Monitoring microglial phenotype in diabetic retinopathy models.

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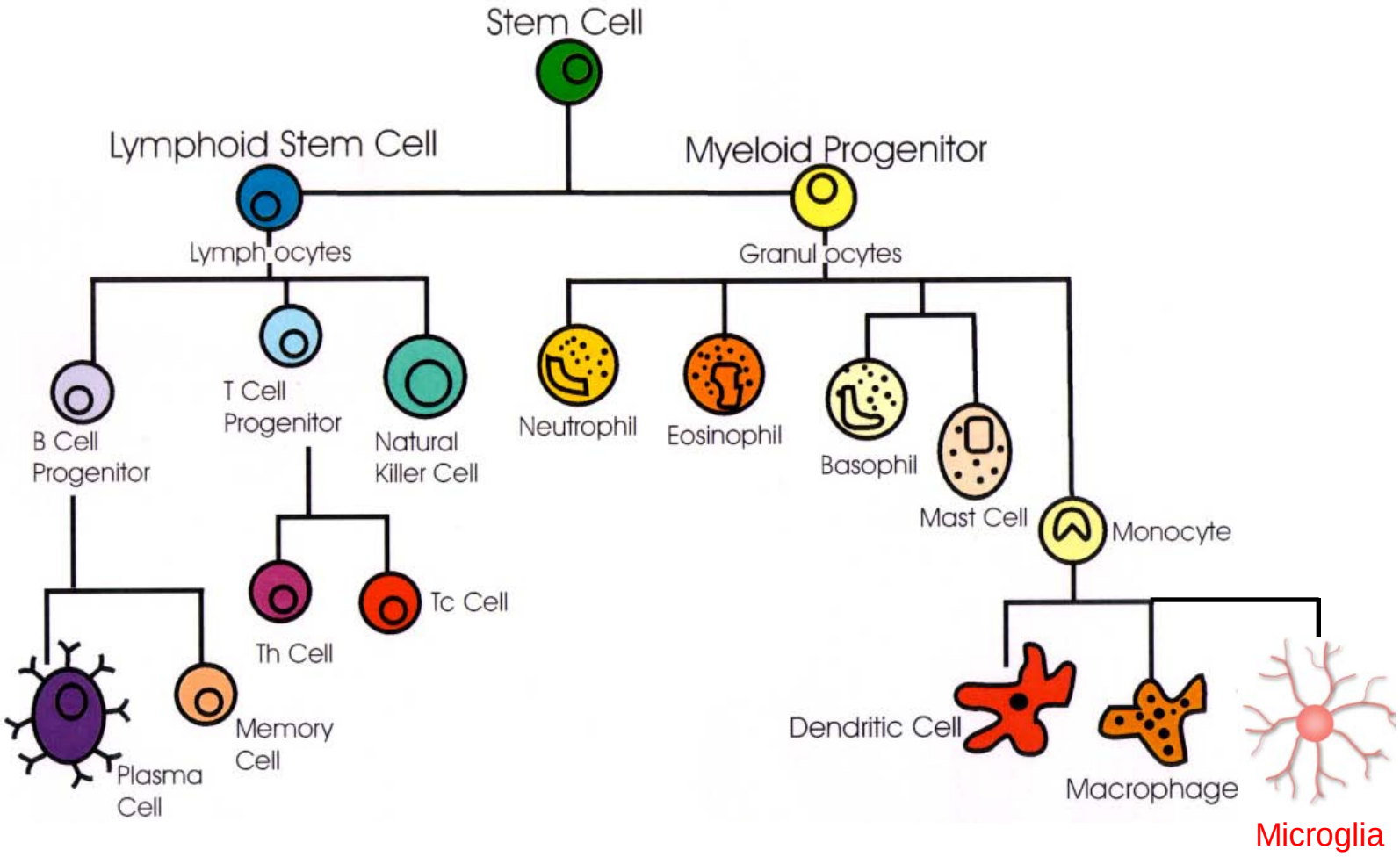
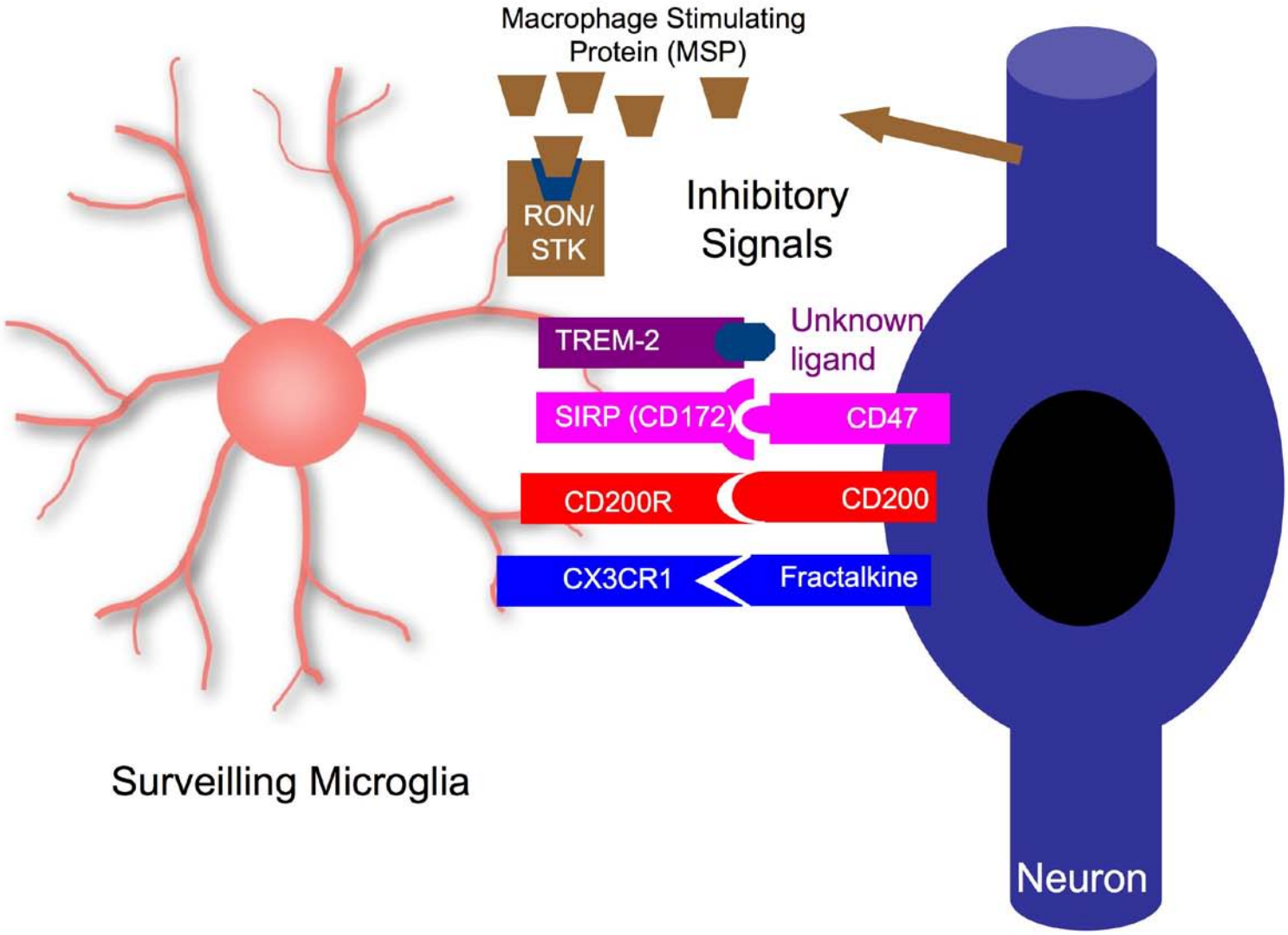
Microglia are monocytic cells that develop from bone marrow stem cells and reside in nervous tissue



