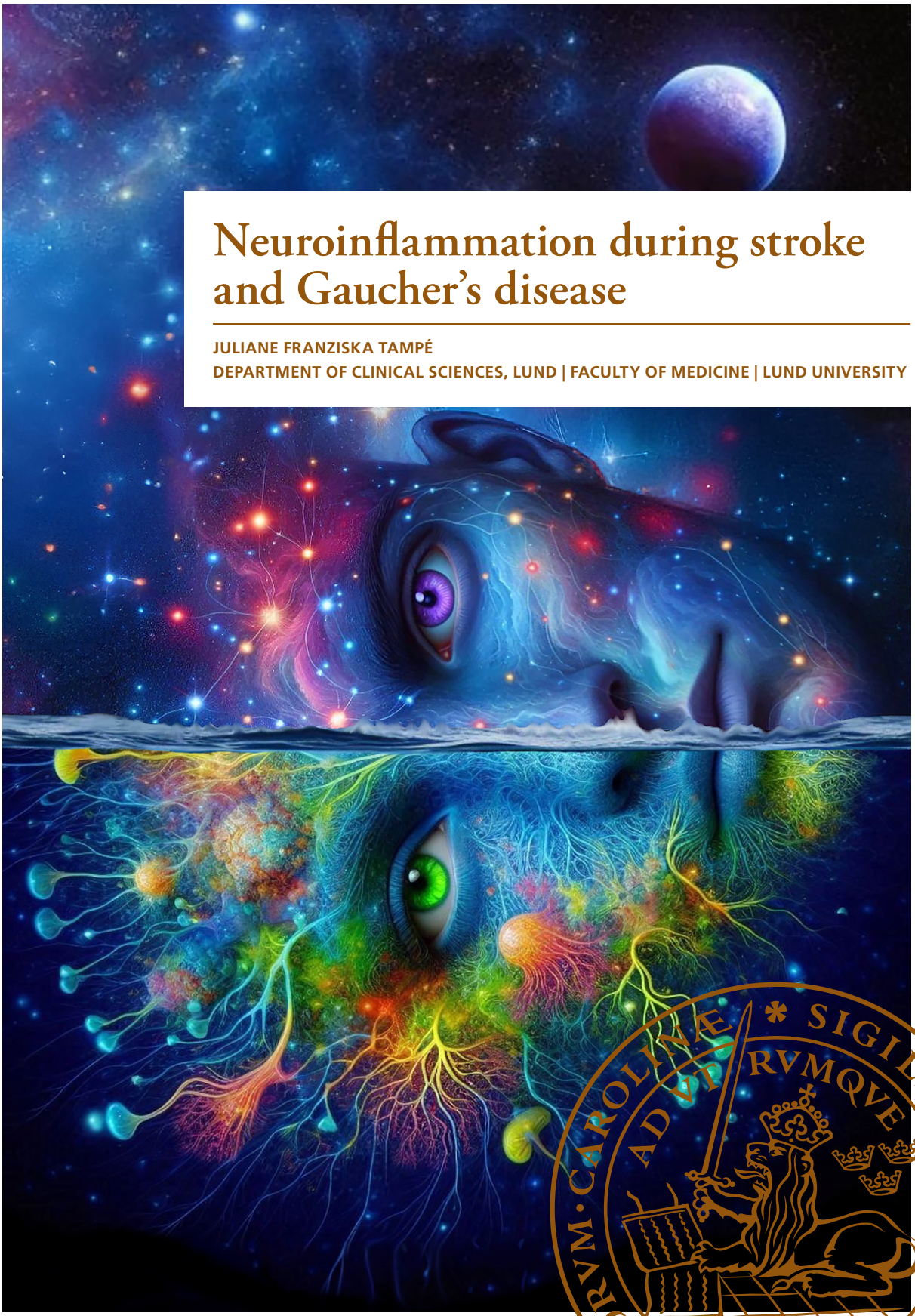
Neuroinflammation during stroke and Gaucher's disease

How monocyte-derived macrophages and microglia shape the inflammatory environment of the brain

Tampe, Juliane

2025

Document Version:
Publisher's PDF, also known as Version of record



Neuroinflammation during stroke and Gaucher's disease

JULIANE FRANZISKA TAMPÉ
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

Neuroinflammation during stroke and Gaucher's disease

Neuroinflammation during stroke and Gaucher's disease

How monocyte-derived macrophages and microglia shape the inflammatory environment of the brain

Juliane Franziska Tampé

LUND
UNIVERSITY

DOCTORAL DISSERTATION
Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 18th of September at 09.00 in Segerfalksalen, Sölvegatan 17, Lund, Sweden

Faculty opponent
Prof. Kate Lykke Lambertsen, University of Southern Denmark

Organization: LUND UNIVERSITY
Document name: DOCTORAL DISSERTATION **Date of issue:** 18.9.2025
Author(s): Juliane Franziska Tampé
Title and subtitle: Neuroinflammation during stroke and Gaucher's disease
- How monocyte-derived macrophages and microglia shape the inflammatory environment of the brain

Abstract:
The human brain is a complex and finely tuned organ, constantly monitored and maintained by its resident immune cells. Among these, microglia are the primary regulators of the brain immune environment under physiological conditions, and monocyte-derived macrophages contribute mainly to pathological states such as ischemic stroke and neuronopathic Gaucher's disease (GD). These two diseases, although different in origin, one acute and vascular, the other chronic and genetic, share a common feature: a disruption in immune homeostasis marked by neuroinflammation.

Using flow cytometry, gene expression profiling, and induced pluripotent stem cell (iPSC)-derived cell modeling, this work demonstrated that monocytes and microglia are dynamically regulated by intrinsic factors and extrinsic stimuli. During human aging, circulating monocyte subsets undergo sex-dependent transcriptional remodeling, with females exhibiting stronger anti-inflammatory aging signatures. This was particularly evident in the upregulation of ANXA1 in female non-classical monocytes. Additionally, age-associated increases in the scavenger receptor CD36 were observed across all monocyte subtypes.

In ischemic stroke patients, monocyte frequency and gene expression patterns predict recovery, particularly through the expansion of intermediate monocytes and the downregulation of inflammatory mediators. A panel of recovery-associated markers: CD91, CD36, TGM2, SLC24A4, and CD38, were identified, with CD36 in intermediate monocytes emerging as a novel marker of recovery.

In neuronopathic GD, human iPSC-derived microglia (hiMG) exhibit intrinsic impairments in cytokine signaling and lysosomal function under immune stimulation, indicating chronic neuroimmune dysfunction. These changes suggest a brain-specific immune phenotype distinct from peripheral macrophages and support a role for microglia in GD-associated neurodegeneration.

In summary, this thesis explores how peripheral monocytes and brain-resident microglia, both part of the mononuclear phagocyte system, shape the neuroinflammatory processes during aging, ischemic stroke, and GD. It highlights how immune responses are influenced by age, sex, and disease state, providing mechanistic insight and models for developing future immune-targeted therapies for neurological diseases.

Key words: Monocytes, Stroke, Gene expression, Neuroinflammation, Recovery, iPSC-derived microglia, Gaucher's disease, Lysosomal dysfunction, Disease modeling

Language: English, German, Swedish **Number of pages**: 107

ISSN and key title: 1652-8220

ISBN: 978-91-8021-744-6
Recipient's notes Price Security classification

Signature  **Date** 2025-08-05

Neuroinflammation during stroke and Gaucher's disease

How monocyte-derived macrophages and microglia shape the inflammatory environment of the brain

Juliane Franziska Tampé

LUND
UNIVERSITY

Faculty of Medicine
Department of Clinical Sciences, Lund

ISBN 978-91-8021-744-6
ISSN 1652-8220
Lund University, Faculty of Medicine Doctoral Dissertation Series 2025:91

Printed in Sweden by Media-Tryck, Lund University
Lund 2025

To all who supported me on this journey
– especially my family

"I knew that the future would depend on this journey."
- Bertha Benz

Table of Contents

Papers included in the thesis

Paper I

Human monocyte subtype expression of neuroinflammation- and regeneration-related genes is linked to age and sex

Juliane F. Tampé, Emanuela Monni, Sara Palma-Tortosa, Emil Brogårdh, Charlotta Böiers, Arne G. Lindgren, and Zaal Kokaia.

PLoS One 2024 Vol. 19 Issue 10 Pages e0300946

Paper II

Temporal dynamics of monocyte subtypes and gene expression reveal predictive immune signatures of ischemic stroke recovery

Juliane F. Tampé, Emanuela Monni, Yu-Ping Shen, Emil Brogårdh, Arne G. Lindgren, and Zaal Kokaia.

Manuscript in preparation

Paper III

Human iPSC-derived microglia reveal inflammatory and lysosomal vulnerabilities in Gaucher disease

Juliane F. Tampé, James Crowe, Oskar Zetterdahl, Altea Gjurgjaj, Emanuela Monni, Zaal Kokaia, and Isaac Canals

Manuscript in preparation

Papers not included in this thesis

Human cortical neurons rapidly generated by embryonic stem cell programming integrate into stroke-injured rat cortex.

Raquel Martinez-Curiel, Mazin Hajy, Oleg Tsupykov, Linda Jansson, Natalia Avaliani, **Juliane F. Tampé**, Emanuela Monni, Galyna Skibo, Olle Lindvall, Sara Palma-Tortosa, and Zaal Kokaia.

Stem Cells, sxaf049.

Author's note

As long as I can remember, I was always curious, maybe a little too curious. There are many family lores from my early years, one of which involves myself as a baby, attempting to inspect an electrical socket far too closely. While in those early days my curiosity was mostly met with a "No!", my parents later welcomed it: Perhaps the most defining memory comes from a Halloween birthday party, when my dad brought home dry ice to fill the bathroom with swirling, mystical fog. It was a hit with everyone, but for me, it was something more – I was instantly drawn to it. The reaction, the transformation, the mystery of it all felt magical. In that moment, my fascination with science was sealed.

As I grew older, I spent countless hours outdoors, especially at the shore, completely mesmerized by tide pools that felt like hidden worlds. Indoors, I had a different kind of lab: Fischer Technik sets and long afternoons of invention with my grandfather, who always encouraged tinkering. As a teenager, I continued chasing science wherever I could find it: I spent school breaks at science camps, from chemistry-in-the-microwave experiments to DIY radios and even sleeping overnight in museums. Each holiday, camp, tiny breakthrough felt like peeling back another layer of how the world works.

Later, when it came to choosing my field of study, I landed on biochemistry. Its structure and logic felt like a safe harbor, offering a way to understand life in detail, from veterinary medicine all the way down to X-ray crystallography. However, the deeper I went, the more I found myself drawn to one question: how do simple physical switches, chemical gradients, and cellular programs scale up to something as profound as movement, emotion, memory, or even consciousness?

I dove headfirst into the unknown of neuroscience and never looked back. It's been a journey of endless wonder, where I found the freedom to explore everything from sociability assays and computational modeling to stem cells, inflammation, immunology, and hematology. It reminded me of the time I first programmed Conway's Game of Life: how simple rules could create self-organized complexity. Neuroscience felt just like that: an intricate puzzle with limitless ways to explore it.

Now, as I reach the end of my PhD journey, I look back and realize that the same mesmerizing curiosity I felt as a child is still very much alive. Time and time again, I get to lose myself in the intricate, elegant machinery of our most fascinating organ, the brain, and every time, it feels surreal, like an astronaut looking at planet Earth.

Abstract

The human brain is a complex and finely tuned organ, constantly monitored and maintained by its resident immune cells. Among these, microglia are the primary regulators of the brain immune environment under physiological conditions, and monocyte-derived macrophages contribute mainly to pathological states such as ischemic stroke and neuronopathic Gaucher's disease (GD). These two diseases, although different in origin, one acute and vascular, the other chronic and genetic, share a common feature: a disruption in immune homeostasis marked by neuroinflammation.

Using flow cytometry, gene expression profiling, and induced pluripotent stem cell (iPSC)-derived cell modeling, this work demonstrated that monocytes and microglia are dynamically regulated by intrinsic factors and extrinsic stimuli. During human aging, circulating monocyte subsets undergo sex-dependent transcriptional remodeling, with females exhibiting stronger anti-inflammatory aging signatures. This was particularly evident in the upregulation of ANXA1 in female non-classical monocytes. Additionally, age-associated increases in the scavenger receptor CD36 were observed across all monocyte subtypes.

In ischemic stroke patients, monocyte frequency and gene expression patterns predict recovery, particularly through the expansion of intermediate monocytes and the downregulation of inflammatory mediators. A panel of recovery-associated markers: CD91, CD36, TGM2, SLC24A4, and CD38, were identified, with CD36 in intermediate monocytes emerging as a novel marker of recovery.

In neuronopathic GD, human iPSC-derived microglia (hiMG) exhibit intrinsic impairments in cytokine signaling and lysosomal function under immune stimulation, indicating chronic neuroimmune dysfunction. These changes suggest a brain-specific immune phenotype distinct from peripheral macrophages and support a role for microglia in GD-associated neurodegeneration.

In summary, this thesis explores how peripheral monocytes and brain-resident microglia, both part of the mononuclear phagocyte system, shape the neuroinflammatory processes during aging, ischemic stroke, and GD. It highlights how immune responses are influenced by age, sex, and disease state, providing mechanistic insight and models for developing future immune-targeted therapies for neurological diseases.

Popular science summary

As we grow older, our bodies change in many ways, as does our immune system. The cells that once helped to protect us from illness can start behaving differently, at times making us more vulnerable to inflammation and disease. One group of these cells, called monocytes, plays a particularly important role in how our bodies respond to disease conditions such as stroke, which happens when blood flow to the brain is interrupted. Interestingly, not only age but also whether a person is male or female can influence how these cells behave.

This research examines how monocytes from people of different ages and sexes respond to inflammation. We found that some important neuroinflammatory genes in these cells change more dramatically with age, especially in women. In people who have experienced a stroke, we discovered that early changes in these immune cells, just days after the event, could predict how well patients will recover.

However, immune cells are not just important in stroke. Another part of the thesis focuses on Gaucher disease, a rare genetic disorder that can affect the brain. Using a technique in which we convert regular skin cells taken directly from a patient with Gaucher disease into brain immune cells (called microglia), we were able to study how these brain immune cells behave. We found that in response to stress, they do not work properly especially when it comes to managing waste inside the cell and immune signaling, which may explain part of the disease process.

Altogether, this research helps us understand how the immune system changes with age and how those changes can affect recovery after a stroke or lead to brain problems in individuals with genetic diseases such as Gaucher syndrome. By learning how these immune cells work and sometimes fail, we hope to create better treatments that are tailored to each person's age, sex, and genetic background.

The brain's symphony: Inflammation in health and disease

Imagine the brain as a grand symphony orchestra, tirelessly performing the soundtrack of our lives. Every emotion we feel, every movement we make, every thought we think are all part of this rich and complex music. However, unlike the melodies we hear through our ears, this music is composed of **chemical signals**, such as **cytokines** and **neurotransmitters**, that guide how we think, act, and feel.

At the center of this orchestra are brain cells, called **neurons**. Among them, **excitatory neurons** take the role of the brass section: bold and energetic, pushing the melody forward with power and precision. In contrast, **inhibitory neurons** play the part of the woodwinds, adding subtlety, restraint, and balance to the overall harmony. We might think of **modulatory neurons** as the string section, weaving emotional depth and

resonance throughout the performance and tying the whole ensemble together. This intricate ensemble behind scenes is supported by **astrocytes**, which act as rhythm sections: essential, and often invisible. They make sure the tempo stays steady, that the instruments are nourished, and that the music does not spiral into disarray. Without them, the orchestra risks losing cohesion.

In this orchestral setting, **microglia** take on the role of conductors, interpreting the body's cues and shaping the brain's response. They are vigilant and dynamic, directing the cellular changes and inflammatory signals that influence the entire ensemble. They are also quiet custodians of harmony, trimming away dissonant notes and tuning neural instruments, ensuring that the symphony remains both beautiful and precise. However, they are not alone: **monocyte-derived macrophages** (**MDMs**), immune cells called from the bloodstream, can join the scene like soloists or guest performers who sway the conductor's direction, sometimes amplifying the crescendos of inflammation, other times softening the score toward resolution. Together, these immune players are central to **neuroinflammation**, a critical process that determines whether the brain's music recovers from disruption or spirals into dissonance.

Sometimes, the performance is abruptly disrupted: a string snaps midpiece, signaling the sudden and jarring crescendo of a **stroke**, throwing the orchestra into chaos. Other times, the disruption creeps in slowly, as in **Gaucher's disease**, where instruments gradually fall out of tune, the tempo becomes unstable, or players become tired, and the harmony frays over time. In both cases, **microglia and MDMs** step into the spotlight, not just reacting but also reshaping the entire inflammatory composition that follows. Their signals, **cytokines,** like emotional swells in the musical score, can guide healing but can also amplify damage. The **genes** provide the musical score, the instructions behind every move, while the immune cells decide how and when to perform it.

This thesis explores the complex roles of microglia and MDMs in the brain's inflammatory response, particularly during **stroke** and **Gaucher's disease**, and how factors like **age** and **sex** can influence this response. Through experimental models and molecular techniques, researchers have sought to understand how these immune conductors and guest players influence the melody of health and disease and whether they might be guided toward harmonizing rather than destabilizing the most delicate compositions of the brain.

By learning to listen more closely to the players, the instruments, the notes, and the score, we may one day become composers of recovery, restoring harmony when the brain's music falters and guiding it back to its full, breathtaking symphony.

Populärvetenskaplig sammanfattning

När vi blir äldre förändras våra kroppar på många sätt, och det gör även vårt immunförsvar. De celler som en gång hjälpte till att skydda oss från sjukdomar kan börja bete sig annorlunda, vilket ibland gör oss mer sårbara för inflammation och sjukdom. En grupp av dessa celler kallas monocyter, och de spelar en särskilt viktig roll för hur våra kroppar reagerar på sjukdomstillstånd som exempelvis stroke, vilket inträffar när blodflödet till hjärnan avbryts. Intressant nog kan inte bara ålder, utan även om en person är man eller kvinna, påverka hur dessa celler beter sig.

I denna forskning undersöks hur monocyter från personer i olika åldrar och av olika kön reagerar på inflammation. Vi fann att vissa viktiga gener i dessa celler blir mer aktiva med åldern, särskilt hos kvinnor. Hos personer som drabbats av stroke upptäckte vi att tidiga förändringar i dessa immunceller, bara några dagar efter händelsen, kan förutsäga hur väl patienterna kommer att återhämta sig.

Men immuncellerna är inte bara viktiga vid stroke. En annan del av avhandlingen fokuserar på Gauchers sjukdom, en sällsynt genetisk sjukdom som kan påverka hjärnan. Med hjälp av en teknik där vi omvandlar vanliga hudceller från en patient som drabbats av Gauchers sjukdom till en typ av immunceller som finns i hjärnan (så kallade mikroglia), blev det möjligt för oss att studera hur sådana immunceller beter sig annorlunda hos Gauchersjuka patienter. Vi fann att de som svar på stress inte fungerar som de ska: särskilt när det gäller att hantera avfall inuti cellen och immunsignalering, vilket kan förklara en del av sjukdomsprocessen.

Sammantaget hjälper denna forskning oss att förstå hur immunsystemet förändras med åldern, och hur dessa förändringar kan påverka återhämtningen efter en stroke eller leda till hjärnproblem vid genetiska sjukdomar som Gauchers sjukdom. Genom att lära oss hur dessa immunceller fungerar, och ibland misslyckas, hoppas vi en dag kunna skapa bättre behandlingar som är skräddarsydda för varje persons ålder, kön och genetiska bakgrund.

Hjärnans symfoni: Inflammation i hälsa och sjukdom

Föreställ dig hjärnan som en stor symfoniorkester som outtröttligt spelar soundtracket till våra liv. Varje känsla vi känner, varje rörelse vi gör, varje tanke vi tänker - allt är en del av denna rika och komplexa musik. Men till skillnad från de melodier vi hör genom våra öron består denna musik av **kemiska signaler**, som **cytokiner** och **neurotransmittorer**, som styr hur vi tänker, agerar och känner.

I centrum för denna orkester finns hjärnceller, så kallade **neuroner**. Bland dem spelar **excitatoriska neuroner** brassektionens roll - djärva och energiska, som driver melodin framåt med kraft och precision. De **hämmande neuronerna** spelar däremot träblåsarnas

roll och bidrar med subtilitet, återhållsamhet och balans till den övergripande harmonin. Vi kan tänka på **modulerande neuroner** som stråksektionen, som väver in känslomässigt djup och resonans genom hela föreställningen och knyter ihop hela ensemblen. Bakom kulisserna finns **astrocyterna**, som fungerar som en rytmsektion - stabila, viktiga och ofta osynliga. De ser till att tempot är jämnt, att instrumenten får näring och att musiken inte hamnar i oordning. Utan dem riskerar orkestern att förlora sin sammanhållning.

