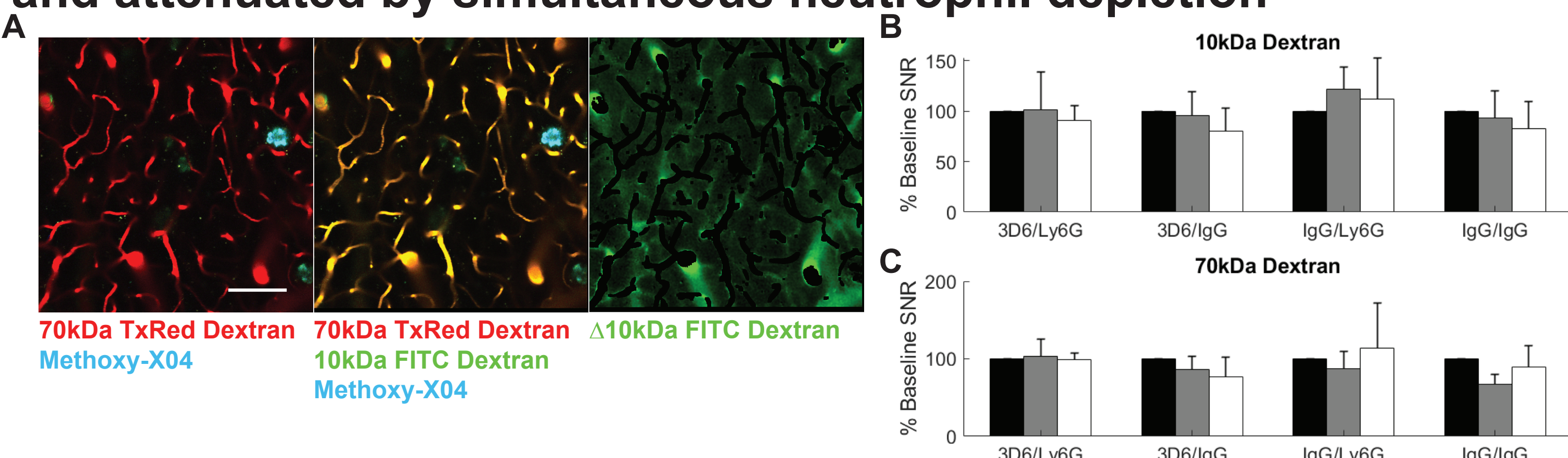
Assessment of chronic neutrophil depletion efficacy using flow cytometry and α -Ly6G antibody. A) Flow cytometry gating scheme. Singlets are selected using FSC-A and FSC-H. Dying cells are excluded by excess scattering and size to remove high autofluorescent signals. Due to antigen masking (bottom row) by using the same antibody target for neutrophil detection, intracellular labeling using 0.1% polysorbate-20 allowed for reliable neutrophil quantification (rightmost column). B) Fraction of the baseline neutrophil composition over the time course of the study. Error bars represent the mean $\pm$ SEM.

Amyloid plaque dense core growth is slowed by 3D6 antibody, but is not altered by neutrophil depletion