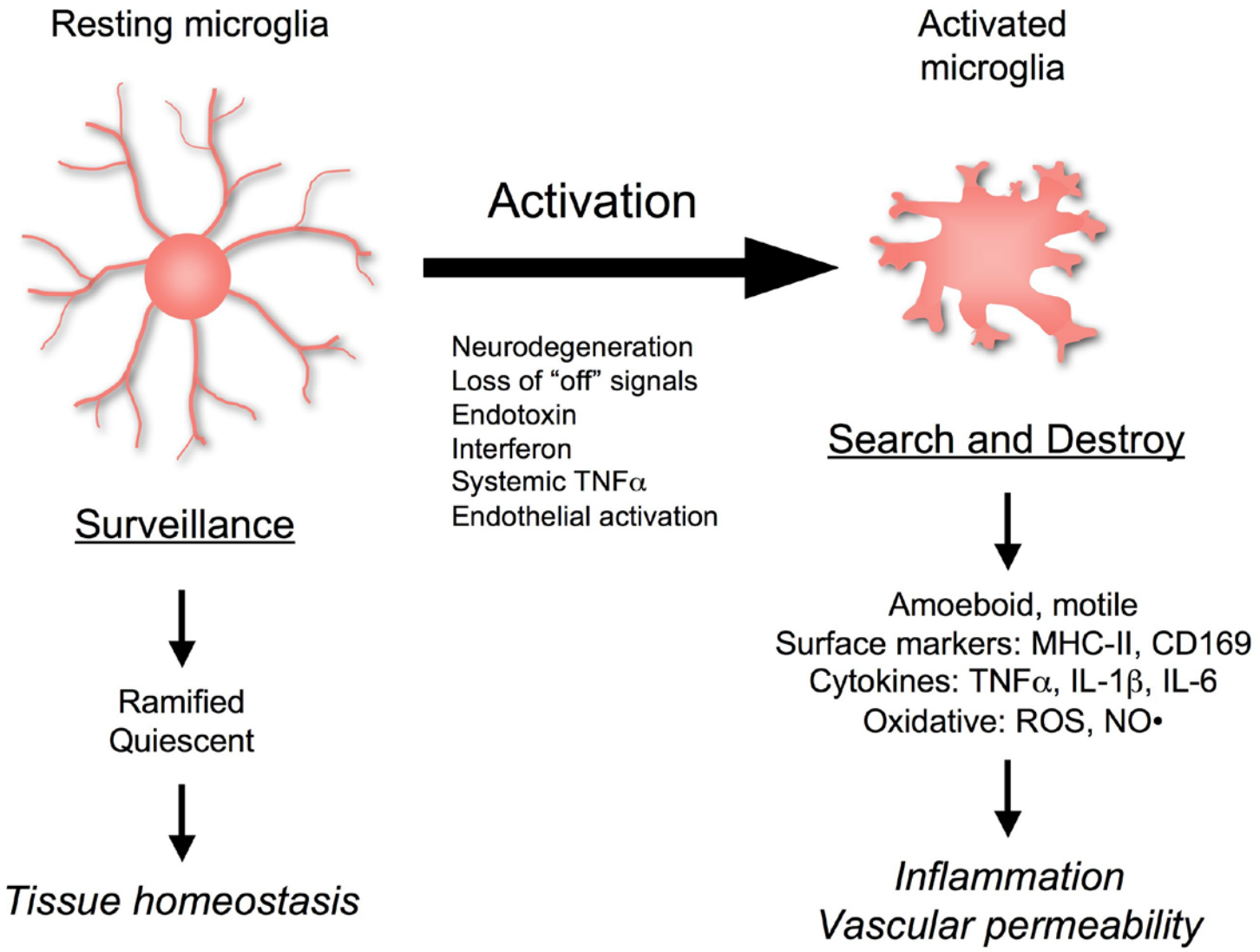
Microglia represent the innate immune system in the retina.