I detta orkestrala sammanhang är **mikroglia** dirigenterna. De tolkar kroppens signaler och formar hjärnans svar. De är vaksamma och dynamiska, styr cellulära förändringar och inflammatoriska signaler som påverkar hela ensemblen. De är också vårdare av harmonin: de justerar, rensar bort feltoner och finjusterar instrumenten. Men de är inte ensamma. **Monocytderiverade makrofager** (**MDM**), immunceller som kallas in från blodomloppet, kan träda in på scenen som solister eller gästartister som påverkar dirigentens riktning: ibland förstärker de inflammationens crescendon, andra gånger mjukar de upp partituret mot upplösning. Tillsammans spelar dessa immunceller en nyckelroll i **neuroinflammation**, en avgörande process som avgör om hjärnans musik återhämtar sig eller faller in i dissonans.

Ibland störs föreställningen abrupt: en sträng brister mitt i stycket: en **stroke** inträffar som en chockerande crescendo och kastar orkestern i kaos. Andra gånger smyger sig störningen på, som vid **Gauchers sjukdom**, där instrumenten gradvis faller ur stämning, tempot blir instabilt, musikerna tröttnar och harmonin långsamt vittrar bort. I båda fallen kliver mikroglia och MDM fram i rampljuset: inte bara som svar, utan som omformare av den inflammatoriska komposition som följer. Deras signaler, **cytokiner**, som känslomässiga svallvågor i partituret, kan leda till läkande, men kan också förvärra skadan. Våra **gener** är notbladen, instruktionerna bakom varje rörelse, medan immunförsvarets celler bestämmer hur och när de ska spelas.

I denna avhandling utforskas **mikroglias och MDM** komplexa roller i hjärnans inflammatoriska respons, med särskilt fokus på stroke och Gaucher's sjukdom, samt hur faktorer som **ålder** och **kön** kan påverka detta. Med hjälp av experimentella modeller och molekylära tekniker utforskas hur dessa immundirigenter och gästartister påverkar hälsans och sjukdomens melodi, och om de kan ledas till att harmonisera snarare än att destabilisera hjärnans mest känsliga kompositioner.

Genom att lära oss lyssna noggrannare på musikerna, instrumenten, tonerna och partituret, kan vi kanske en dag bli läkandets kompositörer och återställa harmonin när hjärnans musik fallerar, och sakta leda den tillbaka till sin fulla, episka symfoni.

Populärwissenschaftliche Zusammenfassung

Wenn wir älter werden, verändert sich unser Körper in vielerlei Hinsicht, so auch unser Immunsystem. Die Zellen, die uns früher vor Krankheiten geschützt haben, verhalten sich nun anders, was uns anfälliger für Entzündungen und Krankheiten macht. Eine Gruppe dieser Zellen, die so genannten Monozyten, spielt eine besonders wichtige Rolle dabei, wie unser Körper auf Krankheiten wie einen Schlaganfall reagiert, der entsteht, wenn die Blutzufuhr zum Gehirn unterbrochen wird. Interessanterweise kann nicht nur das Alter, sondern auch die Tatsache, ob eine Person männlich oder weiblich ist, das Verhalten dieser Zellen beeinflussen.

In dieser These wird untersucht, wie Monozyten von Menschen unterschiedlichen Alters und Geschlechts auf Entzündungen reagieren. Wir fanden heraus, dass sich einige für das Gehirn wichtige Gene in diesen Zellen mit zunehmendem Alter dramatischer verändern, insbesondere bei Frauen. Bei Menschen, die einen Schlaganfall erlitten haben, konnten wir feststellen, dass frühe Veränderungen in Monzyten, nur wenige Tage nach dem Ereignis, vorhersagen können, wie gut sich Patienten erholen werden.

Doch Immunzellen spielen nicht nur bei Schlaganfällen eine Rolle. Ein anderer Teil dieser These befasst sich mit der Gaucher-Krankheit, einer seltenen genetischen Störung, die das Gehirn beeinträchtigen kann. Mithilfe einer Technik, bei der wir Hautzellen von einer Patientin mit Gaucher-Krankheit in sogenannte Mikroglia (Immunzellen des Gehirns) umwandeln, konnten wir untersuchen, wie sich diese Zellen verhalten. Dabei zeigte sich, dass sie in ihrer Funktion unter Stress gestört sind: insbesondere bei der Weiterleitung von Immunsignalen und Entsorgung von Abfallstoffen innerhalb der Zelle. Diese Fehlfunktionen könnten einen Teil des Krankheitsprozesses im Gehirn erklären.

Insgesamt hilft uns diese Forschung zu verstehen, wie sich das Immunsystem mit dem Alter verändert und wie diese Veränderungen die Genesung nach einem Schlaganfall beeinträchtigen oder bei genetischen Krankheiten wie Gaucher zu Störungen im Gehirn führen können. Indem wir lernen, wie diese Immunzellen arbeiten und manchmal versagen, hoffen wir, eines Tages bessere Behandlungen zu entwickeln, die auf das Alter, das Geschlecht und den genetischen Hintergrund jeder Person zugeschnitten sind.

Die Symphonie des Gehirns: Entzündung in Gesundheit und Krankheit

Stellen Sie sich das Gehirn als ein großes Symphonieorchester vor, das unermüdlich den Soundtrack unseres Lebens spielt. Jede Emotion, jede Bewegung, jeder Gedanke, sind alle ein Teil dieser reichen und komplexen Musik. Doch anders als die Melodien,

die wir mit unseren Ohren hören, besteht diese Musik aus **chemischen Signalen** wie **Zytokinen** und **Neurotransmittern**, die unser Denken, Handeln und Fühlen steuern.

Im Zentrum dieses Orchesters stehen die Gehirnzellen, die sogenannten **Neuronen**. Unter ihnen übernehmen **erregende Neuronen** die Rolle der Blechbläser: kühn und energisch, die Melodie mit Kraft und Präzision vorantreibend. **Hemmende Neuronen** hingegen wirken wie Holzbläser: sie bringen Subtilität, Zurückhaltung und Ausgewogenheit in die Harmonie. Die **modulatorischen Neuronen** lassen sich mit Streichern vergleichen, die emotionale Tiefe und Resonanz verleihen und das Ensemble zusammenhalten. Unterstützt wird dieses komplexe Zusammenspiel von den **Astrozyten**, die als Rhythmusgruppe fungieren: beständig, unerlässlich und oft im Hintergrund. Sie halten das Tempo, versorgen die Zellen mit Nährstoffen und sorgen dafür, dass die Musik nicht aus dem Gleichgewicht gerät.

In diesem „Gehirn-Orchester" sind die **Mikroglia** die Dirigenten, die die Signale des Körpers interpretieren und die Reaktion des Gehirns formen. Wachsam und dynamisch lenken sie zelluläre Veränderungen und Entzündungsprozesse, stimmen die neuronalen Instrumente und schneiden dissonante Töne heraus, stets bemüht, die Harmonie zu wahren. Doch sie sind nicht allein: **Monozyten-Makrophagen** (**MDMs**), Immunzellen aus dem Blutkreislauf, treten als Solisten oder Gastmusiker auf. Sie können die Richtung des Dirigenten beeinflussen, indem sie die Crescendos der Entzündung verstärken oder die Partitur in Richtung Auflösung mildern. Gemeinsam sind diese Immunakteure zentrale Gestalter der **Neuroinflammation**, eines entscheidenden Prozesses, der darüber entscheidet, ob sich die Musik des Gehirns nach einer Störung erholt oder in Dissonanz verfällt.

Manchmal wird die Aufführung abrupt unterbrochen: eine Saite reißt mitten im Stück: das plötzliche, erschütternde Crescendo eines **Schlaganfalls** stürzt das Orchester ins Chaos. In anderen Fällen schleicht sich die Störung langsam ein, wie bei der **Gaucher-Krankheit**: Die Instrumente verstimmen sich allmählich, das Tempo wird instabil, die Spieler ermüden, und die Harmonie zerfasert mit der Zeit. In beiden Fällen sind es die Mikroglia und MDMs, die ins Rampenlicht treten und nicht nur reagieren, sondern die gesamte nachfolgende entzündliche Komposition neugestalten. Ihre Signale, **Zytokine**, wie emotionale Dynamiken in der Partitur, können die Heilung steuern, aber auch den Schaden verstärken. Die **Gene** liefern die Partitur, die Anweisungen für jeden Schritt, während die Immunzellen entscheiden, wie und wann sie ihn ausführen.

Diese These untersucht die komplexe Rolle von Mikroglia und MDMs in der Entzündungsreaktion des Gehirns, insbesondere bei Schlaganfall und Gaucher Krankheit – und wie Faktoren wie **Alter** und **Geschlecht** diese Prozesse beeinflussen können. Mithilfe experimenteller Modelle und molekularer Techniken erforschen wir, wie diese Immun-Dirigenten und -Gastspieler die Melodie von Gesundheit und

Krankheit formen, und ob sie dazu gebracht werden können, selbst die empfindlichsten Kompositionen des Gehirns zu harmonisieren, statt sie zu destabilisieren.

Indem wir lernen, den Spielern, den Instrumenten, den Noten und der Partitur genauer zuzuhören, könnten wir eines Tages zu Komponisten der Genesung werden: fähig, die Harmonie wiederherzustellen, wenn die Musik des Gehirns ins Stocken gerät, und sie zu ihrer vollen, atemberaubenden Symphonie zurückzuführen.

Abbreviations

BBB	blood-brain-barrier
CCL2	C-C motif chemokine ligand 2
CCR2	C-C chemokine receptor type 2
CD	cluster of differentiation
CNS	central nervous system
CX3CR1	CX3C chemokine receptor 1
ERT	Enzyme replacement therapy
GCase	glucocerebrosidase
GD	Gauchers disease
GM-CSF	granulocyte-macrophage colony-stimulating factor
hiMG	human iPSC-derived microglia
hiNs	human iPSC-derived neurons
HSCs	hematopoietic stem cells
IL	interleukin
iPSC	induced pluripotent stem cell
MCAO	middle cerebral artery occlusion
MDMs	monocyte-derived macrophages
RT-qPCR	reverse transcription quantitative polymerase chain reaction
rtTA	tetracycline transactivatior
tetO	tetracycline operator
TGFβ	transforming growth factor-beta
TLR	toll-like receptor
TNFα	tumor necrosis factor-alpha
VEGF	vascular endothelial growth factor

Introduction

Rationale

Neurological diseases are among the leading causes of disability and death globally, with a growing prevalence linked to an aging population. Despite decades of scientific advancement, effective treatments remain unavailable for many patients (Feigin et al. 2025). Its impact is not only biological but also deeply societal: survivors often experience long-term motor, cognitive, and emotional impairments that diminish quality of life and impose considerable burdens on caregivers and healthcare systems. Similarly, although rare, neuronopathic GD places a severe and progressive neuroinflammatory burden on patients, leading to early mortality and causing substantial hardship for both individuals and their families. Together, these disorders highlight the urgent need for a deeper understanding of disease mechanisms and the development of novel therapeutic targets.

Despite intensive research efforts, treatment options for both common and rare neurological disorders remain limited. Existing therapies often focus on managing symptoms rather than addressing the underlying drivers of disease. One such driver, increasingly recognized, is neuroinflammation — the immune-mediated response of the central nervous system (CNS) to injury, disease, or homeostatic disruption. Once considered a secondary byproduct of pathology, neuroinflammation is now understood to play a central role in both acute CNS injury (e.g., ischemic stroke) and chronic degenerative processes (e.g., lysosomal storage disorders such as GD). This paradigm shift has positioned neuroinflammation at the forefront of translational neuroscience, unlocking new avenues of research into how immune cells influence brain health, drive disease progression and inspire novel therapeutic strategies.

Microglia and MDMs are key regulators of the neuroimmune landscape. These immune cells, which are traditionally studied in isolation, either as CNS-resident or blood-derived populations, are increasingly recognized to function in cooperation, responding to local signals and systemic conditions such as aging, metabolic stress, and vascular injury. Microglia, the resident macrophages of the CNS, originate from yolk sac progenitors and maintain homeostatic surveillance under physiological conditions but are rapidly activated following trauma or stress. In contrast, in animal models, monocytes circulate in the periphery and infiltrate the brain upon injury, where they

differentiate into MDMs that contribute to both early damage and long-term repair. The functional roles of these cells are highly plastic and context-dependent, influenced by factors such as biological sex, age, genetic predisposition, and environmental cues (Spychala et al. 2017, Marino Lee et al. 2021, So et al. 2021).

While preclinical models have advanced our knowledge of these immune cell types, there are substantial gaps in our understanding of their behavior in human disease, particularly in relation to transcriptional regulation, phenotypic heterogeneity, and disease-specific activation states. For example, although animal models highlight the involvement of microglia in GD pathology, they fall short of replicating the complexity of human neuroimmune interactions (Brunialti et al. 2021, Boddupalli et al. 2022). To address these challenges, induced pluripotent stem cells (iPSCs) offer a powerful approach, enabling the generation of patient-specific neural and immune cells that retain the genetic and phenotypic characteristics of disease (Inoue and Yamanaka 2011, Kumar et al. 2018). Recent protocols allow for the efficient derivation of human microglia and monocytes from iPSCs through mesodermal differentiation and hematopoietic lineage commitment (Cao et al. 2019, Speicher et al. 2019). Understanding how immune cells integrate systemic cues (e.g., aging, sex hormones) with local microenvironmental signals in the injured or diseased brain could yield important insights into the timing and direction of inflammation, resolution, and neurorepair processes.

This thesis was motivated by the need to investigate these immune mechanisms across a range of human-relevant contexts. Specifically, these studies aim to explore how monocytes and microglia contribute to neuroinflammation during aging, stroke, and GD, three conditions in which innate immune dysfunction plays a critical yet incompletely understood role. Special emphasis is placed on the influence of biological sex, monocyte subtype identity, and context-dependent immune activation via a combination of ex vivo analysis, gene expression profiling, and iPSC-derived microglial modeling.

By examining peripheral immune changes across the adult lifespan, the role of monocytes in post-stroke recovery, and microglial dysfunction in a genetic neurodegenerative disorder, this thesis seeks to bridge basic and translational immunology. The ultimate goal is to identify candidate biomarkers and mechanisms that could guide future therapeutic strategies aimed at modulating immune responses in both acute and chronic neurological conditions.

Neuroimmunology

Neuroimmunology is an interdisciplinary field that explores the interactions between the immune and nervous systems: two highly specialized and deeply interconnected networks. It aims to understand how these systems respond to trauma, maintain homeostasis, and coordinate during development, as well as in chronic neurodegenerative and metabolic conditions. While an incomplete understanding of neuroimmune mechanisms limits therapeutic advances in diseases such as neuronopathic GD, which is characterized by progressive neuroinflammation, stroke currently lacks both effective treatments and reliable prognostic tools.

In pathological conditions such as ischemic stroke or GD, immune activation is a hallmark feature. The local activation of microglia, alongside the recruitment of peripheral immune cells from the bloodstream, represents a tightly regulated yet critical component of the injury response (Boddupalli et al. 2022). During this response, myeloid cells, including blood-derived (infiltrating monocytes) and CNS-resident (microglia) macrophages, play essential roles. These cells not only orchestrate the inflammatory cascade but also contribute to its resolution by clearing debris and promoting repair (Colonna and Butovsky 2017). A deeper understanding of how these cell types interact and cooperate could improve prognostic models and open new avenues for inflammation-targeted therapies.

Monocytes - infiltrating immune cells from the blood

Monocytes are among the first responders to sites of tissue injury in the body. These cells are central players in the innate immune system and represent the main circulating precursors of macrophages. During embryogenesis, human monocytes originate from hematopoietic stem cells (HSCs) in the fetal liver (Auffray et al. 2009), which serves as the primary site of definitive hematopoiesis before the bone marrow takes over after birth. Postnatally, monocytes are continuously replenished from HSCs in the bone marrow.

Under normal physiological conditions, monocytes patrol the bloodstream; however, in response to tissue damage or infection, they are recruited to the site of injury, including the CNS. In the context of neurological pathologies such as stroke or lysosomal storage disorders such as neuronopathic GD, circulating monocytes can infiltrate the brain parenchyma. This recruitment is chemokine-driven, with gradients such as C-C motif chemokine ligand 2 (CCL2) attracting monocytes across the blood-brain barrier (BBB). Upon tissue infiltration, monocytes differentiate into MDMs, which are crucial for initiating the immune response. The MDMs contribute to pathogen clearance, removal of damaged cells, cytokine secretion, and antigen presentation (Chiu and Bharat 2016). Once established within tissue, MDMs may

persist for weeks through local self-renewal and cooperate with resident immune cells to regulate inflammation through both pro- and anti-inflammatory pathways (Krzyszczyk et al. 2018).

Human monocytes are classified into three functionally distinct subtypes on the basis of the expression of the surface markers CD14 and CD16: classical (CD14+/CD16-), intermediate (CD14+/CD16+), and non-classical monocytes (CD14-/CD16+), each with unique functions and molecular profiles (Ziegler-Heitbrock and Hofer 2013). Classical monocytes make up the majority of circulating monocytes. They emerge rapidly from the bone marrow in response to inflammatory signals, a process largely dependent on C-C chemokine receptor type 2 (CCR2) signaling, and circulate for approximately 24 hours (Tsou et al. 2007, Shi and Pamer 2011). Those not recruited into tissue mature over a period of several days into intermediate and then non-classical monocytes, which persist in circulation for up to seven days (Patel et al. 2017). This differentiation trajectory results in a steady distribution of approximately 85% classical, 5% intermediate, and 10% non-classical monocytes in the blood (Passlick et al. 1989, Wong et al. 2011). CD91 serves as a pan-monocyte marker and is expressed consistently across all subsets and developmental stages (Hudig et al. 2014).

Classical monocytes express high levels of CCR2, are strongly phagocytic and serve as principal contributors to early-stage inflammatory responses, particularly in acute CNS injury. They are rapidly mobilized from the bone marrow in response to CCL2 gradients, facilitating their recruitment to sites of injury (Hsieh et al. 2014, Chou et al. 2018). Intermediate monocytes exhibit strong pro-inflammatory properties, produce high levels of TNFα and IL-1β, and are thought to play a role in antigen presentation (Zawada et al. 2011). Non-classical monocytes exhibit high levels of CX3C chemokine receptor 1 (CX3CR1) and are involved in endothelial surveillance and tissue repair (Evans et al. 2014). All three subpopulations circulate in the blood and can transmigrate across the endothelial layer to infiltrate tissue upon injury or inflammation. Factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) are essential for their migration across the endothelial cells of the BBB (Vogel et al. 2015). Additionally, the expression of the scavenger receptor CD36 is upregulated on infiltrating monocytes, increasing their phagocytic and pro-inflammatory potential, which, if left unchecked, can exacerbate secondary tissue damage (Cuschieri et al. 2023). The combined expression levels of CCR2, CX3CR1, and CD36 influence how monocytes are recruited and functionally adapt within the inflamed CNS. Monocyte infiltration begins within 24 hours after stroke and increases gradually, peaking at four days (Planas 2018). Following recruitment and differentiation into MDMs, they modulate their pro- and anti-inflammatory functions in response to local cues, orchestrating the immune response tailored to specific needs of the injured, promoting effective repair while limiting excessive inflammation that exacerbates damage (Austermann et al. 2022).