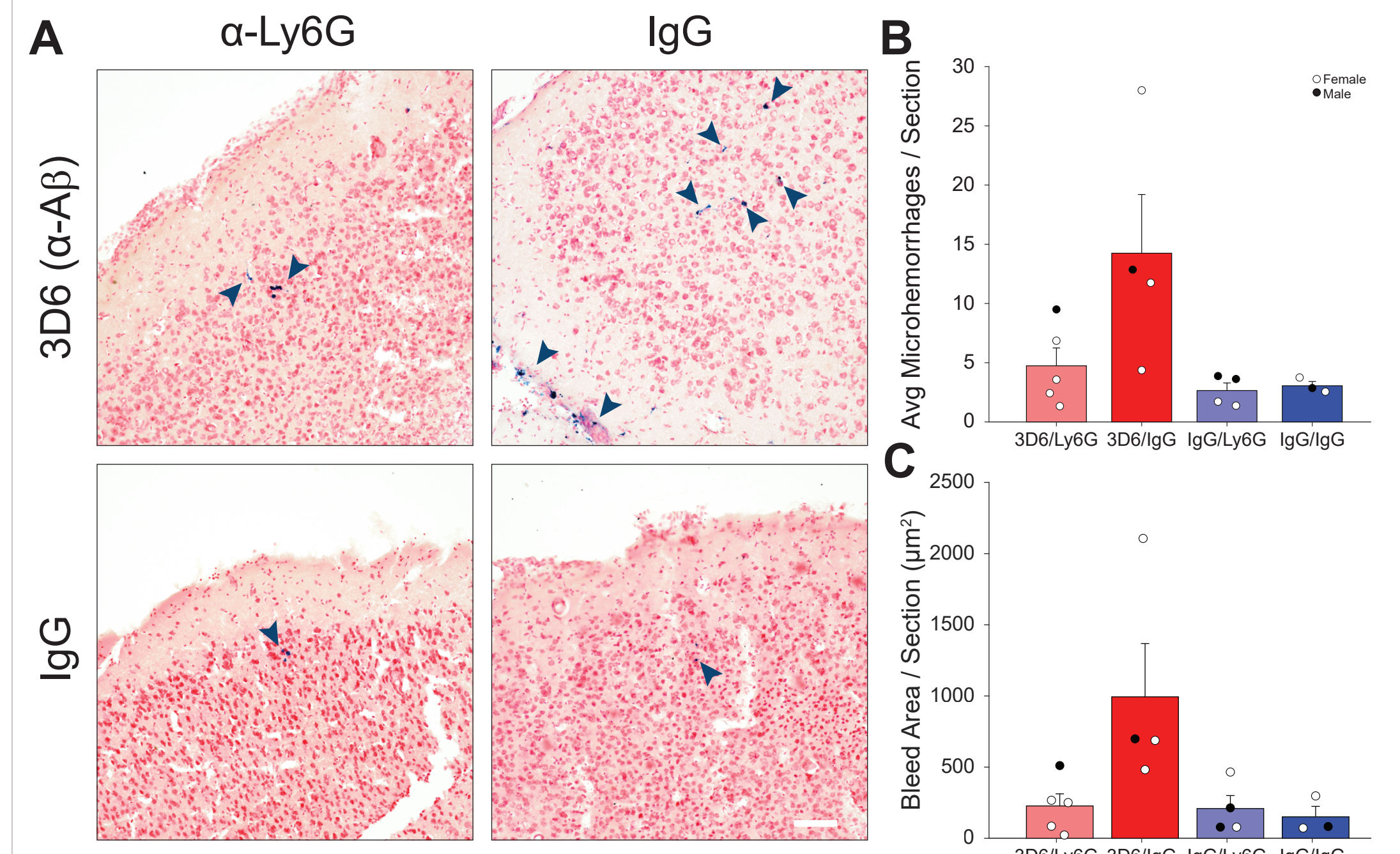
Tracking of dense amyloid plaque growth *in vivo* using multiphoton microscopy. A) Multiphoton image of amyloid dense core plaques labeled using methoxy-X04. Scale bar is 100 μ m. B) Subsequent image stack taken one month from baseline and registered to compare plaque growth. Yellow arrows indicate growing regions with a light blue outline. C) Change in median dense core plaque size within the same acquired volume. Error bars represent the mean $\pm$ SD.

Vascular leakage, by 10kDa FITC dextran, is increased with 3D6 and attenuated by simultaneous neutrophil depletion



Vessel integrity was assessed *in vivo* using a leakage assay with multiphoton microscopy. A) Vascular image taken with Texas Red dextran (left) as ground truth, Texas Red dextran and FITC dextran to assess leakage (middle), and map of the intensity differences with vessels masked out (right). B-C) Signal to noise of the 70kDa Texas Red dextran (B) and 10kDa FITC dextran (C) normalized to baseline imaging. As background levels increase due to leakage, the signal-to-noise would be expected to decrease, seen with 3D6/IgG treatment and to a lesser extent in the other conditions. Error bars represent the mean $\pm$ SD.

The number of cortical microhemorrhages induced by 3D6 administration was greatly reduced by chronic α -Ly6G treatment.



Microhemorrhages were detected using Prussian Blue staining. A) Representative images of cortical microhemorrhages for each group. Arrows indicate presence of microhemorrhages. Scale bar is 100 μ m. B) The average number of cortical microhemorrhages per section per mouse. C) The average total bleed area per section per mouse. Error bars represent the mean $\pm$ SEM.

Conclusions

- Murinized antibody against Ly6G was able to suppress the neutrophil fraction of leukocytes out to 8 weeks with IgG-treated mice but less effectively with 3D6-treated mice.
- The administration of 3D6 slowed the growth of dense core plaques while α -Ly6G had little additional effect.
- Vascular SNR of fluorescent dextrans was decreased with 3D6 administration, indicating impaired vessel integrity, but the impact was reduced by α -Ly6G antibody.
- 3D6-treated mice exhibited greater microhemorrhage number and total bleed area than IgG-treated mice, but the impact was greatly reduced when mice were treated with α -Ly6G.

Future Directions

- Complete data analysis on preliminary results shown
- Examine total A β , dense core plaques, and vascular amyloid by α -A β IHC and Congo Red
- Evaluate glial response by GFAP (astrocytes) and Iba-1 (microglia)
- Measure neutrophil blood plasma protein analytes
- Confirm presence or absence of neutrophils within larger microhemorrhages

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