- As they develop, microglia move from bone marrow to nervous tissue, including the retina.
- Microglia populate nervous tissue early in life.
- Microglia are the major component of the immune system that resides in the retina.
- Microglia are long-lived and turn over very slowly - e.g. brain microglia take 5-8 months to be replaced.
- Microglia can be identified by marker antigens e.g. Iba-1, CD45, CD68, CD11b.

Healthy neurons may send inhibitory signals to microglia.



Simple View: Microglia become “activated” in response to tissue damage or infection.

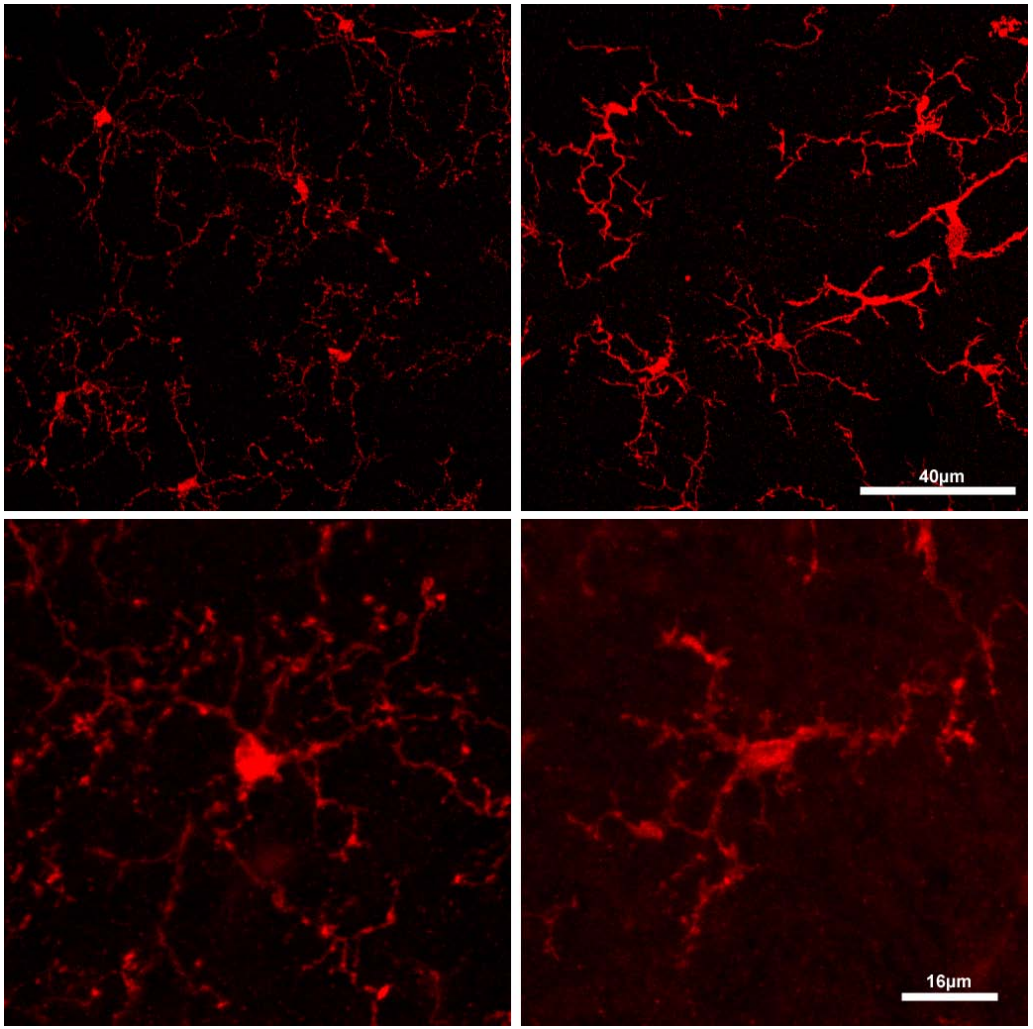


Activated microglia are present in regions of retinas from Ins2^{Akita} diabetic mice.