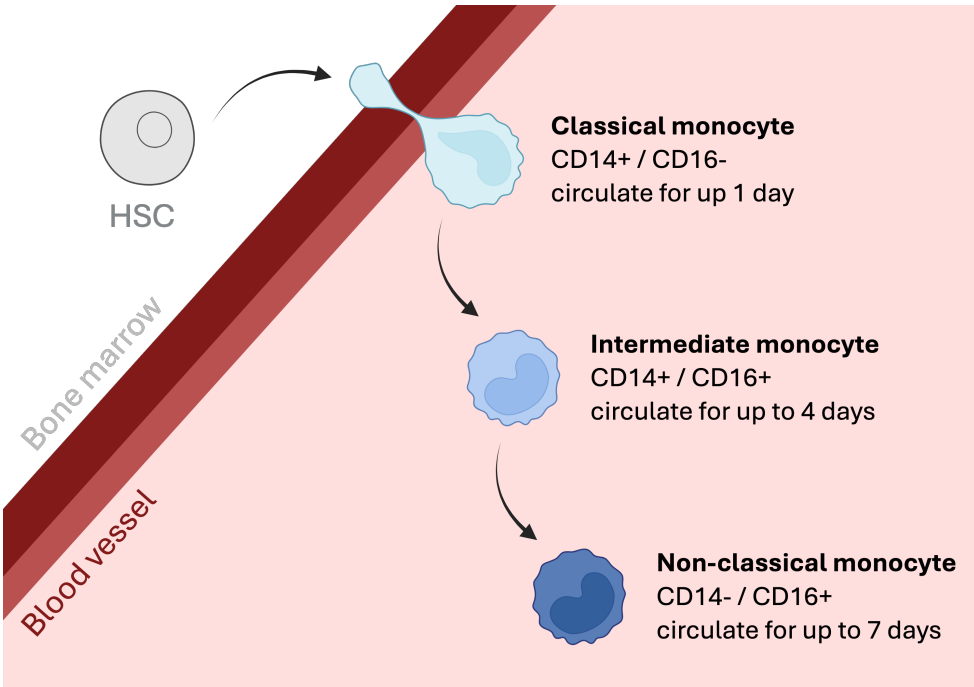


Figure 1 | Differentiation and circulation dynamics of human monocyte subsets. HSCs (gray) give rise to classical monocytes ($CD14^+/CD16^-$), which circulate briefly before maturing into intermediate ($CD14^+/CD16^+$) and non-classical ($CD14^-/CD16^+$) subsets. The figure illustrates their sequential maturation and lifespan (blue) in peripheral blood. Created in BioRender. Tampe, J. (2025) https://BioRender.com/63al4ny

Microglia - resident immune cells of the brain

As the resident macrophages of the brain, microglia are key players in the innate immune defense of the CNS. Unlike monocytes and other macrophages derived from HSCs in the bone marrow, microglia originate from primitive erythromyeloid progenitors in the extra-embryonic yolk sac during early embryogenesis. These precursors migrate into the developing brain, where local signals shape them into a distinct and self-sustaining cell population (Huber et al. 2004, Gomez Perdiguero et al. 2015).

Once established, microglia maintain themselves throughout life via *in situ* proliferation, independent of peripheral hematopoietic input under physiological conditions, even following acute depletion (Cronk et al. 2018, Huang et al. 2018). In the healthy adult brain, microglia play an essential role in maintaining homeostasis. They are involved in various functions, such as synaptic pruning, angiogenesis, immune surveillance, and the phagocytosis of cellular debris and pathogens, depending on inputs from surrounding molecules and cells (Croese et al. 2021, Mosser et al. 2021).

Microglia are characterized by high motility and morphological plasticity, allowing them to continuously survey the CNS microenvironment. Like MDMs, they can activate pro-inflammatory or anti-inflammatory pathways, depending on the nature and context of surrounding stimuli (Nayak et al. 2014). Importantly, microglia respond to virtually all forms of CNS injury, including minor, sterile insults, in a manner entirely independent of circulating immune cells (Cuadros et al. 2022). Their immediate activation makes them the first line of defense within the brain.

Although essential for homeostatic functions, sustained or dysregulated activation of microglia can become detrimental, contributing to neuronal injury or death. Prolonged pro-inflammatory signaling from microglia has been implicated in various neurological disorders, including neurodegenerative and metabolic diseases (Nayak et al. 2014). This dual role, which is protective under homeostatic conditions but harmful when chronically activated, positions microglia as central regulators of neuroinflammatory processes that are functionally aligned with MDMs.

Immune regulation by macrophages

Microglia and MDMs are essential players in the innate immune system. They respond to both internal and external stimuli, adopting activation states that shape inflammation, tissue repair, and homeostasis depending on the local microenvironment and differentiation status (Grage-Griebenow et al. 2001, Prinz 2014). Their crucial functions include recognizing pathogens, initiating immune responses, resolving inflammation, and modulating tissue remodeling (Taylor et al. 2003).

Pro-inflammatory cues drive the activation of monocytes and microglia, differentiating them into macrophages in response to infection or injury. This response is mediated through pattern recognition receptors, like Toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns and activate downstream signaling cascades. Activated cells secrete pro-inflammatory cytokines, including interleukin-1 (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNFα), which contribute to early immune defense (Sochocka et al. 2017). In parallel, phagocytic activity increases, enabling efficient clearance of pathogens and cellular debris.

To prevent excessive or chronic inflammation, anti-inflammatory signals counterbalance immune activation and promote the resolution of the response. This includes the secretion of anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGFβ), which downregulate the production of pro-inflammatory mediators and inhibit immune cell activation (Oswald et al. 1992, Travis and Sheppard 2014). Additionally, anti-inflammatory signals facilitate the clearance of apoptotic cells and debris, contributing to tissue repair and homeostasis (Sieweke and Allen 2013). Thus, anti-inflammatory signals are essential for regulating

the intensity and duration of immune responses and preventing collateral tissue damage.

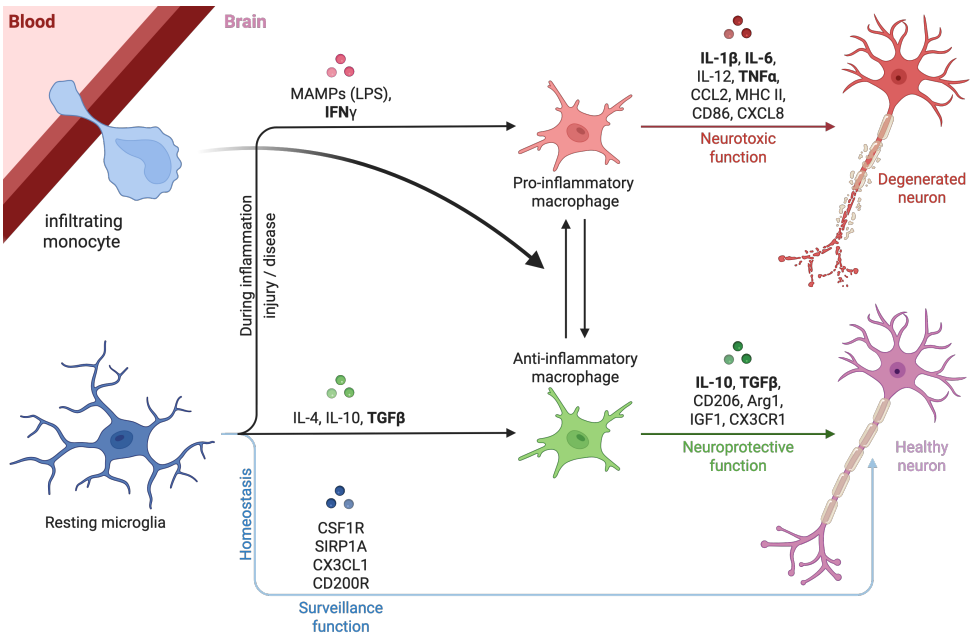


Figure 2 | Monocyte infiltration and macrophage/microglia polarization in the brain during homeostasis or injury. The schematic illustrates how circulating monocytes (blue) and brain-resident microglia (navy blue) respond to inflammation, injury, or disease. Both cell types can differentiate into macrophages with either neurotoxic (red) or neuroprotective (green) functions, depending on the local environment. In the healthy brain, resting microglia contribute to homeostasis through surveillance functions. Created in BioRender. Tampe, J. (2025) https://BioRender.com/zirrkxe

Following the resolution phase, macrophages adopt a reparative phenotype, producing trophic factors, extracellular matrix proteins, and pro-angiogenic molecules that promote healing. These include collagen, vascular endothelial growth factor (VEGF), and signals that recruit fibroblasts and support extracellular matrix deposition (Jaipersad et al. 2014). This phenotypic switch is critical for the structural and functional recovery of damaged tissue.

Distinguishing MDMs from microglia during inflammation remains technically challenging, as they share overlapping markers and functional profiles. IBA1 has been widely used as a pan-microglial marker due to its consistent expression in both resting and activated microglia but is also expressed by MDMs (Lier et al. 2021). However, recent advances have identified the transmembrane protein TMEM119 as a specific and reliable marker for human microglia, enabling improved discrimination between these closely related populations in both health and disease (Vankriekelsvenne et al. 2022).

The interplay between pro-inflammatory, anti-inflammatory, and tissue repair signals is crucial for maintaining tissue homeostasis. Both activated MDMs and microglia are heterogeneous populations that switch rapidly between these highly plastic phenotypes depending on environmental cues (Jurga et al. 2020). This fine-tuned balance ensures that immune responses effectively eliminate threats while minimizing collateral damage to healthy tissue. The orchestrated transition from pro-inflammatory to anti-inflammatory and reparative states underscores the remarkable plasticity of immune cells in adapting to changing microenvironments. Understanding the regulatory mechanisms that initiate these shifts is essential for designing therapeutic strategies that promote resolution and repair while avoiding chronic inflammation or immune-mediated damage.

Monocyte-driven neuroinflammation and recovery after stroke: Insights from preclinical and translational studies

Stroke triggers a robust inflammatory response: within hours of ischemic insult, the brain initiates a coordinated immune cascade involving the activation of resident microglia and astrocytes, along with the infiltration of peripheral immune cells, particularly monocytes. Cytokines, chemokines, and endothelial adhesion molecules guide the recruitment of these hematopoietic cells to the injured brain, establishing a dynamic immune network that evolves over days to weeks and shapes the outcome of recovery (Kleinig and Vink 2009).

Due to the inherent plasticity of the brain, both humans and animal models of stroke can exhibit some degree of spontaneous functional recovery, depending on the location and extent of the injury (Cramer and Riley 2008, Wei et al. 2013). In previous studies in mice, our group showed that depletion of monocytes during the acute phase after stroke and the resulting decrease in MDMs in the stroke brain impaired the recovery of sensorimotor deficits after stroke (Wattananit et al. 2016). Furthermore, intraventricular transplantation of monocytes primed toward an anti-inflammatory fate, improved motor and cognitive outcomes in a mouse model of middle cerebral artery occlusion (MCAO)(Ge et al. 2017). Building on these studies, our group also showed that depleting circulating monocytes early after stroke enhanced striatal neurogenesis. Notably, astrocyte activation was reduced, although the lesion volume and neurogenesis of iPSC-derived human neuronal progenitor cells were unaffected (Laterza et al. 2017). Together, these studies demonstrate the essential role of circulating monocytes in recovery after stroke.

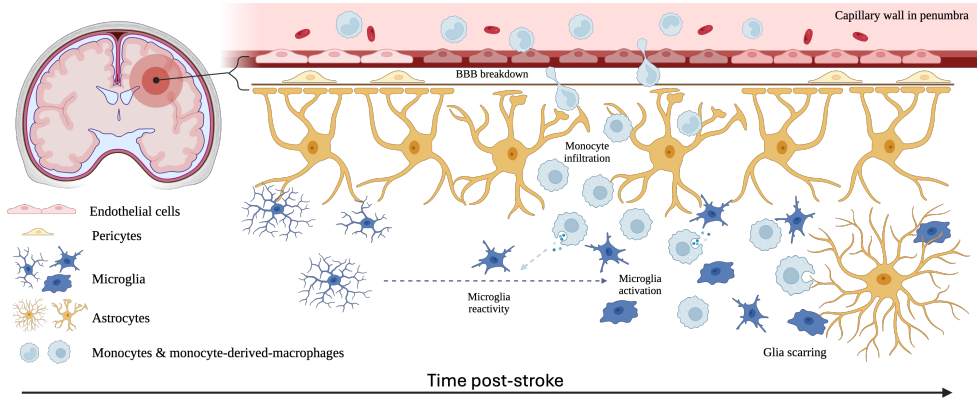


Figure 3 | Schematic overview of infiltrating monocytes and local microglia in the brain following stroke. The top panel shows a cross-sectional view of the brain with a highlighted ischemic region. The middle panel zooms into the capillary wall within the penumbra, depicting key cellular players involved in the post-stroke response, including endothelial cells (pink), pericytes (yellow), microglia (Navy blue, ramified), astrocytes (orange), and infiltrating monocytes and monocyte-derived macrophages (blue). The bottom timeline outlines the temporal progression of pathological events, including BBB disruption, microglial reactivity, monocyte infiltration, microglial activation, and the formation of glial scarring. Created in BioRender. Tampe, J. (2025) https://BioRender.com/6wvo46c

Additional preclinical studies have explored immune modulation approaches aimed at shifting monocyte and macrophage activity toward anti-inflammatory and reparative states. For example, IL-13 treatment was shown to promote anti-inflammatory MDM polarization, reduce infarct volume, and dampen the inflammatory milieu (Kolosowska et al. 2019, Li et al. 2019). These primed MDMs not only secrete higher levels of IL-10 and TGFβ but also lower levels of pro-inflammatory IL-1β and may influence neighboring microglia to adopt similar protective phenotypes (Ye et al. 2019). As a result, outcomes included reduced neuronal apoptosis, improved blood-brain barrier integrity, enhanced cortical perfusion, and improved neurological function (Xu et al. 2021).

Attempts to translate these findings to human stroke have yielded partial parallels. Clinical studies have revealed that monocyte subtype dynamics are correlated with stroke severity and recovery. For example, elevated TLR4 expression on circulating monocytes has been associated with worse clinical outcomes. Moreover, although total monocyte counts also increase with stroke severity, the proportion of TNFα-expressing monocytes decreases during the acute phase (Urra et al. 2009). Additional studies confirmed an increase in total monocyte count between days 2 and 7 post-stroke, with poor outcomes linked to shifts in monocyte subtype composition (Haeusler et al. 2008). At admission, an increased proportion of classical monocytes correlated with clinical severity and poorer functional outcomes. By 90 days post-stroke, reductions in intermediate and non-classical monocyte subpopulations are predictive of mortality

and long-term disability (Haeusler et al. 2008, Urra et al. 2009). These clinical findings mirror those from preclinical studies, where the selective depletion of classical monocytes improved outcomes and was associated with a reduced lesion volume (Dimitrijevic et al. 2007).

Despite these promising findings, clinical trials targeting inflammatory pathways have failed to demonstrate therapeutic benefit. This highlights the need for a more refined understanding of immune dynamics, including the timing of immune responses, the specific roles of monocyte subsets, and the complexity of signaling interactions across the acute and chronic phases of stroke recovery (D'Ambrosio et al. 2004, Lambertsen et al. 2019). Given the rapid phenotypic plasticity of monocytes, further research is essential to elucidate the context-dependent functions of each subset, with the goal of developing effective and precisely timed immunomodulatory therapies.

Stroke

Stroke is an acute cerebrovascular event that leads to neuronal injury, cell death, or sustained persistent impairment of brain function. The resulting neurological deficits typically manifest suddenly and can persist long-term, making stroke one of the leading causes of adult physical and cognitive disability worldwide (Smith et al. 2018). Strokes are classified into ischemic, hemorrhagic, and transient ischemic attacks, each of which poses distinct challenges in terms of etiology, pathophysiology, and clinical management (Sacco et al. 2013).

Ischemic stroke

Ischemic strokes account for approximately 87% of all stroke cases and result from the occlusion of cerebral arteries due to thrombotic or embolic events (Virani et al. 2020). Thrombotic strokes arise from local vessel occlusion and are often associated with atherosclerosis, whereas embolic strokes result from dislodged clots of plaques that originate from extracerebral sources (Ornello et al. 2018). Complete obstruction of blood flow triggers excitotoxicity, oxidative stress, inflammation, and finally, neuronal injury and, ultimately, irreversible neuronal injury. Permanent tissue damage occurs within minutes of full occlusion, whereas partial occlusion may lead to delayed degeneration if reperfusion is not achieved. If blood circulation is restored before brain tissue undergoes necrosis, symptoms may only be transient and reversible (Moskowitz et al. 2010).

This same concept applies to the ischemic penumbra, the region surrounding the infarct core, where cells are functionally impaired and can potentially be rescued if blood flow is restored. Otherwise, the penumbra will ultimately turn necrotic as well.

The extent of cerebral tissue loss is correlated with cognitive and functional impairments (Ermine et al. 2021).

Hemorrhagic stroke

Although less common, hemorrhagic strokes account for approximately 13% of stroke cases and are associated with a high risk of mortality and severe disability. These events occur due to rupture of intracerebral blood vessels, leading to bleeding into the brain parenchyma (intracerebral hemorrhage) or into the subarachnoid space (subarachnoid hemorrhage). The accumulation of blood increases intracranial pressure, expands the hematoma volume, and triggers secondary neuronal injury through inflammation and mechanical stress (Rymer 2011).

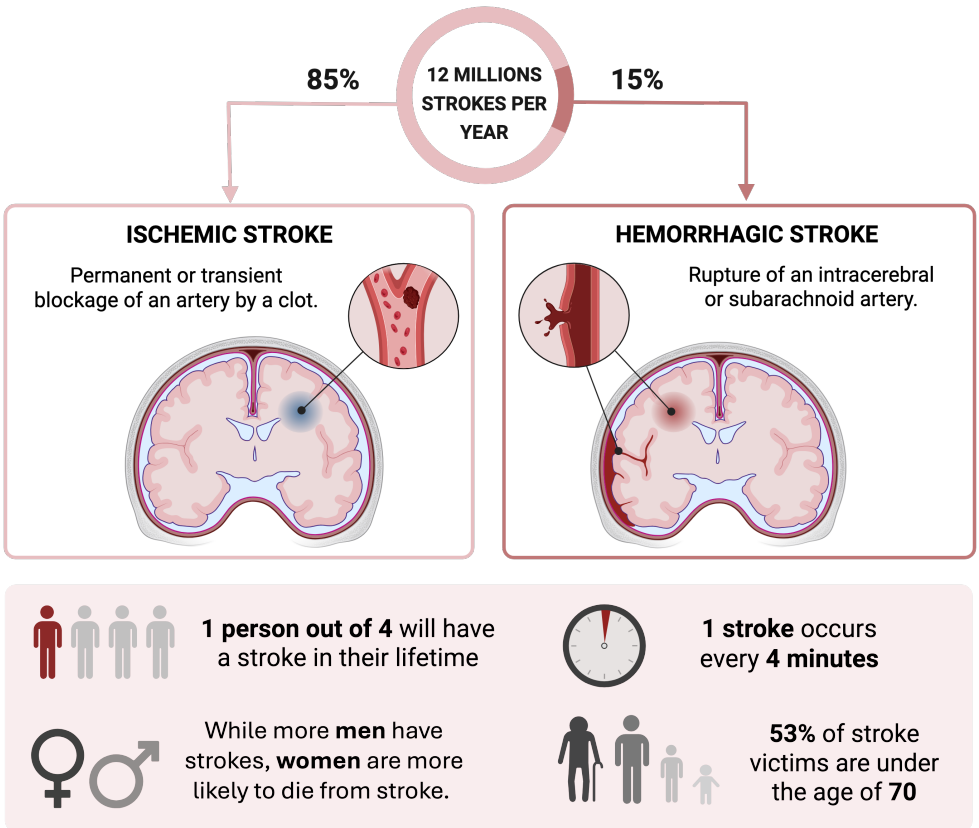


Figure 4 | Overview of stroke types and global epidemiological statistics. The schematic summarizes the two main types of stroke and their relative prevalence and highlights the statistics of the global burden of stroke. Created in BioRender. Tampe, J. (2025) https://BioRender.com/bmfngmc

Transient ischemic attacks

Transient ischemic attacks involve transient reductions in cerebral flow that cause temporary neurological symptoms that resolve within 24 hours. Although no lasting damage occurs, these brief disruptions in cerebral blood flow are considered warning signs of potential future strokes and require monitoring and prophylactic interventions (Sorensen and Ay 2011).

This thesis focuses on ischemic stroke, the most prevalent and clinically significant stroke subtype, reflecting the scope of the experimental work undertaken to contribute to a broader understanding of neuroinflammatory responses and the complex pathophysiology of stroke.

Epidemiology and societal burden

Globally, ischemic stroke is the second leading cause of death and the primary cause of long-term adult disability (Virani et al. 2020, Feigin et al. 2025). It is estimated that one in four individuals over the age of 25 will suffer a stroke in their lifetime (Lindsay et al. 2019). Although its incidence has declined in many high-income countries due to improvements in preventive care and acute treatment (Feigin et al. 2014, Krishnamurthi et al. 2015, Mensah et al. 2015), it has doubled in low-income countries, driven largely by increased obesity and hypertension (Krishnamurthi et al. 2020). Nonetheless, due to an aging population, improved survival, and the limited availability of effective treatment, the number of people living with the consequences of stroke is estimated to increase by more than 25% in the EU during the next 50 years (Wafa et al. 2020).