Iba1 staining at 8 weeks of diabetes

Control Mouse

Ins2^{Akita} Diabetic

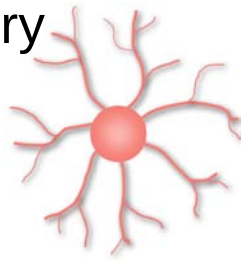


Swollen and retracted processes in microglia of diabetic

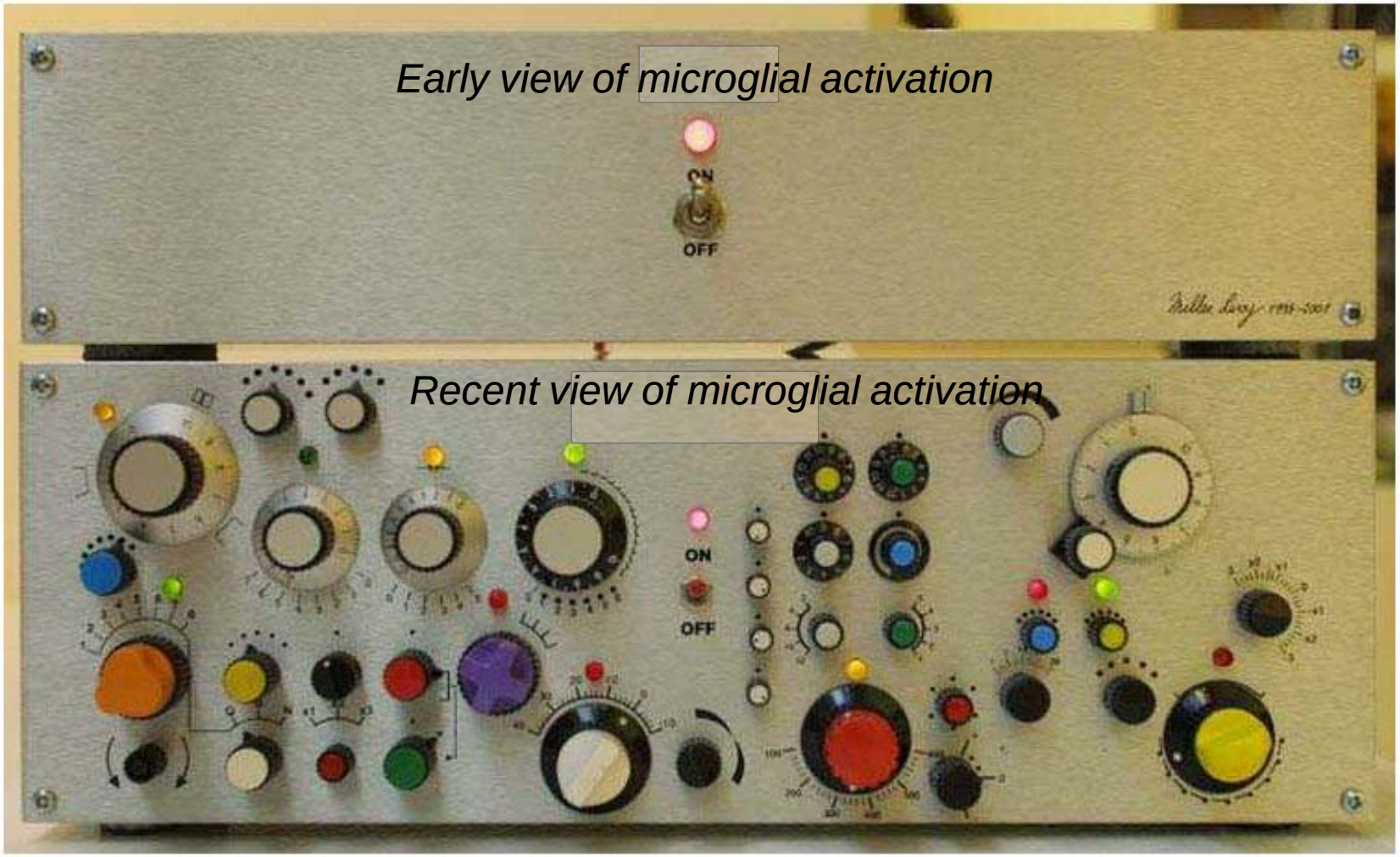
A. J Barber et al., Investigative Ophthalmology and Visual Science. 2005;46:2210-2218

Debate over whether microglia are beneficial or detrimental in neurodegenerative diseases

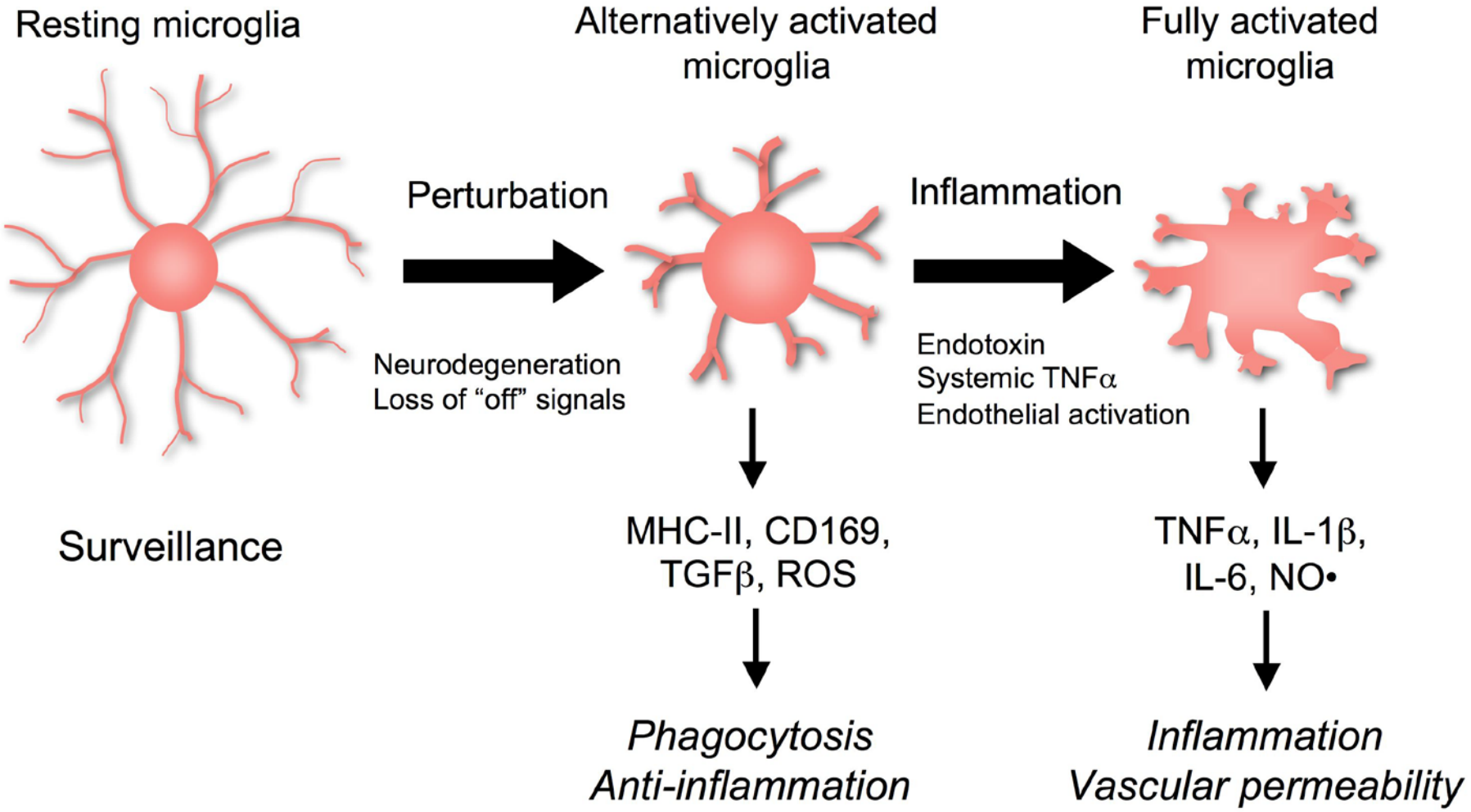
- Detrimental:
  - Inhibition of microglial activation has shown promise in experimental models of several diseases
  - Inhibition of microglial activation reduces collateral damage during acute insults
- Beneficial:
  - Inhibition of microglial activation may delay final resolution of pathology
  - Phagocytic function of microglia may prevent sustained inflammatory stimulus
  - Alternatively activated microglia may be anti-inflammatory