This burden is both medical and economic, as stroke-related disabilities, motor, sensory, and cognitive, place substantial strain on healthcare systems and affected families (Katan and Luft 2018, Feigin et al. 2025). In Sweden, the incidence is approximately 213 per 100,000 individuals, with each stroke case costing an average of 513,800 SEK. Nationally, healthcare costs exceed 12 billion SEK annually (Ghatnekar et al. 2004), with long-term residential and home care costs continuing to rise (Ghatnekar et al. 2014).

Risk factors

A range of modifiable and inherent, non-modifiable factors together contribute to an individual's risk of stroke. Many overlap with those of cardiovascular diseases, including hypertension, dyslipidemia, and smoking. Notably, up to 40% of stroke-related deaths can be prevented through blood pressure control. Tobacco use is also a significant contributor, especially in individuals under 65 years of age (Boehme et al. 2017).

Non-modifiable risk factors include age, biological sex, genetics, and ethnicity. Age remains the strongest determinant, with the majority of strokes occurring in individuals over 65 years old (Kelly-Hayes 2010). Aging is associated with vascular changes, a greater prevalence of risk factors, and greater comorbidity, all of which contribute to stroke susceptibility. While men exhibit higher stroke risk at younger ages, women's overall lifetime risk is greater, likely due to longer life expectancies and hormonal factors (Bushnell et al. 2014, Abdu and Seyoum 2022). Pregnancy, oral contraceptive hormone use, and the postmenopausal state are associated with increased stroke risk in women, who also tend to experience more severe outcomes and deficits (Spychala et al. 2017, Yoon and Bushnell 2023). Population-specific disparities in stroke risk reflect the interplay of genetic predispositions and socio-environmental exposures, underscoring the importance of ethnically informed approaches in stroke prevention and research (Feigin et al. 2007, Morgenstern et al. 2013).

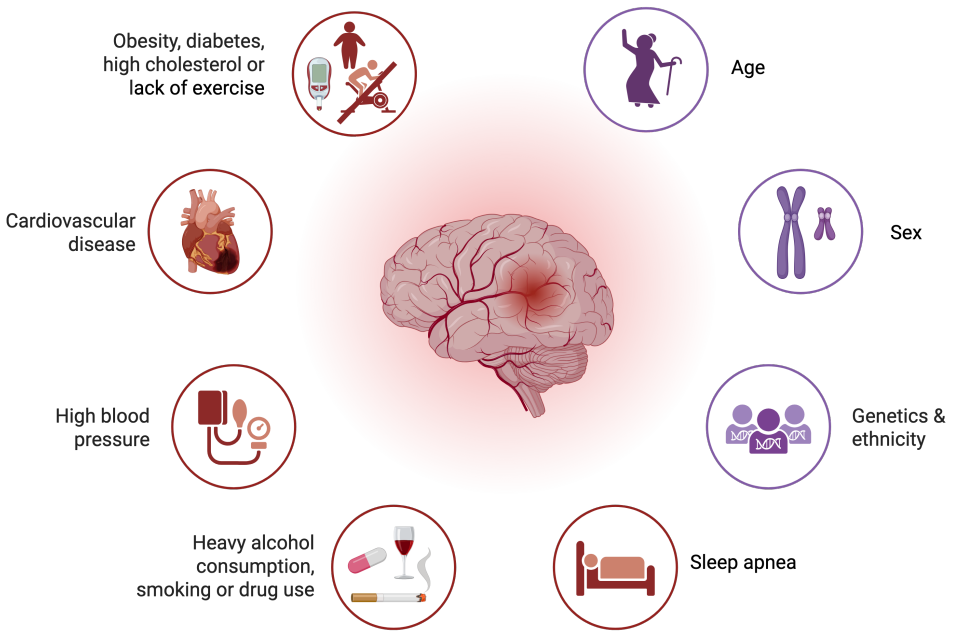


Figure 5 | Overview of major risk factors for stroke. The schematic illustrates key modifiable and non-modifiable risk factors associated with stroke: modifiable factors include obesity, diabetes, high cholesterol, and physical inactivity; cardiovascular disease; hypertension; substance use, such as heavy alcohol consumption, smoking, or drug use; and sleep apnea. Non-modifiable factors include genetic predisposition, ethnicity, sex, and age. Created in BioRender. Tampe, J. (2025) https://BioRender.com/jv8sr41

As the individual risk of a stroke can still vary widely, comprehensive risk assessment, along with lifestyle factors and medical history, is essential before preventive strategies

and interventions are recommended (Yan et al. 2016). Despite progress in awareness and prevention, the global incidence of stroke continues to rise, primarily due to the aging population and increasing incidence of hypertension, obesity, and dyslipidemia (WHO 2017, Feigin et al. 2025).

Symptoms, recovery, and therapeutic gaps

Ischemic stroke symptoms vary depending on the brain region affected and can result in a wide range of neurological impairments. These deficits range from coma and death to visual, speech, and motor deficits. Common clinical presentations of acute stroke include asymmetric facial or limb movements, often accompanied by sudden, severe headache, general weakness, disorientation, memory loss, or nausea and vomiting (Blumenfeld 2010). These latter symptoms have been most common but not exclusively reported in women (Jerath et al. 2011).

Despite advances in acute stroke care, no current interventions can restore lost neurological function. Thrombectomy and thrombolysis aim to restore blood flow to ischemic regions but are limited to a narrow therapeutic window within the first hours after onset and can therefore be applied to only a small fraction of patients (Hurd et al. 2021). However, these treatments do not directly address tissue repair and post-stroke inflammation.

Stroke recovery involves a highly complex cascade of biological responses to ischemic injury. While the infarct core undergoes irreversible cell death, the penumbra retains partially functional tissue that may recover. Due to the high plasticity of the human brain, surviving neurons and neurons in the contralateral hemisphere can compensate for lost functions (Dancause and Nudo 2011). This adaptation is facilitated through synaptogenesis, changes in dendritic ramification and spine morphology, and the modulation of synaptic strength. Recovery involves not only the intrinsic capacity of neurons to reorganize but also the coordinated response of resident immune cells and circulating blood cells (Moskowitz et al. 2010, Ma et al. 2021). Among these, circulating monocytes and their derived macrophages play pivotal roles by modulating inflammation, clearing cellular debris, and supporting tissue remodeling (Jin et al. 2010, Ritzel et al. 2015). Despite growing evidence for the beneficial role of monocytes in post-stroke recovery, translation into clinical interventions remains limited. Most therapeutic strategies have focused on acute reperfusion, whereas immune-targeted approaches to promote long-term functional restoration remain underdeveloped (Jin et al. 2010, Doyle et al. 2015, Wattananit et al. 2016, García-Bonilla et al. 2018). One of the key barriers is the incomplete understanding of how monocyte subsets – classical, intermediate, and non-classical – respond to stroke over time and how their phenotypic transitions are related to neurological outcomes.

This thesis addresses this gap by investigating the dynamic responses of circulating monocytes in stroke patients and identifying temporally regulated changes in cell frequency and gene expression linked to functional impairment and post-stroke recovery. By resolving monocyte behavior at the subset and transcriptional levels, this work provides new insight into how innate immune modulation contributes to stroke repair and how these immune signatures may serve as predictive biomarkers or therapeutic targets. Given that monocytes, not microglia, constitute a central axis of post-stroke neuroinflammation, this thesis advances our understanding of immune plasticity in brain injury and highlights new avenues for immunomodulatory intervention in stroke rehabilitation.

Gaucher's disease

Gaucher's disease is the most common lysosomal storage disorder caused by a deficiency in the glucocerebrosidase (GCase) enzyme due to mutations in the GBA gene. Enzyme deficiency leads to the intracellular accumulation of glucocerebroside and related metabolites within lysosomes, resulting in cellular dysfunction. This accumulation primarily affects the spleen, liver, bone marrow, and, in some cases, the CNS, including early-onset and progressive neurodegenerative phenotypes (Stirnemann et al. 2017). While the somatic manifestations of GD are well characterized, the origin of its neurological symptoms remains poorly understood, posing major challenges to the development of effective neurologically directed therapies.

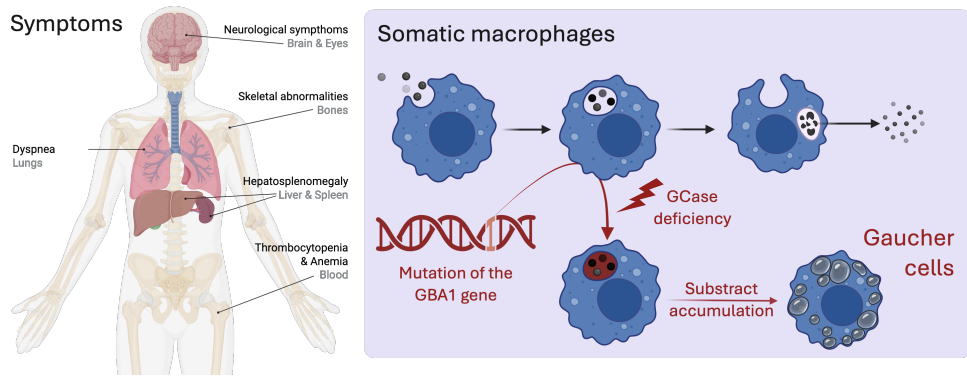


Figure 6 | Pathophysiology and organ involvement in patients with Gaucher disease. (Left) Anatomical illustration of organs affected by Gaucher cells, including the brain and eyes (neuropathies in types 2 and 3), bones and blood, lungs, liver, and spleen. The accumulation of Gaucher cells leads to organ swelling and dysfunction. (Right) Schematic representation of the cellular mechanism underlying GD. A mutation in the GBA gene results in GCase deficiency, causing substrate accumulation within macrophages and their transformation into Gaucher cells. Created in BioRender. Tampe, J. (2025) https://BioRender.com/4pa47mu

Genetics and classifications

Gaucher's disease is inherited in an autosomal recessive manner, requiring both parents to be carriers for their child to inherit mutations in both alleles and subsequently develop the disease. The GD phenotype is rare in the general population, with an estimated prevalence of approximately one in 40,000 to one in 100,000 individuals, and affects men and women equally (Alaei et al. 2019). However, among certain groups, such as Ashkenazi Jews, the prevalence is notably higher, with a carrier frequency of approximately one in 15 to one in 18 individuals. The specific GBA mutation N370S is very prevalent in this group. In Sweden, while not as prevalent as in Ashkenazi Jewish populations, GD still occurs with higher carrier frequencies than in the general population (Conradi et al. 1984). In the northernmost region of Sweden, Norrbotten, there is a unique genetic variant known as the Norrbottnian type of GD, characterized by the L444P missense mutation in the GBA gene (Svennerholm et al. 1982), with a reported prevalence of approximately 1 in 17,500 individuals in this region (Tsitsi et al. 2022). There are three main types of GD, each differing in severity and symptoms:

Type 1 – non-neuronopathic

This type is the most common form, accounting for 95% of all cases, and is highly prevalent in Ashkenazi Jews (1 in 800 - 1,000), who typically lack neurological symptoms, presenting with hepatosplenomegaly, anemia, thrombocytopenia, fatigue, and skeletal complications such as bone pain and pathological fractures.

Type 2 – acute neuronopathy

Type 2 is the most severe and rapidly progressive form, emerging in infancy and prominently featuring severe neurological impairments, including seizures, brainstem dysfunction, feeding difficulties, and profound developmental delays. It typically leads to death by two to three years of age.

Type 3 – chronic neuronopathy

Type 3 is particularly associated with the L444P mutation, is intermediate in severity and presents both systemic and neurological symptoms. Common neurological symptoms include seizures and cognitive decline, ataxia, and skeletal abnormalities, with lifespan varying on the basis of symptom severity (Sidransky and Lopez 2012).

Diagnosis and treatment

The diagnosis of GD typically involves a combination of clinical evaluation, laboratory testing, and genetic analysis. Clinically, healthcare providers assess symptoms such as

hepatosplenomegaly, anemia, thrombocytopenia, and skeletal abnormalities. Laboratory tests confirm the diagnosis by measuring GBA enzyme activity levels in white blood cells or identifying GBA gene mutations through genetic testing (Hughes and Pastores 1993).

Treatment for GD often involves enzyme replacement therapy to supplement the deficient enzyme GBA, along with supportive care to manage symptoms and complications. Medical care involves regular infusions of the deficient enzyme, enzyme replacement therapy (ERT), to help break down accumulated glucocerebroside in cells and tissues. While ERT alleviates somatic symptoms, it cannot cross the blood-brain barrier, leaving the neurological manifestations untreated (Shemesh et al. 2015). In these cases, alternative treatments, such as substrate reduction therapy, which reduces glucocerebroside production, or bone marrow transplantation, which introduces new enzyme-producing cells, potentially provide neurological benefits (Somaraju and Tadepalli 2017).

Early diagnosis, supported by screening and education of carriers, genetic counseling, and close monitoring of at-risk individuals, is crucial for initiating appropriate treatment interventions promptly, as it can help manage symptoms and potentially slow disease progression.

Effect on the mononuclear phagocyte system

In GD, tissue-resident macrophages, which are responsible for clearing cellular debris and foreign substances, are significantly affected. Due to the deficiency of the enzyme GBA, macrophages cannot properly degrade glucocerebroside, a lipid that accumulates within cells (Aflaki et al. 2014). As a result, macrophages accumulate lipid-filled vesicles, becoming enlarged and dysfunctional, which contributes to the enlargement of organs such as the liver and spleen and other systemic manifestations observed in GD (Stirnemann et al. 2017). The accumulation of these swollen cells in various tissues contributes to the many phenotypes of the disorder, impacting not only the immune system but also the skeletal system, blood, and, in some cases, the nervous system.

Recent studies have used iPSC-derived neuronal models to investigate the effects of enzyme deficiency on neurons and their networks (Tiscornia et al. 2013, Schöndorf et al. 2014). Although macrophage-related pathology is well documented in peripheral tissues, the role of the brain's resident immune cells, microglia, has not been studied in human models (Boddupalli et al. 2022). Microglia play crucial roles in maintaining brain homeostasis by scavenging plaques, damaged neurons, synapses, and pathogens. Their phagocytic capacity is highly dependent on functional lysosomes and enzymatic activity, both of which are impaired in individuals with GD.

The development of a human iPSC-derived microglial model from patients with neuronopathic GD represents a critical advance toward understanding the immune mechanisms driving neurodegeneration. Such models enable characterization of disease-specific microglial phenotypes and direct investigation of disease-related microglial lysosomal dysfunction and may serve as platforms for testing CNS-targeted therapies, including gene editing. Ultimately, these tools may support the development of targeted treatments not only for neuronopathic GD but also for other lysosomal and neuroinflammatory disorders with overlapping neurodegenerative features.

Induced pluripotent stem cells

Generation and development

Induced pluripotent stem cells are a type of pluripotent cell generated from somatic cells by reprogramming them into an embryonic-like state. This groundbreaking technology was first established by Shinya Yamanaka, who demonstrated that the introduction of a defined set of transcription factors within adult mouse fibroblasts could induce pluripotency (Takahashi and Yamanaka 2006). Earlier foundational work by John Gurdon demonstrated the feasibility of somatic nuclear transfer, an approach that later culminated in the cloning of the sheep "Dolly" (Gurdon 1962, Wilmut et al. 1997). Together, these discoveries lay the groundwork for the development of iPSC-based models as ethically viable alternatives to embryonic stem cells.

The establishment of long-term human pluripotent cultures was later made possible by advances in maintaining stem cell identity, building on foundational work that demonstrated how to sustain mouse embryonic stem cells in an undifferentiated, pluripotent state and paved the way for culturing pluripotent cells once they had been generated (Smith et al. 1988). Human iPSCs were independently generated by two research groups using combinations of factors such as OCT4, SOX2, KLF4, and c-MYC, with OCT4 and SOX2 later shown to be essential for reprogramming (Takahashi 2007, Wernig et al. 2007, Yu et al. 2007).

Once generated, iPSCs can be differentiated into virtually any somatic cell type, including neural and immune lineages, through the stepwise activation of lineage-specific gene expression programs using small molecules, growth factors, cytokines, and environmental cues such as coculture systems and transcription factors. This capacity underpins their application in disease modeling, drug screening, and regenerative medicine.

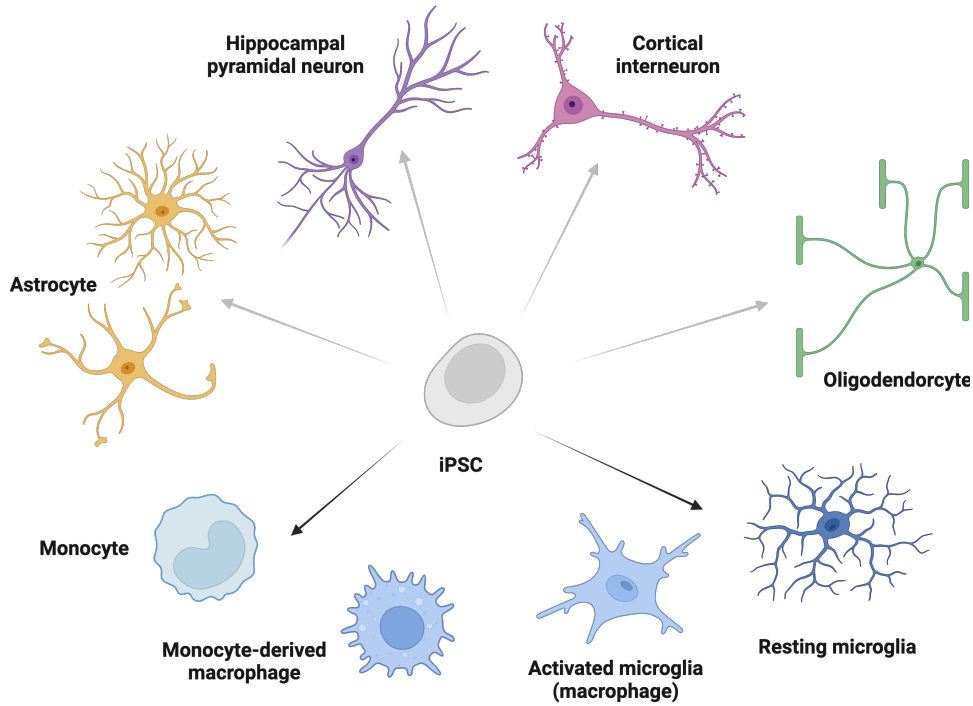


Figure 7 | Differentiation potential of human iPSCs into neural and immune cell types. The schematic illustrates the capacity of iPSCs to differentiate into a range of central nervous system and immune-related cell types. Neural derivatives include hippocampal pyramidal neurons (purple), cortical interneurons (pink), oligodendrocytes (green), and astrocytes (orange). The immune-related lineages included resting and activated microglia, monocytes, and monocyte-derived macrophages (blue). This model system enables the study of cell type-specific functions and interactions in both physiological and pathological contexts. Created in BioRender. Tampe, J. (2025) https://BioRender.com/ysklg0b

Disease modeling

The development of iPSC technology has revolutionized the study of human diseases by enabling the generation of patient-specific cell types that retain the genetic background of the donor. This approach allows for the modeling of both rare monogenic disorders and complex multifactorial conditions in a human, genetically defined context. To model neuronopathic GD, iPSCs can be derived from patient fibroblasts and subsequently differentiated into relevant CNS cell types, such as microglia and neurons. Previous studies have employed a range of reprogramming strategies, including polycistronic DNA cassettes, episomal plasmids, and lentiviral vectors, to generate GD iPSC lines (Tiscornia et al. 2013, Aflaki et al. 2014, Sun et al. 2015). Lentiviral vector systems are widely used because of their efficiency and reproducibility in delivering reprogramming factors, although they carry a risk of genomic integration (Somers et al. 2010, Takahashi et al. 2014).

Once established, patient-specific iPSCs can be directed to differentiate into microglia or cortical-like neurons via established protocols. These iPSC-derived cell types closely recapitulate many of the transcriptional and functional profiles of their *in vivo* counterparts and offer a tractable system to explore disease mechanisms that are otherwise inaccessible in living patients. The iPSC-derived microglia enable the study of lysosomal dysfunction in neuronopathic GD, while coculture with human iPSC-derived neurons (hiNs) from the same genetic background provides a physiologically relevant model for examining neuron-microglia interactions, inflammatory signaling, and neuronal vulnerability.

More broadly, iPSC-based models offer significant advantages for disease research, including the ability to study cell-intrinsic pathophysiology, gene-environment interactions, and patient-specific phenotypes such as protein aggregation, cytokine dysregulation, or neurodegeneration (Li et al. 2018). These platforms also support high-throughput drug screening and the testing of gene-editing technologies such as CRISPR/Cas9 to correct pathogenic mutations (McTague et al. 2021). For translational applications, however, attention must be given to ensuring reproducibility and minimizing inter-line variability to obtain reliable and generalizable results.

Collectively, these advancements highlight the importance of iPSC-derived models in neuroscience and neuroimmunology, offering critical mechanistic insights and translational opportunities, particularly given the limited accessibility of brain tissue.

iPSC-derived microglia

The hiMG is a powerful tool for investigating the role of resident immune cells in neurological disease. These cells closely recapitulate key transcriptional, morphological, and functional features of their *in vivo* counterparts, including a dependency on brain-specific environmental cues for full maturation (Abud et al. 2017). Their development *in vitro* enables mechanistic insights into human microglial biology that are otherwise inaccessible.

In neuronopathic GD, where microglial dysfunction contributes to progressive neurological decline, the specific roles of these cells remain poorly defined. Patient-derived iPSCs offer a personalized platform to generate microglia carrying disease-causing mutations, thus allowing for the study of cell-intrinsic phenotypes in a genetically matched background. To direct iPSCs toward a microglial lineage, transcriptional programming using inducible lentiviral vectors encoding PU.1 and CEBPA has emerged as an effective approach (Chen et al. 2021, Chen and Wong 2023). This approach supports synchronized and reproducible differentiation into cells exhibiting hallmark microglial markers and phenotypes by day 14 of culture.

The resulting hiMG constitutes a relevant model for analyzing GD-associated alterations in inflammatory signaling and lysosomal function. Their use in monoculture and coculture formats enables functional studies under controlled conditions while maintaining genetic specificity. As such, hiMG provide a critical link between genetic etiology and the cellular phenotype in GD-related neuroinflammation.

iPSC-derived neurons and coculture with microglia

To investigate how disease-associated microglial phenotypes influence neuronal integrity, we established a coculture system in which hiMG were paired with excitatory cortical-like neurons. Neurons are generated through inducible expression of the transcription factor NGN2, which allows rapid and uniform neuronal differentiation from human iPSCs. This strategy yields a population of functionally active neurons suitable for modeling neuron-glia interactions.

To promote neuronal maturation after five days of induction, neurotrophic factors were added to the culture medium. Moreover, microglia that had also been induced for five days were introduced. Coculture of microglia and neurons derived from the same iPSC donor enables a physiologically relevant system that mimics aspects of the brain's cellular microenvironment and permits the study of autologous cellular interactions. Within this platform, hiMG appear to adopt more mature morphologies compared to monoculture, reflecting the supportive influence of neuronal cues during maturation (Muffat et al. 2016).

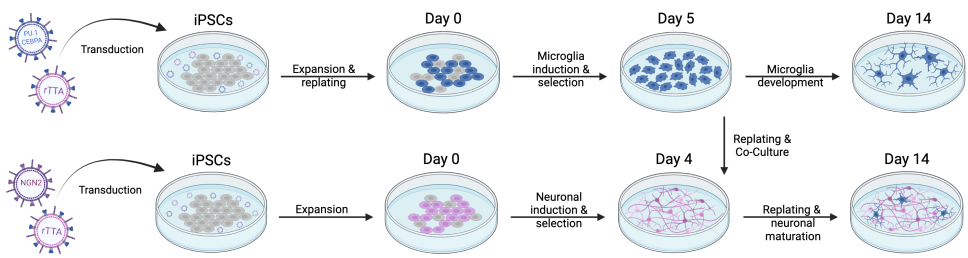


Figure 8 | Differentiation workflows for generating microglia and neurons from human iPSCs. The schematic shows parallel 14-day protocols for generating microglia (top) and neurons (bottom) from iPSCs. The key steps include transduction, induction, selection, and maturation, enabling the production of functionally relevant cell types for *in vitro* modeling. Created in BioRender. Tampe, J. (2025) https://BioRender.com/99o990n

Importantly, this model allows for detailed interrogation of how patient-derived microglia affect neuronal health, synaptic function, and inflammatory tone. In the case of GD, it provides a unique opportunity to study the interplay between genetic lysosomal dysfunction and neuroinflammation in a human genetic context. Moreover,

it serves as a preclinical system for evaluating potential therapeutic strategies, including gene editing and anti-inflammatory interventions, in a genetically defined and human-specific context.

Ethical considerations

The 3Rs concept

The 3Rs, replacement, reduction, and refinement, provide a comprehensive framework to minimize animal use and suffering while ensuring high-quality, ethically sound, and translationally relevant research. Replacement prioritizes non-animal methods, such as the use of human iPSCs to model diseases and drug responses, thereby reducing reliance on animal models and improving translational relevance (**Paper III**). Reduction seeks to lower the number of animals used by integrating results from preclinical studies with human data through meta-analyses and cross-species validation (**Papers I and II**). Refinement focuses on improving experimental protocols to reduce pain, stress, and distress in animal subjects, thereby enhancing animal welfare and scientific outcomes (**Paper III**).

Clinical samples from human donors

In **Papers I and II**, primary blood samples from human donors were used to characterize immune cell populations and transcriptional signatures in the context of aging, biological sex, and ischemic stroke. These studies were designed to investigate the biological variability of circulating monocytes under physiological and pathological conditions, with the goal of ensuring clinical applicability and building on insights gained from prior experimental models.

All procedures involving human subjects were conducted in accordance with the Declaration of Helsinki and approved by the Regional Ethical Review Board (Dnr 2016/179; 2017/357; 2017/879). Written informed consent was obtained from all participants prior to sample collection, and protocols were implemented to ensure compliance with ethical and legal standards regarding confidentiality, autonomy, and the right to withdraw. To ease participation and minimize burden, sample collection was coordinated by specifically trained research staff at the hospital or arranged at local health care centers. Patient confidentiality and anonymity were maintained at all stages, with strict adherence to data protection regulations.

Control samples were obtained from healthy individuals matched for age and sex to the stroke cohort, enabling controlled comparisons across experimental groups. The use of

human-derived clinical material in these studies was essential to establish the immunological relevance of peripheral monocytes in aging and stroke. By integrating high-quality clinical sampling with rigorous ethical oversight, the studies aim to generate findings with direct translational value while adhering to the highest standards of ethical responsibility and participant care.

Patient-derived iPSC lines

The use of patient-derived iPSC lines obtained from the Coriell Institute of Medical Research was conducted in accordance with established ethical guidelines and regulatory standards. All the cell lines were deidentified and provided with informed consent for research use. This approach minimizes the need for direct patient involvement and reduces reliance on animal models, supporting ethical and human-relevant translational research practices.

Aim of the thesis

This thesis aims to explore how monocytes and microglia contribute to neuroinflammation across three distinct yet interrelated contexts: aging, ischemic stroke, and neuronopathic GD. Special attention is given to how biological sex, monocyte subset identity, and the inflammatory state shape peripheral immune responses, alongside CNS-specific insights from microglial dysfunction in GD.

- **Paper I** characterizes age- and sex-dependent changes in the distribution of human monocyte subtypes - classical, intermediate, and non-classical – focusing on neuroinflammation and regeneration-related gene expression, with an emphasis on aging-related immune remodeling and sex-specific anti-inflammatory signatures.
- **Paper II** investigates monocytes and their subtype frequency dynamics during stroke recovery, identifying temporal patterns and gene expression profiles associated with clinical outcome.
- **Paper III** develops a patient-specific iPSC-derived microglia model to characterize inflammatory and lysosomal defects in GD and introduces a microglia–neuron coculture system to explore disease mechanisms and therapeutic strategies.

Together, these studies demonstrate how innate immune cells, both circulating monocytes and CNS-resident microglia, contribute to context-specific yet overlapping neuroinflammatory responses across aging, ischemic stroke and GD. By characterizing transcriptional programs, monocyte subset dynamics and functional impairment under distinct biological conditions, this work highlights how monocyte and microglia responses are contextually regulated and, in the case of ischemic stroke, identifies candidate biomarkers of recovery. These findings support the future development of targeted therapeutic strategies aimed at modulating innate immune function in both acute and chronic neurological diseases.

Summary of key results

This chapter highlights the key findings from three studies that collectively shed more light on neuroinflammation, focusing on how peripheral and CNS-resident immune cells contribute to pathological or reparative processes. Both monocytes and microglia are dynamic players in the regulation of neuroinflammation, and their responses are shaped by age, sex, and disease context. Through complementary techniques, including flow cytometry, gene expression profiling, and iPSC-based modeling, these studies aimed to provide a comparative view of immune aging and disease-specific alterations in monocyte and microglial phenotypes. Together, these genes may serve as both biomarkers and therapeutic targets in neuroimmune disorders.

Paper I: Human monocyte subtype expression of neuroinflammation- and regeneration-related genes is linked to age and sex

In this study, we investigated how aging and biological sex influence the distribution and gene expression profiles of peripheral blood monocyte subtypes in healthy individuals. Monocytes are key regulators of inflammation and tissue repair, and their phenotypic plasticity is increasingly recognized as a contributor to age-related immune dysfunction and disease susceptibility. While prior studies have described age-associated changes in immune cell function, the impact of aging and sex on monocyte subset-specific expression of neuroinflammation- and regeneration-related genes remains poorly understood.

To address this gap, we investigated how aging and biological sex influence the distribution and gene expression profiles of peripheral blood monocyte subtypes in healthy individuals. We hypothesized that age and sex shape monocyte phenotypes in ways relevant to neuroinflammation and tissue repair.

PBMCs were collected from 44 healthy adult donors (26 males, 18 females) aged 28 to 98 years. Flow cytometry was used to quantify immune cell populations and isolate monocyte subtypes (classical, intermediate, and non-classical) based on the expression

of CD14 and CD16. Monocyte subsets were assessed for the expression of 40 genes associated with neuroinflammation and tissue regeneration.

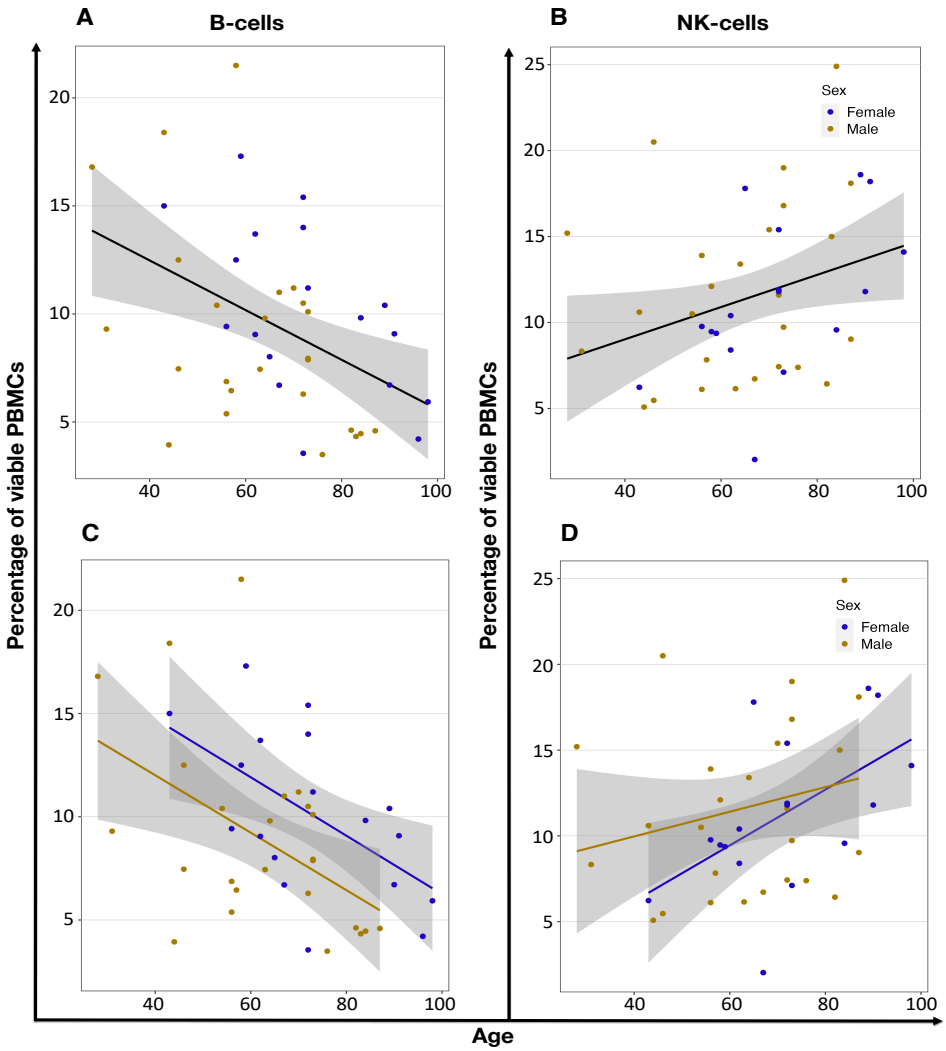


Figure 9 | Age-related correlation of PBMC ratios. Only cells/groups with significant p-values are shown. (A) Shows the decrease (r=-0.44) in the percentage of B-cells and (B) an increase (r=0.31) in the percentage of NK-cells among viable PBMCs. (C) Analysis stratified by sex revealed a decreased B-cell ratio and (D) increased (rf=0.54, P=0.025) proportion of female NK-cells among viable PBMCs from female (rf=-0.56, P=0.016) and male (rm=-0.49, P=0.013) donors. Each dot represents an individual donor, with orange dots indicating males and blue dots indicating females. The black, orange (for males), and blue (for females) lines represent linear regression, and the gray area represents the 95% confidence interval.

Linear regression analysis of blood mononuclear cell subset frequencies revealed that the overall proportions of total monocytes and monocyte subtypes remained relatively stable with age. However, significant shifts were observed in lymphocyte populations. Specifically, we found an age-associated decline in B-cell frequencies, which was consistent across both sexes. In contrast, the proportion of NK cells increased with age, but this trend reached significance only in females, highlighting the sex-specific effects of immune aging. These findings highlight sex-specific patterns of immune aging that could underlie differences in immune function.

Age-associated monocyte gene expression dynamics

Using Fluidigm-based multiplex qPCR, we identified age-associated changes in the expression of genes related to neuroinflammation and repair in all monocytes and their three subtypes. Analysis of gene expression in all monocytes revealed that aging was associated with the upregulation of ANXA1 and CD36.

The pro-inflammatory CD36 gene expression increased with age across all monocytes, suggesting a shift toward a more pro-inflammatory state. This increase may reflect a heightened basal inflammatory state or impaired resolution capacity. The concomitant rise in ANXA1 expression may partially offset this increase.

The anti-inflammatory gene ANXA1, which encodes annexin A1, was significantly upregulated with age, not only in all monocytes but also in intermediate and non-classical monocytes. Females largely drove this effect. Its increased expression with age suggests a shift toward resolution-promoting functions, potentially as a compensatory mechanism for chronic inflammation.

Further subtype analysis revealed that classical monocytes exhibited age-associated downregulation of the pro-inflammatory genes TLR8 and S100A8. Intermediate monocytes exhibited increased levels of the anti-inflammatory factor TGFβ and decreased levels of the pro-inflammatory factors TNFα and TLR8. In non-classical monocytes, CD91, another gene implicated in immune regulation, was upregulated, whereas TLR8 was downregulated. These findings indicate a complex, subtype-specific modulation of monocyte neuroinflammatory gene expression during aging, with a notable predominance of shifts in females. This highlights the importance of cell subtype-specific analyses, as pooled monocyte measurements may obscure subtype-specific dynamics.

Table 1 | Differentially expressed genes associated with age. All genes whose expression levels significantly changed in primary human monocytes and their subtypes associated with age via Pearson correlation (P-value < 0.05). An upward arrow (↑) indicates increased gene expression, whereas a downward arrow (↓) indicates decreased gene expression. The regression coefficient and its corresponding 95% confidence interval quantify the relative increase in gene expression with each year of age.

Subtype	Gene	Function	p-value	N		r	r (95% CI)	
All	CD36	Pro-inflammatory, scavenger receptor	0.042	44	↑	0.0019	0.0001	0.0038
All	ANXA1	Anti-inflammatory	0.012	43	↑	0.0116	0.0027	0.0206
Classical	TLR8	Pro-inflammatory	0.025	44	↓	- 0.0001	- 0.0003	- 1.8843
Classical	S100A8	Pro-inflammatory	0.022	44	↓	- 0.0317	- 0.0587	- 0.0047
Intermediate	TNFα	Pro-inflammatory	0.014	43	↓	- 0.0001	- 0.0002	- 0.0000
Intermediate	TLR8	Pro-inflammatory	0.008	42	↓	- 0.0002	- 0.0003	- 0.0001
Intermediate	ANXA1	Anti-inflammatory	0.026	44	↑	0.0075	0.0009	0.0141
Intermediate	TGFβ	Anti-inflammatory	0.033	44	↑	0.0163	0.0014	0.0312
Non-classical	TLR8	Pro-inflammatory	0.014	44	↓	- 0.0001	- 0.0002	- 0.0001
Non-classical	ANXA1	Anti-inflammatory	0.004	44	↑	0.0081	0.0028	0.0134
Non-classical	CD91	Immune modulator	0.031	44	↑	0.0001	0.0000	0.0002

Sex-specific gene expression changes with aging

Transcriptomic profiling of female classical monocytes revealed a significant decline in the expression of pro-inflammatory genes. In classical monocytes, decreased expression of TNFα and IL-1β, two key pro-inflammatory cytokines implicated in neurodegeneration and systemic inflammation, was revealed with age. Intermediate monocytes presented decreased expression of TLR8 and TNFα, accompanied by upregulation of TGFβ, which further supports an anti-inflammatory shift. The gene TSPO, which encodes a mitochondrial membrane protein linked to microglial activation and brain inflammation, was upregulated in female non-classical monocytes with age. The adhesion molecule CD91 was found to be upregulated in intermediate and non-classical monocytes, exclusively in female donors. These transcriptional changes did not mirror those in males, suggesting a sex-dependent rewiring of monocyte function with age.

In male non-classical monocytes, aging is associated with significant downregulation of TLR8, a pattern recognition receptor critical for sensing viral RNA and promoting antiviral immunity. Two additional genes, CD33 and MARCO, also showed significant age-associated downregulation in intermediate and classical monocytes, respectively. Overall, age-related transcriptional changes were both gene- and subtype-specific and were often more pronounced in female donors.

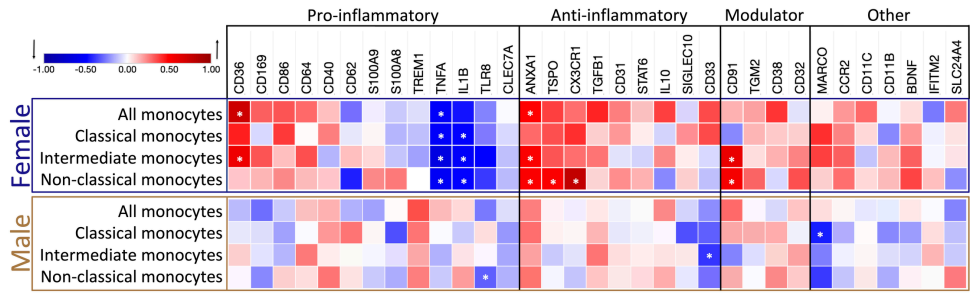


Figure 10 | Heatmap of the relationships between age and gene expression in the different monocyte subtypes, separated by biological sex. The strength of the association of gene expression with age is represented by the Pearson correlation coefficient (r), ranging from -1, a perfect, negative correlation (blue); 0, no correlation; and 1, a perfect, positive correlation (red) of gene expression relative to ACTβ expression with increasing age. Genes are grouped by their most prevalent inflammatory function. Modulators have been linked to both pro- and anti-inflammatory functions. The female correlations are framed in blue (N=18), and the male correlations are framed in orange (N=26). Significant genes, defined by a p-value less than 0.05, are marked with an asterisk (*).