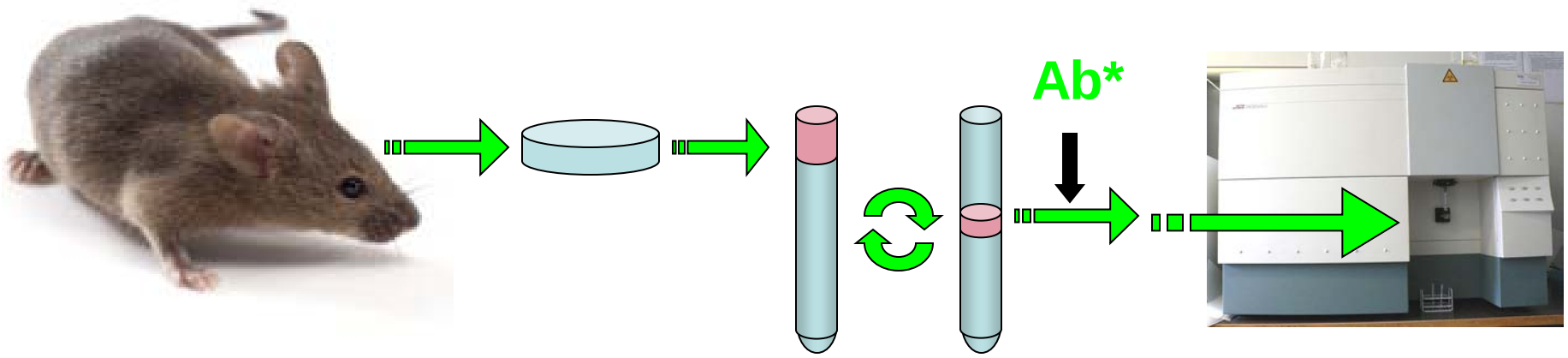
Microglia activation may not be so simple…

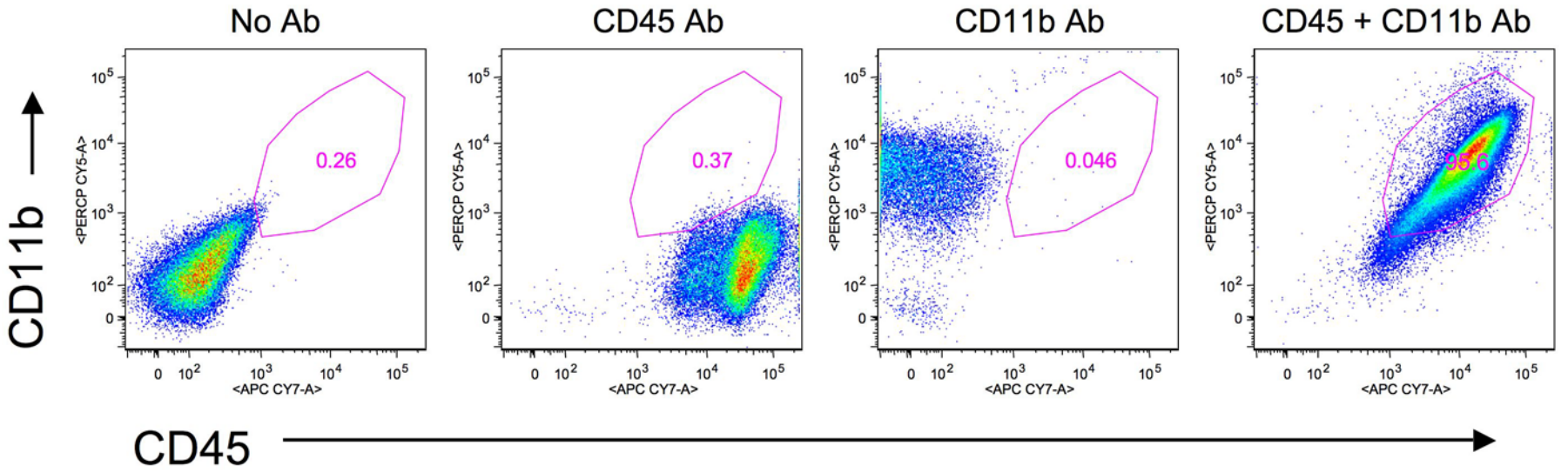


Microglia can assume various states of activation.

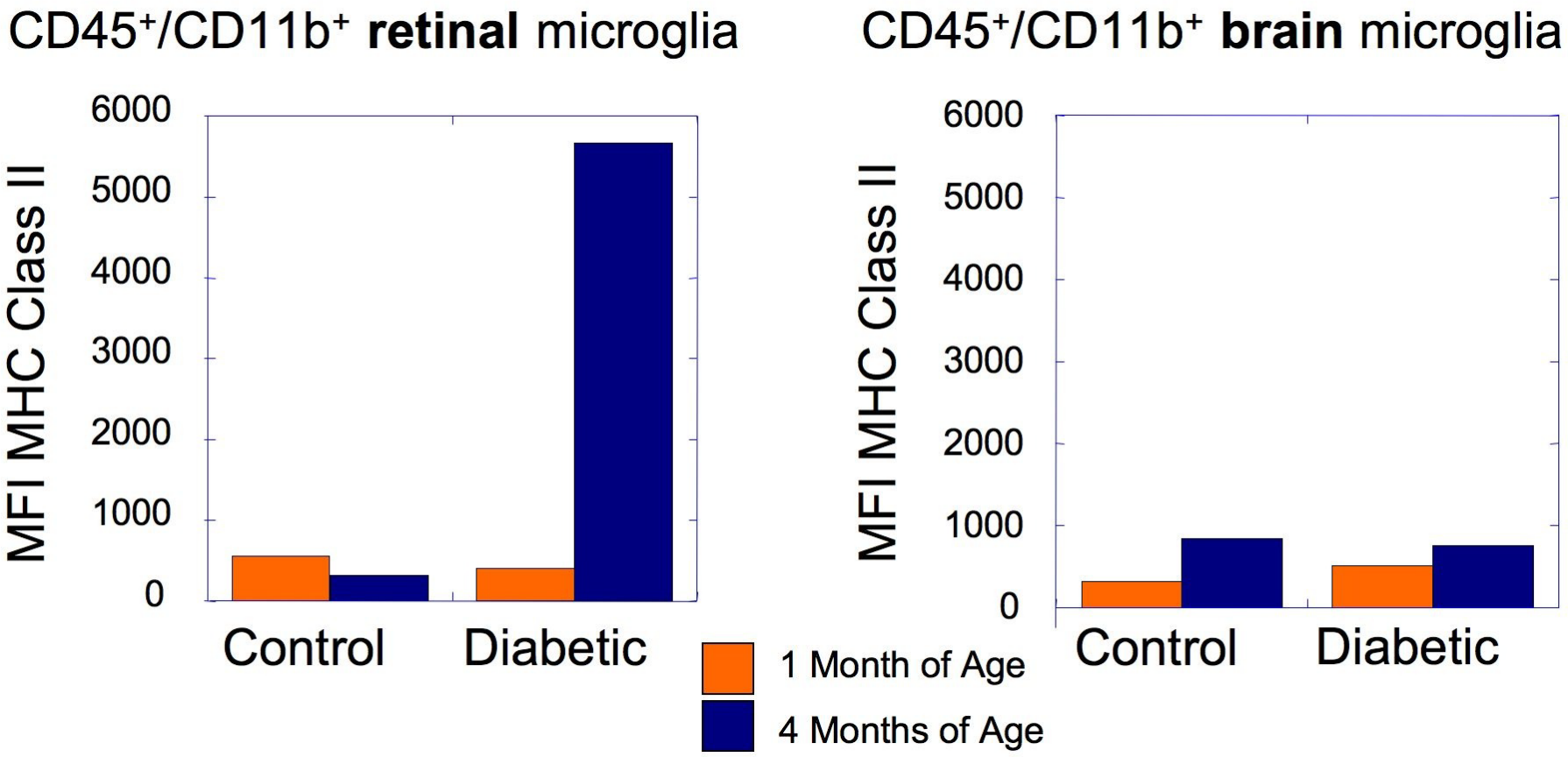


Ex vivo microglial analysis by flow cytometry

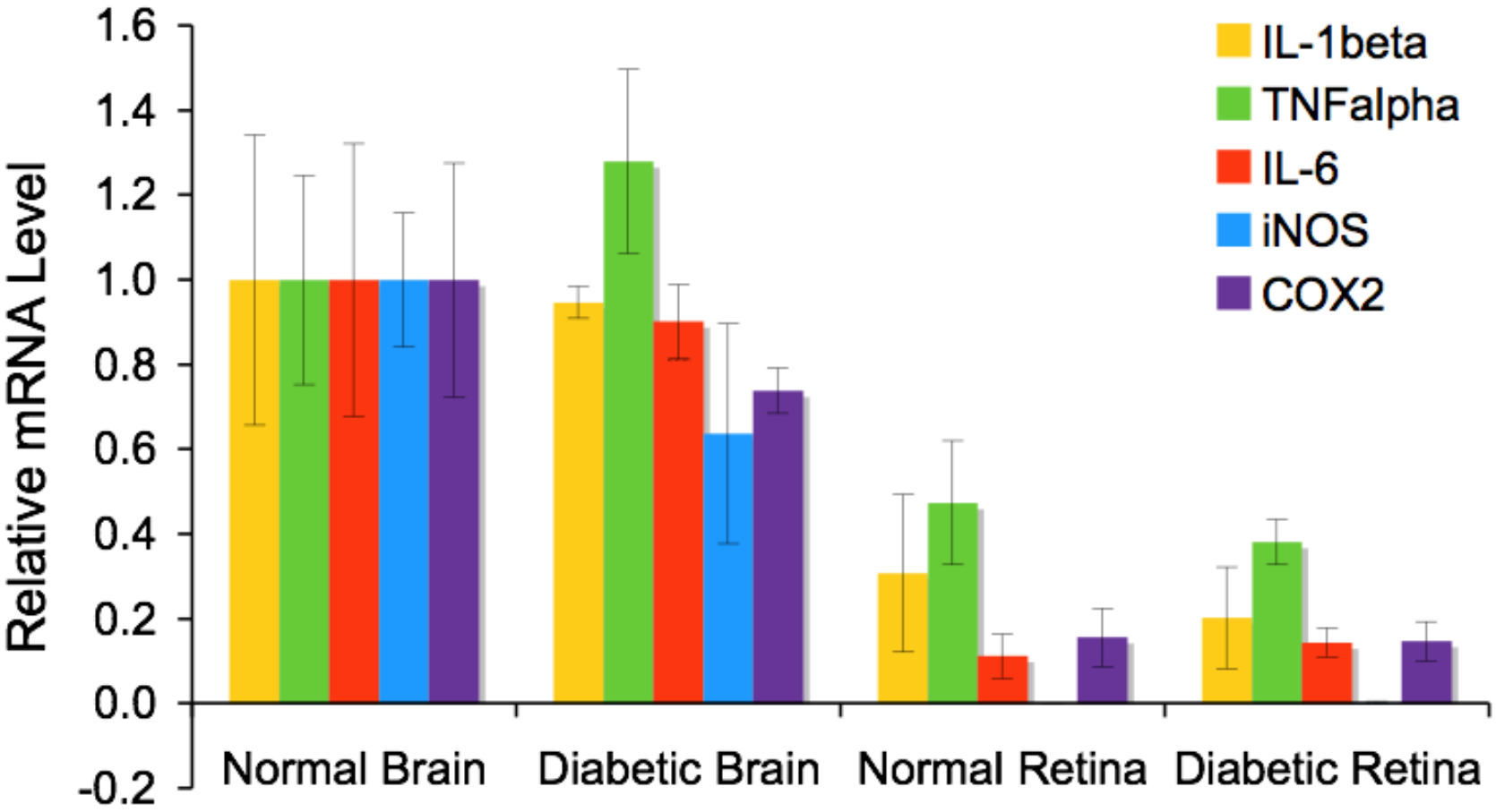




$CD45^+/CD11b^+$ subset of retinal microglia show antigenic signs of activation in $Ins2^{Akita}$ diabetic mice



No indication of increased inflammatory gene expression in retinal microglia isolated from Ins2^{Akita} diabetic retinas