Conclusion

Together, our findings suggest that aging triggers coordinated transcriptional shifts in human peripheral monocytes, which exhibit clear subtype-specific and sex-dependent patterns. In all monocytes, the expression levels of CD36 and ANXA1 increased with age. While classical monocytes exhibited modest changes, intermediate and non-classical subsets presented dynamic transcriptional alterations. Subtype-specific analysis revealed that ANXA1 was also upregulated in intermediate and non-classical monocytes, particularly in females. In contrast, CD36 was upregulated with age across all monocyte subsets. Female monocytes exhibited a broader and more pronounced transcriptional response to aging, including the downregulation of pro-inflammatory genes such as TNFα, IL-1β, and TLR8 and the upregulation of anti-inflammatory or regulatory genes, including ANXA1, TGFβ, CD91, and TSPO. In contrast, male monocytes presented fewer significant changes, with notable downregulation of TLR8 in non-classical monocytes. These results demonstrate that age-related changes in gene expression in monocytes are both gene- and subset-specific, with more extensive alterations observed in female donors.

Paper II: Temporal dynamics of monocyte subtypes and gene expression reveal predictive immune signatures of ischemic stroke recovery

Building upon the findings of Paper I, which demonstrated that aging and biological sex distinctly modulate monocyte subset phenotypes and inflammatory gene expression, we further investigated in **Paper II** how these factors affect immune responses to acute brain injury. Stroke is a leading cause of disability and mortality worldwide, with immune responses playing a dual role: contributing to early tissue damage while also supporting neurorepair. Given the age-associated shifts in the monocyte phenotype observed in healthy individuals, understanding how these baseline immune characteristics may influence or predict post-stroke outcomes is essential.

In this study, we investigated longitudinal changes in monocyte subset frequencies and gene expression after ischemic stroke. We hypothesized that both the frequency and molecular profile of monocytes would correlate with functional recovery and that sex differences may influence immune trajectories.

We collected PBMCs from patients with confirmed ischemic stroke at three time points: 24 hours, 3-5 days, three months and 1 year post-stroke. Patients were stratified by sex and recovery, defined by changes in the NIHSS score from baseline to three months. Flow cytometry and multiplex RT-qPCR were used to assess monocyte subset distribution and gene expression.

Dynamic changes in monocyte subtypes after stroke are linked to sex and recovery

Flow cytometry analysis of monocytes and their subtypes revealed that the total monocyte frequency significantly increased at 24 hours and 3-5 days post-stroke. This response was consistent in male patients and those with favorable recovery outcomes. By three months, monocyte levels returned to baseline in most patients but remained significantly elevated in the recovery groups. Classical monocytes decreased at 24 hours in males and recovery patients and further declined at three months in the no recovery group. Intermediate monocytes increased at 3-5 days in patients who later recovered, especially in males. Non-classical monocytes did not show consistent changes overall, but were reduced in female patients and those with no recovery. These findings demonstrate that monocyte subtype distributions are dynamically regulated following stroke and are influenced by biological sex and recovery status, with early shifts in classical and intermediate monocytes potentially serving as indicators of functional outcome.

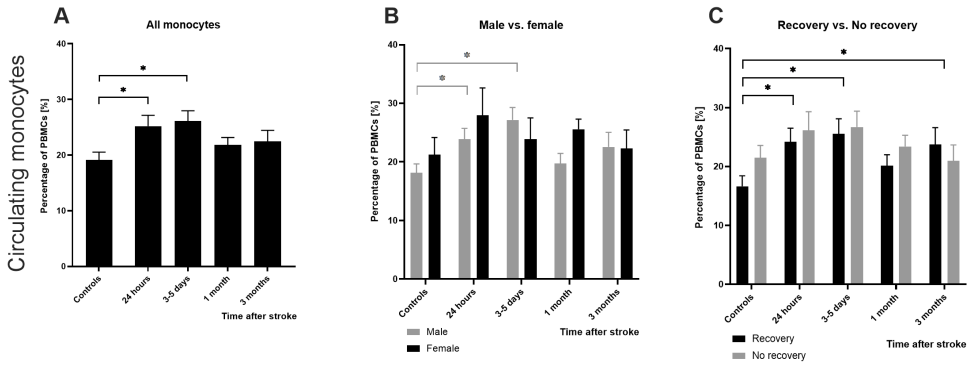


Figure 11: Temporal changes in peripheral monocytes after ischemic stroke. (A) The proportion of all circulating monocytes within isolated, viable PBMCs was compared to age- and sex-matched controls at acute (24 h), sub-acute (3–5 days), and chronic (3 months) timepoints after stroke (N=37). (B) The percentage of all monocytes (same time points), split by biological sex, is compared (N(Male)=25; N(Female)=12). (C) The proportion of all monocytes (same time points), split by the occurrence of recovery (defined by the NIHSS score in the chronic phase (N(Recovery)18; N(No recovery)=19). Peripheral monocyte frequencies are presented as a percentage of all viable PBMCs. The data are shown as the means ± SEMs, were compared by multiple unpaired t-tests (Wilcoxon), and were generated using PRISM software.

Temporal dynamics of gene expression are linked to outcome

To investigate the relationship between peripheral monocyte neuroinflammatory gene expression and neurological recovery, we analyzed the mRNA expression profiles of monocyte subtypes in relation to the NIHSS score at three stages post-stroke: acute (<24 hours), subacute (3-5 days), and chronic (3 months). Across all monocytes, early expression of the inflammation-associated gene TSPO was strongly negatively correlated with the NIHSS score, suggesting a link to early neurological improvement. Additionally, after 3-5 days, the expression of CD86 and IL-1β was similarly associated with better outcomes. In contrast, at 3 months, elevated CCR2 and STAT6 expression correlated with persistent neurological impairment, whereas downregulation of SLC24A4 was associated with improved function.

Monocyte subtype analysis revealed temporally distinct expression profiles, with TSPO expression remaining strongly associated with early improvement in classical monocytes. At the same time, 3-month upregulation of CCR2 and downregulation of SLC24A4 and CD91 suggested a shift toward persistent inflammation or recovery-related remodeling. Intermediate monocytes showed a subacute reduction in CD33 expression and chronic upregulation of CCR2 and CD11b in individuals with greater deficits, indicating subtype-specific inflammatory reprogramming. Non-classical monocytes exhibited acutely increased TLR8 expression, whereas reduced CD40 levels were observed in more impaired patients at 3-5 days post-stroke. At 3 months, negative correlations were detected between the NIHSS score and the expression of CD91, IL-

10, and SLC24A4, suggesting the involvement of anti-inflammatory and regenerative pathways in favorable recovery trajectories.

Together, these findings underscore the contributions of dynamic and subtype-specific monocytes to stroke outcome, highlighting transcriptional programs linked to both persistent inflammation and tissue repair.

Table 2 | Differentially expressed genes associated with higher NIHSS scores at different time points post-stroke (PS). This table presents genes whose expression levels in primary human monocytes and their subtypes are significantly correlated with NIHSS scores, based on Pearson correlation analysis (P-value < 0.05). An upward arrow (↑) denotes increased gene expression, while a downward arrow (↓) indicates decreased expression with increasing NIHSS score.

Gene	Function	Subtype	Time PS	p-value		r
TSPO	Anti-inflammatory	all	24 h	0.004	↓	-0.106
TSPO	Anti-inflammatory	classical	24 h	0.003	↓	-0.095
TLR8	Pro-inflammatory	non-classical	24 h	0.032	↑	0.060
CD86	Pro-inflammatory	all	3-5 d	0.010	↓	-0.089
IL-1β	Pro-inflammatory	all	3-5 d	0.018	↓	-0.105
CD33	Anti-inflammatory	intermediate	3-5 d	0.027	↓	-0.064
CD40	Pro-inflammatory	non-classical	3-5 d	0.022	↓	-0.083
22CCR2	Monocyte infiltration	all	3 mo	0.002	↑	0.360
SLC24A4	cellular calcium ion homeostasis	all	3 mo	0.049	↓	-0.255
STAT6	Anti-inflammatory	all	3 mo	0.021	↑	0.306
CCR2	Monocyte infiltration	classical	3 mo	0.010	↑	0.334
CD91	Immune modulator	classical	3 mo	0.049	↓	-0.372
SLC24A4	cellular calcium ion homeostasis	classical	3 mo	0.044	↓	-0.199
CCR2	Monocyte infiltration	intermediate	3 mo	0.049	↑	0.362
CD11b	Monocyte adhesion and migration	intermediate	3 mo	0.022	↑	0.266
CD91	Immune modulator	non-classical	3 mo	0.031	↓	-0.478
IL-10	Anti-inflammatory	non-classical	3 mo	0.035	↓	-0.376
SLC24A4	cellular calcium ion homeostasis	non-classical	3 mo	0.006	↓	-0.509

Monocytic gene expression predicts stroke recovery

To identify predictors of long-term neurological recovery, we performed gene expression analysis of peripheral monocyte subsets at the acute (24 hours) and subacute (3-5 days) stages after stroke, the results of which were correlated with the functional recovery (recovered NIHSS score at 3 months). At 24 hours, recovery was associated with downregulation of CD11c in all monocytes, including classical and intermediate monocytes, and with reduced expression of TGFβ and CD32 in all monocytes, as well as CD169 in intermediate monocytes. In contrast, the upregulation of TGM2 and CD36 in intermediate monocytes and of CD91 across all monocyte subsets, as well as classical and non-classical subsets, was positively associated with recovery.

At 3-5 days, improved recovery was correlated with all monocytes, as well as their subsets, with the downregulation of CCR2, except non-classical, and CX3CR1, except intermediate. Additionally, decreased CD11c expression in classical monocytes and increased IL-10 expression in non-classical monocytes were associated with recovery at 3 months post-stroke. Conversely, increased expression of IFITM2 in all monocytes, SLC24A4 in classical monocytes, CD38 in classical and intermediate subsets, and CD91 in non-classical monocytes was associated with improved NIHSS scores. These findings suggest that distinct, time- and subset-specific immune signatures in monocytes can serve as early biomarkers of stroke recovery, with potential utility for prognosis and individualized treatment planning.

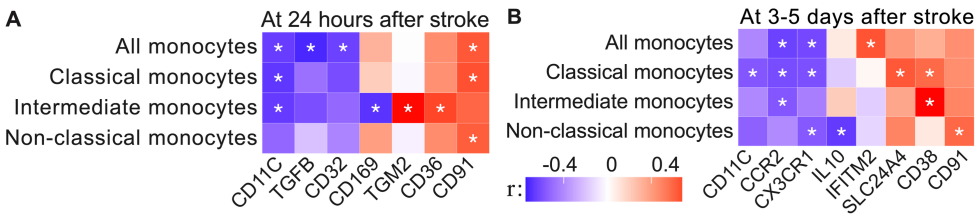


Figure 12: Gene expression correlated with recovery after three months. Heatmap of predictive gene expression for the recovery of NIHSS score at three months. The strength of the association of (A) acute (24 hours) and (B) sub-acute (3-5 days) gene expression with relative recovery by three months post-stroke is represented by the Pearson correlation coefficient (r), which ranges from -1 (perfect, negative correlation; blue), 0 (no correlation), and 1 (perfect, positive correlation; red). The relative recovery was calculated as the change in the NIHSS score between stroke onset and three months post-stroke, normalized to the NIHSS score at stroke onset (0 indicates no recovery, and 1 indicates full recovery). Significant genes, defined by a p-value less than 0.05, are marked with an asterisk (*).

Conclusion

The dynamics and transcriptional profiles of peripheral monocyte subtypes vary following ischemic stroke. A timely shift from pro-inflammatory to reparative phenotypes was observed, particularly in intermediate and non-classical subsets, and was associated with improved functional outcomes. These phenotypic transitions differ by sex, with distinct monocyte responses observed in male and female patients.

Together, our findings demonstrate that stroke induces coordinated, time-dependent changes in monocyte subset frequencies and gene expression, with specific transcriptional signatures associated with recovery. CD91, CD36, TGM2, and SLC24A4 emerged as key genes associated with favorable outcomes, while persistent expression of CCR2 and downregulation of CD91 and SLC24A4 were linked to adverse outcomes. When integrated with findings from **Paper I**, these results highlight that age- and sex-related differences in baseline monocyte phenotypes are reflected in post-stroke immune responses and contribute to variability in recovery.

Paper III: Human iPSC-derived microglia reveal inflammatory and lysosomal vulnerabilities in Gaucher disease

While the first two studies focused on peripheral monocytes in aging and stroke, we next sought to investigate the role of their CNS-resident counterparts, microglia, in the context of chronic neurodegeneration. Specifically, we modeled neuronopathic GD, a lysosomal storage disorder caused by mutations in the GBA1 gene that results in GCase deficiency. Microglial dysfunction and neuroinflammation are increasingly recognized contributors to the neuropathology of GD; however, human-relevant cellular models are scarce and do not adequately address microglial contributions.

In this study, we investigated the inflammatory and lysosomal phenotypes of iPSC-derived microglia generated from a patient with type II GD and a sex-matched healthy control. We hypothesized that disease-associated mutations in GBA1 would alter microglial immune responses and lysosomal function, particularly under inflammatory stimulation.

To address this, we established a novel iPSC-derived microglia model using patient-derived and sex-matched control iPSCs. This system enabled the investigation of intrinsic microglial impairment in cytokine signaling and lysosomal homeostasis in GD, supporting the development of a microglia–neuron coculture platform to study intercellular dynamics relevant to neurodegeneration.

Establishing a human iPSC-derived microglia model of GD

The iPSC lines were generated from the dermal fibroblasts of GD patients using non-integrating mRNA-based reprogramming, ensuring genomic integrity and avoiding insertional mutagenesis. All cell lines maintained a normal karyotype and expressed key pluripotency markers, confirming successful reprogramming. To generate microglia-like cells, we employed a transcription factor–driven differentiation protocol using inducible PU.1 and CEBPA expression (Chen and Wong 2023). After 14 days, both the GD and control iPSC lines yielded cells with characteristic microglial morphology and robust expression of canonical markers, including CD11b, CX3CR1, and TREM2, as confirmed by flow cytometry. No significant differences in marker expression were observed between GD and control hiMG, indicating that GD-associated mutations do not impair microglial lineage commitment. These results validated the generation of a human iPSC-derived microglial model suitable for downstream functional and disease-specific analyses.

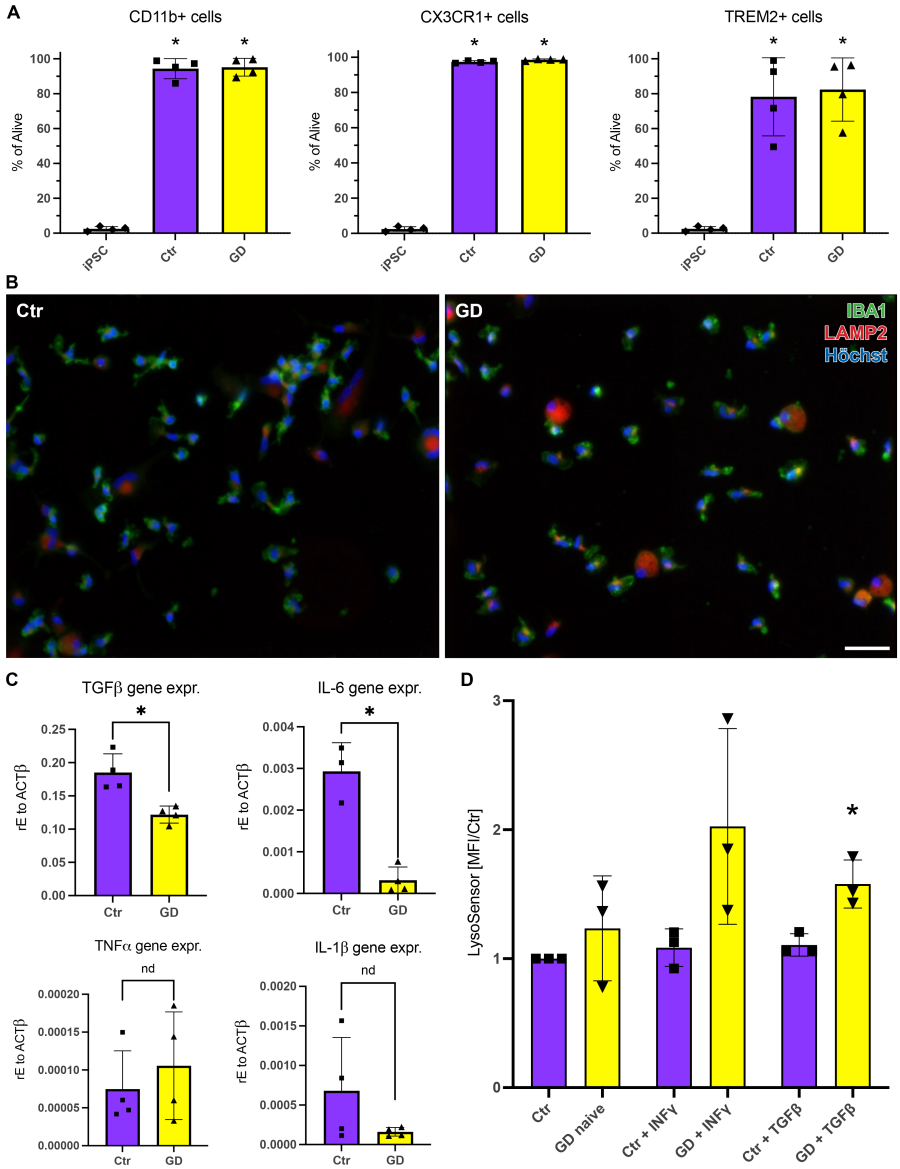


Figure 13 | Characterization of the generated hiMG and GD phenotypes. (A) Flow cytometry analysis quantifying the frequency of CD11b-, CX3CR1-, and TREM2-positive cells, presented as a percentage of total, Draq7-negative viable cells. The following antibody conjugates were used: CD11b (PE-Cy5), CX3CR1 (BV605), and TREM2 (APC). Purple, induced microglia from healthy controls; yellow, induced microglia from GD iPSCs; gray, undifferentiated iPSCs. (B) ICC analysis of the microglia-specific marker IBA1 and the lysosomal-specific marker LAMP2, which are representative of four independent differentiation experiments for both control and GD-derived hiMG (scale bar = 75 µm). (C) Gene expression analysis of TGFβ, IL-6, TNFα, and IL-1β in naïve hiMG to assess differences in cytokine production. (D) Flow cytometry analysis of viable (DraQ7-) induced microglia showing the mean fluorescence intensity (MFI) of the pH-sensitive lysosomal dye LysoSensor Green DND-189. The MFI is presented as a ratio relative to the baseline (naïve control hiMG) for each of the four independent rounds of differentiation. The data are shown as the means ± SEMs and were generated using the software PRISM. A p-value of less than 0.05 was considered significant (*).

Impaired cytokine signaling and lysosomal response in GD microglia

To characterize the disease-specific phenotype of GD microglia, we assessed both inflammatory signaling and lysosomal function. Under basal conditions, GD-hiMG exhibited significantly reduced gene expression of the cytokines TGFβ and IL-6 under naïve conditions. Lysosomal acidity, measured using LysoSensor Green DND-189, was comparable between GD and control hiMG at baseline. However, upon stimulation with TGFβ, GD-hiMG displayed a marked increase in lysosomal acidification. This condition-specific response suggests that lysosomal dysregulation in GD-hiMG is latent under resting conditions but becomes apparent in response to anti-inflammatory challenge. Together, these findings define a distinct microglial phenotype in GD, characterized by impaired cytokine signaling and context-dependent lysosomal dysregulation, which may contribute to the progression of neuroinflammation in this disease.

Establishment of a microglia-neuron coculture platform

To model microglia-neuron interactions in GD, we established a coculture system using independently generated hiMG and hiNs. Microglia were seeded onto neuronal cultures at a physiologically relevant ratio and maintained for 14 days. Both the GD and control hiMG retained their typical morphology within the coculture environment and were observed in close proximity to neurons, suggesting the potential for direct cell-cell interactions. The stable integration of both cell types supports the feasibility of this platform for studying genotype-dependent effects on neuroimmune dynamics. These findings provide a foundation for future investigations into microglia-mediated neuronal dysfunction in GD.

Conclusion

This study demonstrated that GD microglia exhibit intrinsic defects in inflammatory regulation and lysosomal function that become evident upon exposure to immune stress. Our results suggest that restoring lysosomal integrity and immune restraint in microglia may mitigate neurodegeneration in patients with Gaucher syndrome and related disorders, suggesting a potential path toward disease-modifying therapies. These findings align with the overarching theme of this thesis: the adaptability of monocytes and microglia in response to aging, injury, and disease. Unlike stroke or aging, where systemic factors predominate, the Gaucher model highlights cell-autonomous dysfunction in microglia, the CNS-resident macrophages.