The Yin and Yang of Microglial Activation

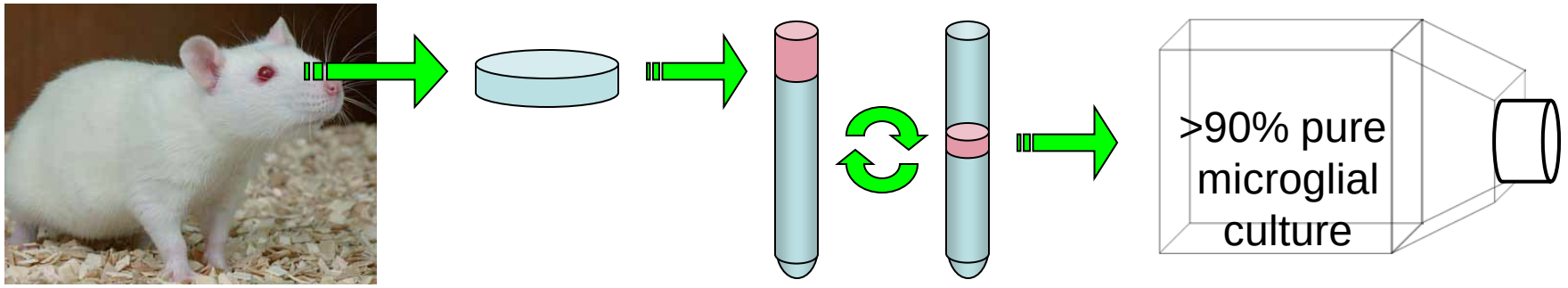
M1 Classical Inflammatory Phenotype

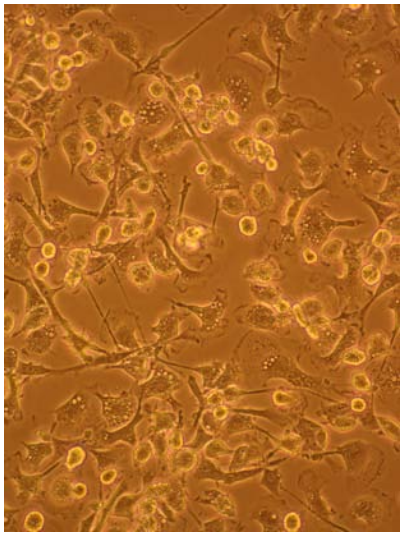
iNOS
TNF-α
IL-1β
IL-6
IL-12
CD169
MHC-II?

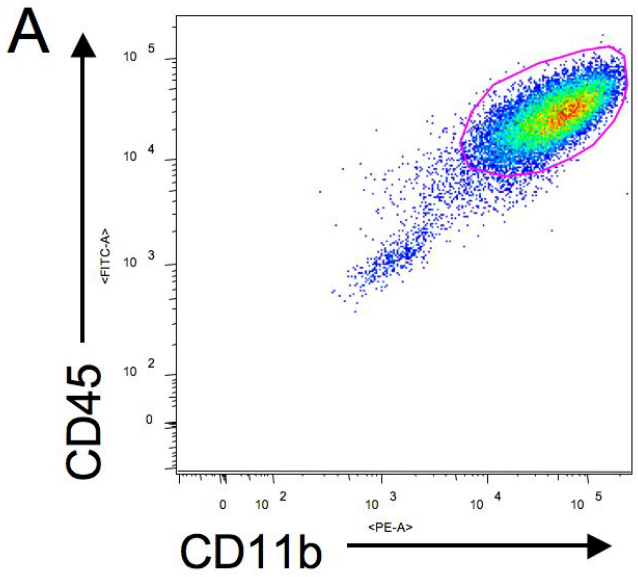
M2 Alternative Anti-Inflammatory Phenotype

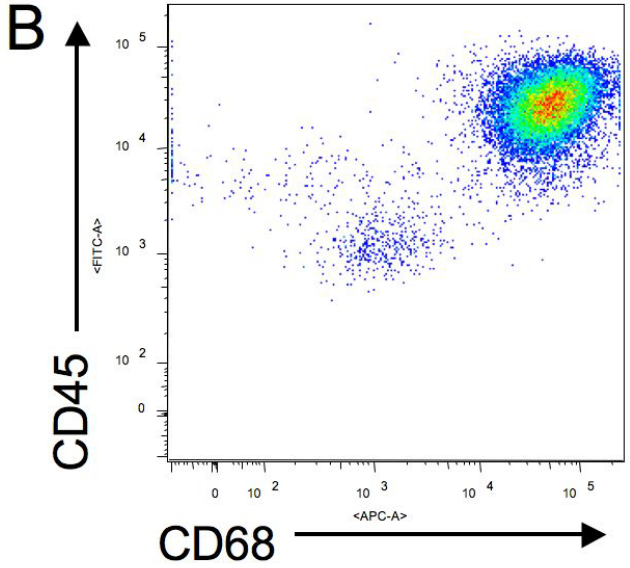
ARG1
TGF-β
IL-1Ra
IL-10
IL-27
CD163
MHC-II?

Flow cytometric analysis of cultured adult rat retinal microglia demonstrates the >95% of cells express microglial markers



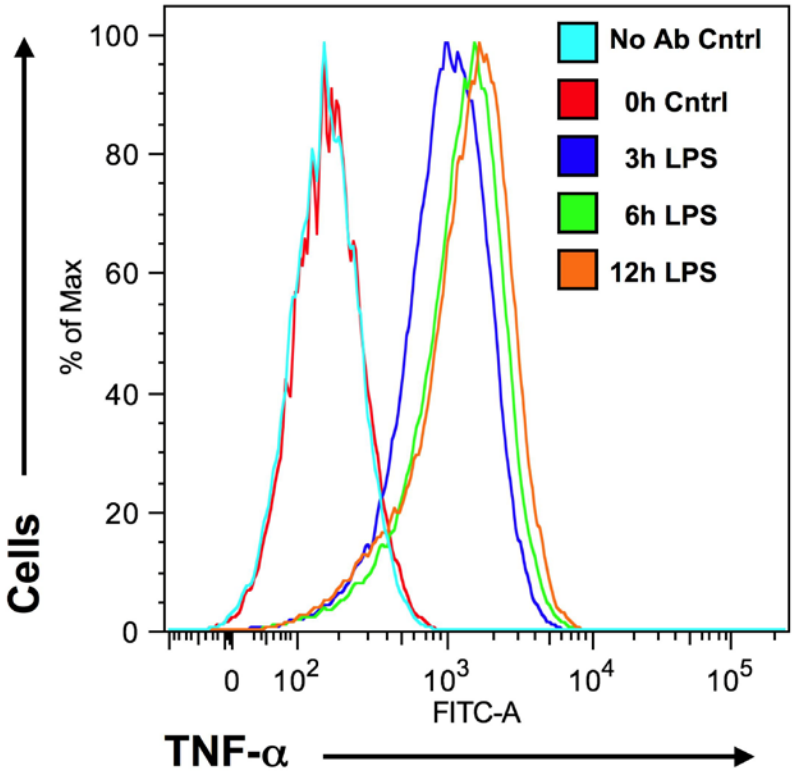


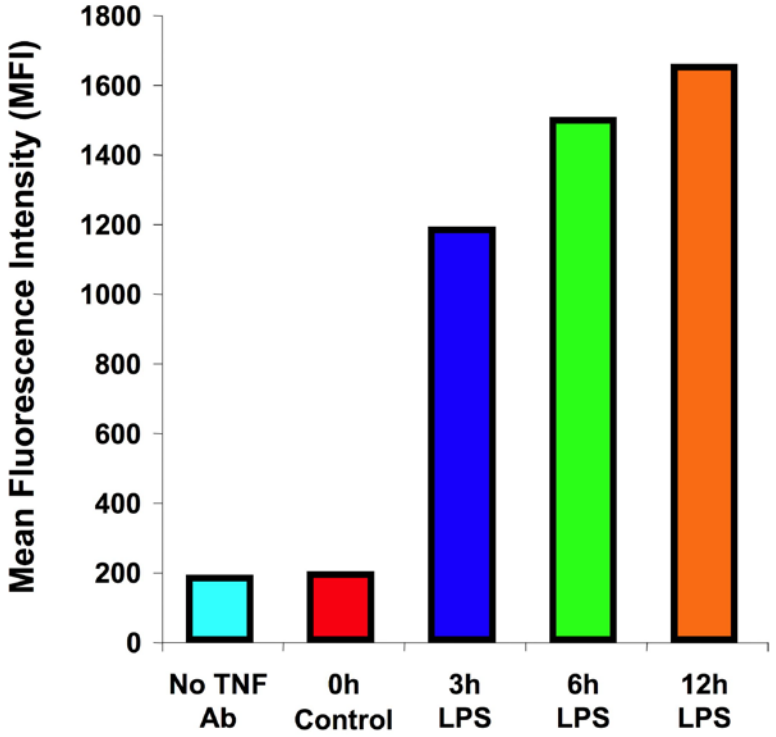




Flow cytometry can be used to quantitate protein levels in single cells and thus in isolated and specific cell population

Intracellular cytokine staining of TNF-α protein levels in CD68^{+} primary microglia following LPS activation





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