Discussion and future perspectives

The studies presented in this thesis focus on the premise that monocytes and microglia, both cells of the macrophage lineage, exhibit marked context-dependent plasticity in orchestrating neuroinflammatory responses. Rather than functioning as static effectors, these cells undergo dynamic phenotypic and transcriptional shifts in response to temporal, biological, and disease-specific cues. Their roles are not uniformly pro- or anti-inflammatory; instead, they adapt according to the nature and timing of the immune challenge, whether driven by age-related immune reprogramming, ischemic injury, or inherited enzyme dysfunction.

This plasticity is particularly evident following ischemic stroke, where coordinated changes in monocyte subset function suggest a role in modulating recovery trajectories. In Gaucher disease, altered microglial responses to immune stimuli point to a chronic, dysregulated inflammatory milieu that may reflect impaired resolution rather than overt activation. Across these conditions, a recurring theme is the importance of immune resolution, not simply the attenuation of inflammation but also the active transition toward tissue repair and homeostasis. These transitions appear to be governed by both intrinsic determinants, such as sex-biased gene expression, and extrinsic signals, including cytokine gradients and cellular stress.

Collectively, these findings emphasize the need to evaluate the adequacy of binary models of neuroinflammation that categorize immune responses as either destructive or protective. Instead, they point to a spectrum of responses influenced by context and timing. They support a framework where timing, the cellular environment, and systemic interactions determine the functional outcome. Addressing such complexity requires integrative methodologies, combining immunophenotyping, transcriptional profiling, and *in vitro* disease modeling to be capable of capturing the multifaceted nature of macrophage responses to stresses in the brain. Macrophage plasticity in neuroinflammation

Monocytes and microglia represent highly plastic immune populations that can rapidly shift their functional phenotype in response to systemic or local cues. Across aging, stroke, and Gaucher disease, these cells adopt distinct phenotypic and functional states shaped by internal programming and external stimuli. The studies presented in this thesis underscore how monocyte and microglia plasticity manifests in context-specific ways and how this adaptability holds both reparative and pathological potential.

Paper I demonstrates that, during aging, circulating monocytes undergo progressive transcriptional remodeling, particularly within the intermediate and non-classical subsets. Age-related upregulation of ANXA1, an anti-inflammatory gene involved in the resolution of inflammation, in all monocytes was primarily confined to these two subsets (Perretti and D'Acquisto 2009, Xu et al. 2021). The increased expression of CD36 across all monocyte populations suggests heightened activation (Cho et al. 2005). However, classical monocytes, which are generally considered the most pro-inflammatory subset, exhibit downregulation of TLR8 and S100A8, suggesting a potential dampening of inflammatory responsiveness with age (Swindell et al. 2013, Wang et al. 2018). The intermediate subset also displayed reduced expression of pro-inflammatory TLR8 and TNFα, while the expression of the anti-inflammatory genes TGFβ and ANXA1 increased alongside. Non-classical monocytes upregulated CD91 and ANXA1 while downregulating TLR8, indicating a shift toward reparative or regulatory phenotypes in aging individuals. These changes were particularly pronounced in females, suggesting that hormonal or genetic factors may modulate immune plasticity post-menopause (Abildgaard et al. 2020, Ryczkowska et al. 2023). This anti-inflammatory trend is further supported by the coordinated downregulation of IL-1β and TNFα in aging monocytes, particularly in classical and intermediate subsets, suggesting a compensatory shift toward immune resolution in the face of chronic low-grade inflammation (De Francesco et al. 2020, Xu et al. 2021).

These results highlight the need to assess monocyte biology in a subset-specific manner, as opposing functions may coexist within the total monocyte pool (Metcalf et al. 2017).

Building on the findings of Paper I, **Paper II** explores how, following ischemic stroke, monocyte plasticity becomes temporally and functionally dynamic. In the acute phase, classical monocytes are rapidly mobilized, likely contributing to initial injury via pro-inflammatory cytokine production (Ritzel et al. 2015). As recovery progresses, their numbers decline, especially in patients with better outcomes, suggesting a resolution of acute inflammation or a shift in subset balance (Kaito et al. 2013). In contrast, intermediate monocytes increase during the subacute phase and are positively associated with recovery, indicating a role in promoting neurovascular repair (López et al. 2014, Wattananit et al. 2016). Non-classical monocytes, which play roles in tissue surveillance and anti-inflammatory signaling, did not significantly change in overall number but were reduced in females and patients with no recovery, suggesting a possible deficiency in immune surveillance or repair capacity in this group (Varghese et al. 2022).

Gene expression profiling revealed distinct temporal patterns associated with neurological recovery following ischemic stroke. In all monocytes, higher TSPO expression within 24 hours post-stroke was strongly associated with lower NIHSS scores, suggesting a link between early upregulation of TSPO and favorable neurological outcomes (Tsuyama et al. 2024). The gene encoding the 18 kDa

translocator protein TSPO is a mitochondrial membrane-associated molecule that is upregulated in activated microglia and monocyte-derived cells during inflammation (Yao et al. 2020). It has been widely used as a biomarker of neuroinflammation across neurological diseases and has recently been employed in PET/MRI imaging to monitor dynamic inflammatory changes within infarcted brain tissue, with signal intensity peaking acutely and resolving by approximately 90 days (D'Anna et al. 2023). These findings support its utility not only as a molecular marker but also as a potential modulator of early reparative responses following stroke.

By 3-5 days post-stroke, downregulation of the pro-inflammatory genes CD86 and IL-1β was associated with increased NIHSS scores, indicating that these early innate immune signals promote functional recovery (Ge et al. 2017). Similar transcriptional trajectories have been observed in peri-infarct cortical regions in animal models, underscoring the importance of temporally coordinated immune activity in recovery processes (Ito et al. 2018). At the chronic stage (3 months), elevated expression of CCR2 was positively correlated with the NIHSS score, indicating a potential association between persistent monocyte infiltration and impaired recovery, which is in line with the chronic inflammation observed in preclinical models (Park et al. 2022). Nevertheless, CCR2 may exhibit dual-phase functionality, as prior studies have shown that its role in mediating both detrimental and reparative monocyte recruitment depends on the temporal context (Pedragosa et al. 2020).

Conversely, the expression of SLC24A4 and CD91 was negatively associated with the NIHSS score at the 3-month time point. SLC24A4, a calcium transporter, and CD91, a receptor involved in immune regulation, may both contribute to the resolution of intracellular stress and calcium dysregulation, which are mechanisms implicated in excitotoxic injury and secondary damage following stroke (Ito et al. 2018, Prabhakar et al. 2019). The downregulation of these genes in patients with poorer outcomes may thus reflect a failure to reestablish homeostasis. Similarly, IL-10 expression was negatively correlated with the NIHSS score, which is consistent with its recognized role as an anti-inflammatory and neuroprotective cytokine; reduced expression in more severely impaired individuals suggests a persistent inflammatory state detrimental to long-term recovery (García-Bonilla et al. 2018).

Taken together, these findings highlight several gene candidates, including TSPO, CD86, IL-1β, CCR2, SLC24A4, CD91, and IL-10, that may serve as molecular indicators of stroke progression or recovery. Their expression patterns reflect distinct temporal roles in immune activation, resolution, and repair and may inform future strategies for stratified therapy and personalized rehabilitation (Włodarczyk et al. 2022, Shafieesabet et al. 2023).

Microglia, the resident immune cells of the brain, also exhibit functional heterogeneity similar to that observed in peripheral monocytes. In **Paper III**, hiMG from patients

with neuronopathic GD exhibited altered cytokine expression and stress-induced lysosomal dysfunction: Using iPSC-derived models, we observed in GD-hiMG decreased gene expression of inflammatory cytokines such as TGFβ and IL-6. Other studies also reported altered cytokine levels but opposite trends, such as increased expression of IL-6 and TNFα (Aflaki et al. 2016). These discrepancies might reflect differences in the cell types studied or in the methods used.

Unlike monocytes, whose peripheral plasticity may be maintained or replenished during disease or aging, GD microglia exhibit a blunted regulatory response, likely contributing to persistent neuroinflammation (Brunialti et al. 2021, Boddupalli et al. 2022). Importantly, while naïve GD-hiMG maintained baseline lysosomal acidity, immune stimulation resulted in increased fluorescence of LysoSensor DND-189, possibly reflecting stress-induced lysosomal swelling, impaired autophagy, or compensatory lysosome biogenesis (Wang et al. 2018, Zhang and Peterson 2020, Chin et al. 2022). Chronic lysosomal stress has been linked to impaired clearance of substrates, neuroinflammation, and neuronal loss in individuals with GD (Li et al. 2016, Dupuis et al. 2022). This altered lysosomal acidity may reflect impaired autophagy and degradation pathways, contributing to microglial dysfunction and neurodegeneration due to disrupted homeostasis (Mindell 2012, Brunialti et al. 2021, Kim et al. 2021, Mondal et al. 2022). These findings are consistent with previous reports of lysosomal dysfunction and neuroinflammation in GD and related lysosomal storage disorders (Vitner et al. 2012, Panicker et al. 2014).

Monocytes and microglia, although distinct in origin and anatomical niche, share a common hallmark: immunological plasticity. Neither cell type is monolithic: both exhibit a remarkable capacity to adopt diverse activation states in response to environmental cues. While traditionally viewed through the lens of M1/M2 polarization, accumulating evidence now supports a more nuanced spectrum of activation that reflects their dynamic and context-dependent roles in homeostasis and pathology (Nayak et al. 2014). Similarly, monocyte subsets, classical, intermediate, and non-classical, demonstrate distinct transcriptional and functional profiles that evolve with age, inflammation, and injury.

Across the conditions examined in this thesis, namely, aging, ischemic stroke, and Gaucher disease, this plasticity has emerged as a double-edged sword. In healthy aging, shifts in monocyte gene expression suggest compensatory responses aimed at preserving immune balance, although these responses vary by biological sex and subtype. In the context of stroke, peripheral monocytes demonstrate a temporally regulated transition from pro-inflammatory to reparative roles, with the balance between subsets correlating with long-term recovery outcomes. In contrast, microglia derived from patients with neuronopathic Gaucher disease have a restricted ability to adapt, marked by persistent lysosomal dysfunction and blunted inflammatory signaling. These fixed deficits

underscore how genetic perturbations may constrain microglial flexibility, leading to prolonged neuroinflammatory stress.

Taken together, these findings emphasize the need for high-resolution, subtype-specific characterization of innate immune cells. Monocyte profiling by subset and sex, alongside disease-specific modeling of microglia using patient-derived iPSCs, offers an essential framework for unraveling immune dynamics in both physiological aging and neurological disease. Clarifying the conditions under which plasticity supports resolution versus dysfunction will be essential for designing targeted immunomodulatory strategies.

The role of biological sex in immune aging and stroke recovery

Biological sex is an increasingly recognized determinant of immune function, with profound implications for aging, inflammation, and neurological recovery. Immune cells such as monocytes respond not only to environmental and disease-specific stimuli but also to intrinsic sex-linked biological factors, including chromosomal differences and hormonal regulation. This thesis highlights how sex-specific immune trajectories influence monocyte phenotype and function, with consequences for aging and stroke recovery.

In **Paper I**, sex emerged as a critical modifier of monocyte gene expression across aging. Compared with male donors, female donors presented a broader and more pronounced transcriptional shift in monocyte subtypes, particularly in genes associated with inflammation and repair. Classical monocytes from females showed age-dependent downregulation of the pro-inflammatory cytokines TNFα and IL-1β, a shift not observed in males. Similarly, intermediate monocytes in females displayed both increased expression of anti-inflammatory genes, such as TGFβ, and reduced levels of pro-inflammatory transcripts, such as TLR8 and TNFα, suggesting a trend toward resolution-supporting profiles with age.

Flow cytometry analysis revealed an age-associated decline in B-cell frequencies, which was consistent across both sexes. In contrast, the proportion of NK-cells increased with age, but this trend reached significance only in females, highlighting the sex-specific effects of immune aging. These findings are consistent with prior observations of lymphocyte remodeling with age and further support the importance of sex as a biological variable (Le Garff-Tavernier et al. 2010, Hirokawa et al. 2013).

In contrast, male monocytes presented fewer age-related transcriptional changes. However, non-classical monocytes from male donors showed a significant

downregulation of TLR8, a pattern recognition receptor that detects viral RNA and contributes to antiviral immunity. TLR8 is located on the X chromosome and escapes inactivation in females, which may confer enhanced innate immune responsiveness in women (Heil et al. 2004). Reduced TLR8 expression in aging males could contribute to increased vulnerability to infections and impaired recovery later in life, aligning with broader patterns of sex-specific immune aging. Another sex-dependent marker identified in our study was TSPO, a mitochondrial membrane protein linked to immune activation and neuroinflammation. TSPO expression increased with age, specifically in non-classical monocytes from female donors. TSPO has been implicated as a PET imaging marker in various neurodegenerative and neuroinflammatory diseases, and its upregulation in peripheral monocytes may reflect heightened mitochondrial stress responses or immune activation in aging females (Conti et al. 2023). These findings support a potential link between peripheral immune activity and central neuroimmune states, particularly in the context of female aging. Sex-specific patterns also extended to CD91: while CD91 expression increased with age in intermediate and non-classical monocytes, this upregulation was observed only in females. CD91 has previously been proposed as a biomarker of biological age and immune surveillance, and its selective induction in aging females may reflect hormonal or epigenetic modulation of monocyte function (De Francesco et al. 2020).

Hormonal transitions, most notably menopause, which typically occur between the ages of 45 to 55 years, likely contribute to the sex differences observed in immune aging in females. The age range of our female cohort (43--98 years) suggested that a substantial proportion of the patients were peri- or post-menopausal. Estrogens are known to modulate immune function by suppressing pro-inflammatory cytokine production and promoting regulatory T-cell responses (Vegeto et al. 2008). The menopausal decline in estrogen may contribute to the immune remodeling observed in our female donors, including shifts toward both anti-inflammatory and maladaptive pro-inflammatory phenotypes (Abildgaard et al. 2020, Ryczkowska et al. 2023). Previous studies have linked the loss of estrogen to increased systemic inflammation and increased risk of neurovascular and neurodegenerative disease (Giannoni et al. 2011).

These findings lay the groundwork for **Paper II**, where we further explored the role of sex in monocyte dynamics after stroke. Monocyte levels rose at 24 hours and 3-5 days after stroke, with greater increases observed in male patients. Interestingly, higher monocyte levels in males during the acute phase were associated with better long-term recovery, suggesting beneficial early immune activation that supports repair (Wang et al. 2021). This may reflect sex-specific differences in monocyte trafficking or responsiveness to chemokine signals, such as CCL2−CCR2 axis activation (Stowe et al. 2012, Roth et al. 2018). At three months post-stroke, male patients presented a more marked reduction in classical monocytes, a subset generally associated with early

inflammation and tissue damage, potentially indicating more effective resolution of inflammation. Interestingly, patients who showed clear recovery had higher levels of monocytes at this stage, particularly in intermediate and non-classical subsets, suggesting that these cells may continue to support tissue repair beyond the acute phase. These dynamics may reflect a more rapid transition from pro- to anti-inflammatory states, potentially influenced by androgen-regulated immune modulation (So et al. 2021). This late-phase expansion was more pronounced in males, which aligns with their stronger association between monocyte dynamics and recovery outcomes (Urra et al. 2009, Wang et al. 2021). In contrast, female patients displayed lower levels of intermediate and non-classical monocytes during recovery, particularly those with no recovery. Since these subsets are generally associated with anti-inflammatory functions and tissue surveillance, we speculate that impaired expansion or persistence of reparative monocyte populations may hinder recovery in females (Ziegler-Heitbrock 2015). Although estrogen loss may drive pro-inflammatory changes in aging women, it may also limit the recruitment or maintenance of regulatory monocytes, compounding the challenge of post-stroke repair (Pelekanou et al. 2016). Moreover, sex-specific gene expression patterns emerged. Paper I, as well as other studies, have reported sex differences in gene expressions, such as reduced levels of IL-10 and CX3CR1 in female non-classical monocytes, potentially limiting anti-inflammatory responses also after stroke (Carmona-Mora et al. 2023, Filippenkov et al. 2023). These studies suggest that sex-linked differences in monocyte gene regulation may underlie divergent recovery trajectories.

Although this thesis did not directly assess sex differences in GD within **Paper III**, recent studies indicate that biological sex may also modulate the clinical phenotype and immune responses in neuronopathic GD. In particular, females with chronic neuronopathic GD have been reported to exhibit slower neurological progression, while males more often present with early-onset systemic symptoms (Siebert et al. 2014, Hopf et al. 2019, D'Amore et al. 2021). The L444P mutation, which is frequently associated with neuronopathic forms, has been linked to more severe phenotypes in females (Rice et al. 1996, Hopf et al. 2019), and murine models further suggest sex-specific differences in inflammatory responses and disease progression (Sun et al. 2011, McNamara et al. 2014). These findings underscore the broader importance of integrating sex as a biological variable across neuroinflammatory disorders, particularly when assessing immune-related biomarkers or designing individualized therapies (Márquez et al. 2020, Varghese et al. 2022).

Collectively, these findings underscore the importance of considering biological sex in both experimental design and clinical intervention. The molecular signatures of aging and stroke recovery differ markedly between males and females, not only in magnitude but also in the direction of gene regulation. Sex-linked genes such as TLR8, hormonal modulators such as estrogen, and immune regulators like TSPO and CD91 define

divergent aging trajectories and immune responses that ultimately shape disease susceptibility and recovery potential. Future research into the intersection of sex, immunity, and neuroinflammation will be essential for developing tailored therapeutic approaches in precision medicine.

Biomarkers, disease models, and therapeutic targets

Understanding the immune landscape of neuroinflammatory conditions requires both the identification of circulating biomarkers and the development of mechanistic models that reflect CNS pathology. In this thesis, two distinct but functionally convergent myeloid populations, circulating monocytes and CNS-resident microglia, were investigated for their roles in aging, stroke, and GD. Although monocytes and microglia differ in their ontogeny and anatomical niches, both can adopt macrophage-like phenotypes in response to inflammatory cues. Monocyte-derived gene signatures, such as ANXA1, CD36, CD91, TSPO, CCR2, and SLC24A4, have emerged as promising candidates for tracking immune aging and stroke recovery. In parallel, patient-specific iPSC-derived microglial models were established to investigate the cellular and molecular dysfunctions underlying neuronopathic GD. Together, these complementary approaches provide translational insight into how distinct myeloid cells, monocytes, MDMs, and microglia contribute to neuroinflammation through shared macrophage-like functions, thereby advancing the potential for personalized diagnostics and targeted therapies.

Paper I identifies several age- and sex-associated monocyte biomarkers relevant to inflammation and immune remodeling. ANXA1, an anti-inflammatory gene involved in resolution pathways, was consistently upregulated with age, particularly in intermediate and non-classical subsets (Perretti and D'Acquisto 2009, Xu et al. 2021). CD36 expression also increased across all subsets, indicating increased monocyte activation (Cho et al. 2005). Conversely, classical monocytes showed downregulation of pro-inflammatory genes such as TLR8 and S100A8 (Swindell et al. 2013, Wang et al. 2018). Intermediate monocytes exhibited reduced TLR8 and TNFα alongside increased ANXA1 and TGFβ, while non-classical monocytes presented upregulation of CD91 and ANXA1 with concurrent TLR8 downregulation. These subset-specific changes collectively suggest a skew toward reparative phenotypes in aging monocytes.

Sex-specific differences further shaped the monocyte transcriptome with aging. In females, CD91 and TSPO were selectively upregulated in intermediate and non-classical monocytes. CD91 has been proposed as a biomarker of immune surveillance and biological aging (De Francesco et al. 2020), whereas TSPO, which is linked to mitochondrial stress and neuroinflammation, may reflect increased peripheral immune activation in aging women (Conti et al. 2023). Reduced TLR8 expression in non-

classical monocytes was observed in both sexes but was more pronounced in males, potentially reflecting sex-linked differences in antiviral immunity (Heil et al. 2004).

These transcriptional profiles, particularly ANXA1, CD36, CD91, TSPO, and TLR8, may serve as valuable indicators of age- and sex-related immune remodeling.

By building on the temporally defined transcriptional patterns identified in monocytes, **Paper II** further explored subset-specific signatures that linked acute immune responses with long-term recovery. At 24 hours post-stroke, downregulation of the CD11c, CD32, and TGFβ genes, which are associated with monocyte activation, phagocytosis, and inflammation, was correlated with improved outcomes across total monocytes, with CD11c expression additionally downregulated in the classical and intermediate subsets. In non-classical monocytes, CD169, a marker of pro-inflammatory macrophages, is also negatively associated with recovery (Arnold et al. 2016, Kim et al. 2022, Chen et al. 2023, Deng et al. 2024). These findings reinforce the protective effect of attenuated early monocyte activation.

Conversely, genes implicated in reparative processes (TGM2, CD36, and CD91) were positively correlated with neurological recovery. Notably, CD36 expression in intermediate monocytes represents a novel human biomarker, which is consistent with its role in efferocytosis and neuroprotection in preclinical models (Woo et al. 2016). CD91 was consistently associated with improved outcomes across both time points and monocyte subsets, highlighting its potential as a robust prognostic marker. While TGM2 has been linked to hypoxia-induced injury, its association here may reflect a reparative function within intermediate monocytes (Filiano et al. 2010).

At 3-5 days, further differentiation of prognostic signals emerged. Downregulation of CCR2, CX3CR1, CD11c, and IL-10 across various monocyte subsets was associated with favorable outcomes, suggesting that prolonged inflammatory signaling during this window may be detrimental. In contrast, the upregulation of IFITM2, SLC24A4, CD38, and CD91 was positively correlated with recovery. In particular, CD38 expression in intermediate monocytes, which was previously shown to exert context-dependent effects in murine stroke models, was identified here as a novel correlate of recovery in human patients (Mayo et al. 2008). These findings position CD38 alongside CD91, CD36, TGM2, and SLC24A4 as part of a candidate gene panel for stroke outcome prediction.

Finally, immunophenotyping revealed that overall monocyte abundance at the acute and subacute stages was associated with recovery, while early classical monocyte predominance predicted poorer outcomes. Sex-specific dynamics were also observed, with males and recovery patients exhibiting early reductions in classical monocytes and a delayed rise in intermediate monocytes, whereas females and no recovery patients presented an elevated non-classical fraction at 3-5 days.

Together, these data underscore the prognostic potential of integrating monocyte subset dynamics with targeted gene expression profiles. Biomarkers such as CD91, CD36, and CD38 may guide personalized therapeutic strategies and enhance recovery monitoring in stroke care.

Paper III aims to better understand neuronopathic GD by generating iPSC lines from patients and healthy sex-matched controls using mRNA reprogramming, which avoids the risks of insertional mutagenesis associated with viral vectors while maintaining high reprogramming efficiency (Soyombo et al. 2013, Kogut et al. 2018). The resulting GD-iPSCs exhibited a normal morphology, karyotype, and expression of pluripotency markers, fulfilling key criteria for disease modeling.

The iPSC-based microglial model adds mechanistic depth to these findings: iPSC-derived macrophages have been widely used in GD research, and hiMG offer greater CNS relevance, enabling exploration of brain-specific immune functions and pathology. Microglia-neuron coculture systems have also been applied to study Parkinson's disease and Alzheimer's disease, and our model extends this framework to Gaucher neuropathology (Garland et al. 2022, Hertz et al. 2024).

In GD-hiMG, reduced TGFβ and IL-6 gene expression indicates a deficient anti-inflammatory profile, whereas stimulus-dependent lysosomal dysfunction suggests impaired degradation capacity. These defects mirror known pathological features of neuronopathic GD and highlight microglial vulnerability to inflammatory stress (Vitner et al. 2012, Panicker et al. 2014, Dupuis et al. 2022). Notably, this downregulation contrasts with elevated IL-6 levels typically observed in peripheral macrophages from GD patients, suggesting a CNS-specific immune signature and emphasizing the need to study microglia independently in GD (Tantawy 2015, Aflaki et al. 2016, Brunialti et al. 2021).

Upon immune stimulation, GD-hiMG presented increased LysoSensor Green DND-189 fluorescence, indicating altered lysosomal acidity and potential lysosomal swelling or biogenesis (Zhang and Peterson 2020, Feng et al. 2023). These findings align with known lysosomal dysfunction in GD, where impaired glucocerebrosidase activity was shown to disrupt autophagy and cellular homeostasis, contributing to neurodegeneration (Sasaki et al. 2014, Mondal et al. 2022).

Notably, the coculture of GD-hiMG with isogenic neurons recapitulated the cellular interactions relevant to neurodegeneration and enabled the study of genotype-specific neuroinflammatory cascades. These systems provide a foundation for high-throughput drug screening, functional assays, and investigations of patient-specific therapeutic interventions (Canals et al. 2023). Future integration of iPSC-derived astrocytes into these cocultures may further elucidate the contributions of microglia to GD pathology, as astrocyte dysfunction is increasingly recognized in neurodegenerative disease models (Benetó et al. 2020).

Altogether, the integration of monocyte biomarkers with iPSC-derived cellular models offers a dual strategy for translational neuroimmunology: diagnostic profiling of peripheral immunity and mechanistic exploration of central immune dysfunction. These platforms could ultimately support individualized treatment approaches, increase disease monitoring, and support the development of targeted immunotherapies tailored to sex, subtype, and disease context.

Methodological considerations and limitations

While this thesis presents novel insights into the immunobiology of neuroinflammation across aging, stroke, and GD, several methodological considerations must be acknowledged to contextualize the findings.

One important limitation lies in the sampling strategy and cohort composition. In **Paper I**, the study of age-related changes in the gene expression of monocyte subsets was conducted in 44 healthy individuals (26 males, 18 females), spanning an age range of 28 to 98 years. Although this wide range allowed for continuous correlation analyses, it may have introduced variability linked to unmeasured confounders such as medication, comorbidities, or lifestyle factors (Ramirez-Santana 2018). The sample size, particularly in analyses stratified by sex and monocyte subset, limits the statistical power to detect subtle expression changes and increases the risk of type II errors. For example, several trends observed in male donors did not reach statistical significance, possibly due to reduced group size and inter-individual variability (Cao et al. 2024). Future studies should consider larger, more balanced cohorts and control for confounding lifestyle or clinical variables.

In **Paper II**, stroke patients were followed longitudinally to examine immune responses at 24 hours, 3-5 days, and 3 months post-stroke. However, patient heterogeneity, including differences in stroke severity, etiology, treatment, and rehabilitation, likely influences immune profiles (Barrowman et al. 2019). Although the use of NIHSS scores and mRS outcomes allowed for some degree of clinical stratification, unquantified factors such as lesion location or prior immunological status could have contributed to variability (Ernst et al. 2018). Pre-stroke immune status, where available, would provide stronger baseline comparisons.

Flow cytometry-based immunophenotyping was employed across **Papers I and II** to identify and quantify peripheral monocyte subsets and assess surface marker expression. While this technique offers high sensitivity and resolution, it is subject to certain limitations. The manual gating strategy can introduce subjective bias, and population definitions may vary across studies. For example, the delineation of intermediate versus non-classical monocytes is particularly sensitive to small variations in CD14/CD16

expression thresholds (Ziegler-Heitbrock et al. 2010, Maecker et al. 2012). In addition, the functional relevance of surface markers such as CD91, TSPO, or TLR8 may not be fully captured through flow cytometry alone, especially when expression is low or inducible.

In the same studies, we employed Fluidigm for targeted bulk-cell gene expression profiling of monocytes rather than large-scale single-cell RNA sequencing approaches. This decision was guided by the availability of predefined gene targets relevant to aging and stroke, allowing for focused, high-resolution analysis within a controlled microfluidic environment. While Fluidigm offers precise capture and reduced doublet rates compared with high-throughput single-cell sequencing platforms such as 10X Genomics, it is limited by lower throughput, higher per-sample cost, and susceptibility to contamination due to the small input volumes and microfluidic handling (Szulwach et al. 2015, Xin et al. 2016, DeLaughter 2018). Single-cell sequencing methods, although more scalable, introduce challenges such as increased doublet formation and variable sequencing depth, which can complicate downstream interpretation (Wu et al. 2013, Macosko et al. 2015). Nonetheless, its specificity for hypothesis-driven analysis and the need for subset-specific resolution make Fluidigm an appropriate choice given the research aims.

A related challenge involves the discrepancy between gene expression and protein-level data. In **Paper III**, gene expression was measured using high-throughput microfluidic qPCR systems and transcriptomic assays. While this allows for the simultaneous quantification of multiple inflammation-related transcripts, mRNA levels do not always correlate directly with protein abundance or activity, particularly for secreted cytokines or membrane receptors. For example, IL-6 and TGFβ are transcriptionally downregulated in GD-hiMG, yet earlier studies in iPSC-derived macrophages reported increased cytokine secretion in the context of GD (Xu et al. 2011, Aflaki et al. 2016). These discrepancies may be explained by methodological factors such as differences in assay timing, stimulation conditions, or detection sensitivity rather than solely by underlying biological variation (Choquet et al. 2025). Future studies should integrate proteomic and functional validation, particularly for cytokines.

The use of *in vitro* models, particularly in **Paper III**, also imposes certain constraints. Although iPSC-derived microglia recapitulate key transcriptional and functional features of brain-resident microglia, their maturation and behavior are influenced by *in vitro* culture conditions (Pandya et al. 2017). While coculture with neurons improved microglial viability and function, the absence of additional CNS cell types, such as astrocytes or endothelial cells, limits the complexity of cellular interactions (Park et al. 2018). Moreover, while our transcription factor-based reprogramming and differentiation protocols were optimized for consistency, iPSC-derived cell systems can exhibit line-specific variability due to donor genetics, epigenetic memory, or passage number (Kilpinen et al. 2017).

Finally, technical constraints in modeling neuroinflammation across disease contexts must be acknowledged. While the iPSC platform enables human-specific modeling of GD-related inflammation, direct comparisons to stroke- or aging-associated changes are complicated by differences in cell type (microglia vs. monocytes), tissue context (brain vs. blood), and disease duration (chronic vs. acute). Comparative analyses should account for these differences in cellular origin and disease dynamics, even when overlapping markers are observed (Rovira-Moreno et al. 2023).

Despite these limitations, the methodological approach adopted in this thesis allowed for integrative analyses across distinct but complementary biological systems. The combined use of flow cytometry, targeted gene expression, and iPSC modeling enabled a multi-layered investigation of neuroinflammation that lays the foundation for more mechanistically precise and clinically relevant follow-up studies.

Future directions

The findings presented in this thesis emphasize the need for a more nuanced understanding of neuroinflammation that considers context and its regulation by monocytes and microglia. Growing evidence suggests that immune responses are shaped by age, sex, and disease state. Therefore, future research should focus on approaches that combine cellular heterogeneity, temporal dynamics, and personalized biology.

One important avenue involves the development of sex-specific and monocyte subtype-targeted interventions. The prominent sex differences observed in **Papers I and II**, including the age-associated increase in ANXA1, CD91, and TSPO in female monocytes and the downregulation of TLR8 in aging males, suggest that monocyte responses are not only sex-dimorphic but also may respond differently to treatment. These findings support a personalized approach that accounts for biological sex differences, such as hormonal and chromosomal influences on immunity. Future therapies might leverage anti-inflammatory agents or immune modulators that selectively influence intermediate or non-classical monocytes, which are associated with recovery-promoting gene signatures in stroke and with anti-inflammatory traits in aging.

Additionally, to capture the full complexity of neuroinflammatory processes, multi-omics approaches should be employed. While this thesis focused on gene expression profiling, proteomics, epigenetics, and metabolomics could provide deeper insight into post-transcriptional regulation and functional protein networks in aging, stroke, and GD contexts. These layers may help explain the observed discrepancies between mRNA expression and protein abundance, particularly for secreted cytokines such as IL-6,

where temporal release patterns and post-transcriptional control often diverge from transcript levels. Integrating transcriptomic, proteomic, and metabolic data across different monocyte subsets or microglial states could clarify how distinct pathways interact to drive inflammatory or reparative phenotypes (Rovira-Moreno et al. 2023). Such approaches could also help explain discrepancies between gene and protein expression levels observed in **Paper III** and other studies.

The use of coculture models and brain organoids represents another powerful strategy for investigating cell-cell interactions under physiologically relevant conditions. In **Paper III**, we established a coculture system of iPSC-derived microglia and neurons to investigate microglial dysfunction in GD, aiming to more accurately reflect the *in vivo* interplay between immune cells and neural networks. Expanding this platform to include astrocytes, endothelial cells, or myeloid lineage cells from aged or diseased donors could provide an organotypic model of neuroinflammation suitable for both mechanistic studies and therapeutic screening (Liu et al. 2020, Supakul et al. 2024). The incorporation of region-specific neuronal subtypes or 3D brain organoids may further enhance the accuracy of these models.

A key translational step will be the clinical validation of identified biomarkers, such as ANXA1, CD36, CD91, TSPO, CCR2, and SLC24A4. These molecules have emerged as potential indicators of monocyte or microglial activation states and are correlated with age, sex, and functional outcomes in stroke patients. Longitudinal studies in larger patient cohorts should evaluate their utility in predicting disease progression or treatment response, particularly in neurodegenerative or vascular conditions. Importantly, flow cytometry-based surface profiling, transcriptomic signatures, or plasma protein assays could be developed as minimally invasive diagnostic tools (Su et al. 2019). Where appropriate, these biomarkers could also inform therapeutic decisions in a sex- and disease-specific manner.

Finally, testing treatments in patient-derived hiMG could be a useful step before clinical trials for evaluating drug efficacy in a patient-specific and disease-relevant context. In **Paper III**, GD-hiMG were shown to exhibit altered lysosomal responses and impaired inflammatory signaling. This vulnerability to immune stress can be harnessed to test compounds that restore microglial function, correct lysosomal pH, or modulate cytokine release. Such approaches could include small molecules, enzyme replacement strategies, or CRISPR/Cas9-mediated gene editing. Moreover, future studies should explore how pre-conditioning hiMG with anti-inflammatory agents or neurotrophic factors modulates their interaction with neurons in coculture, potentially identifying new pathways for neuroprotection in GD and related disorders.

In summary, the next phase of research should focus on integrating personalized, systems-level insights with experimental models that capture the cellular diversity and dynamic nature of neuroinflammation. This integrative strategy has the potential to

bridge mechanistic findings with therapeutic innovations, advancing the goal of individualized treatment for neurological diseases driven by immune dysregulation.

Conclusion

This thesis demonstrates how peripheral monocytes and central microglia contribute to neuroinflammatory processes across the human lifespan and in distinct pathological contexts. Through the integration of high-resolution immunophenotyping, transcriptional profiling, and patient-derived iPSC models, the work presented here underscores the remarkable adaptability and plasticity of innate immune cells in aging, stroke, and GD.

Paper I demonstrated that aging induces transcriptional changes in monocyte subsets in a sex-specific and subtype-dependent manner, with prominent alterations in neuroinflammatory and regenerative gene expression. These findings not only provide a nuanced view of immune aging but also establish a cellular framework for understanding sex-linked vulnerabilities to neuroinflammatory disease. **Paper II** extended this investigation into dynamic immune responses following ischemic stroke, showing that monocyte subset fluctuations and gene expression profiles are temporally regulated and predictive of neurological recovery. The identification of early post-stroke immune signatures linked to long-term outcomes offers new possibilities for prognostication and immune modulation in stroke rehabilitation. **Paper III** introduces a patient-specific iPSC-derived microglial model for neuronopathic GD, revealing disease-associated deficits in inflammatory signaling and lysosomal function. This platform paves the way for personalized interrogation of microglial dysfunction and the development of targeted therapies.

A unifying theme across these studies is the immunological plasticity shared by monocytes and microglia: cells that, while distinct in origin and niche, exhibit remarkable adaptability in response to environmental cues. This plasticity, however, is not uniformly beneficial; it can support repair or perpetuate dysfunction depending on the context. These findings underscore the limitations of binary activation models and highlight the need for high-resolution, subset-specific characterization of innate immune responses. By embracing this complexity, future research can better delineate the conditions under which immune plasticity promotes resilience versus pathology, paving the way for more precise and personalized immunomodulatory strategies.

Collectively, these studies highlight the central role of monocyte and microglia dynamics in regulating the balance between injury and repair in the brain. These findings highlight the critical need for a lifespan-spanning, immune-focused approach to understanding neuroinflammation that considers cellular heterogeneity, sex-specific

regulation, and disease context. They also demonstrate how immune cells not only reflect but also actively shape the trajectory of neurological health and disease, offering critical insight into potential biomarkers and therapeutic targets.

Summary of key methods

This section summarizes the key methods applied throughout this thesis. Full experimental details and practical guidance to support reproducibility and implementation can be found in the materials and methods sections of the corresponding publications.

Primary peripheral blood samples

In **Paper I**, PBMCs from 44 healthy donors (18 females, 26 males) were included to achieve balanced sex distributions and broad age coverage. The samples were used to assess age- and biological sex-related changes in monocyte frequency and gene expression.

For **Paper II**, 37 individuals with a confirmed diagnosis of ischemic stroke (12 females, 25 males) of either minor or moderate severity were enrolled in the study. Age- and sex-matched healthy controls were included for comparison. The samples were used to assess changes in monocyte frequency and gene expression in the context of biological sex and recovery during the acute, subacute, and chronic phases after stroke. Patients were stratified by stroke severity, sex, and recovery status at 3 months and 1 year post-stroke.

Ethical approval and human peripheral blood sample collection

All human studies were approved by the Regional Ethics Review Board in Lund, Sweden, under the Lund Stroke Register project (Ethical permits: 2016/179 and 2017/357) and complied with the Declaration of Helsinki. Written informed consent was obtained from all participants for blood sampling, genetic/protein analyses, and clinical evaluations. The participants were informed of their right to withdraw at any time, and samples from excluded individuals were discarded. If a diagnosis of ischemic stroke could not be confirmed via MRI, the corresponding blood samples were discarded.

The control participants were blood donors who reported being in good health at sampling. The general exclusion criteria included pregnancy, systemic inflammatory or hematologic disorders (**Papers I** and **II**). All samples were anonymized, randomized, and processed in a blinded fashion to minimize bias.

Clinical assessment of stroke severity using thee NIHSS

Neurological function was assessed using the National Institute of Health Stroke Scale (NIHSS) at 24 hours, 3-5 days, 3 months, and 1 year post-stroke (**Paper II**). The NIHSS is a validated tool for quantifying stroke-related neurological deficits (0–42 scale). The stroke severity categories included minor (1-4), moderate (5-15), moderate to severe (16-20), and severe (21+).

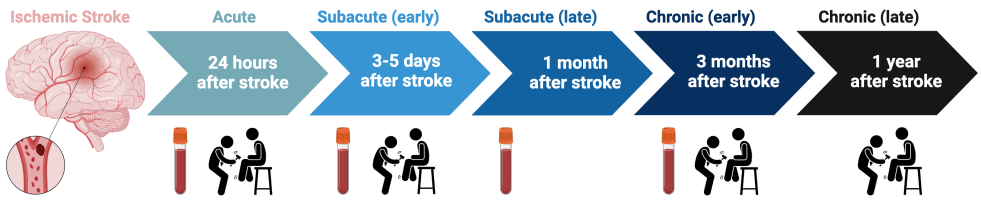


Figure 14 | Workflow of the clinical assessments of stroke patients. After ischemic stroke (and consent to join the study), patients are assessed via neurological tests (NIHSS), and 10 ml of blood is taken. Neurological tests are performed within 24 hours, 3-5 days, 3 months, and 1 year after stroke. Blood samples for flow cytometry and gene expression analysis were collected within 24 hours, 3-5 days, 1 month, and 3 months after stroke. Created in BioRender. Tampe, J. (2025) https://BioRender.com/mm8lopy

Collection of peripheral blood mononuclear cells

Venous blood (10 mL) was collected from stroke patients and healthy controls using EDTA-coated vacutainers (Becton Dickinson). The samples were kept at room temperature and processed within 24 hours. PBMCs were isolated under sterile conditions in a biosafety level 2+ laminar flow hood using SepMate tubes with Lymphoprep (STEMCELL Technologies). All procedures were performed with animal-free reagents to prevent monocyte activation. The cells were cryopreserved in StemCellBanker (Amsbio) at -80°C and then transferred to -150°C or liquid nitrogen for long-term storage.

Flow cytometry and cell sorting

All papers employed flow cytometry for cellular identification:

In **Paper I**, a BD FACSAria II cell sorter flow cytometer was used to assess the frequencies of B-cells, NK-cells, T-cells, monocytes, and their subtypes. The cells were also sorted for bulk gene expression analysisZ with a focus on neuroinflammation and regeneration markers, to evaluate the impact of aging and sex on monocyte populations.

Paper II applied the flow cytometry analysis and sorting approach from Paper I to analyze monocytes and their subtypes following stroke. The frequencies of these populations and their corresponding gene expression levels were compared at various time points post-stroke with those of age- and sex-matched controls. The aim of this study was to identify potential biomarkers early after stroke that are predictive of recovery or no recovery at later stages.

For **Paper III**, BD FACSAria III and Fortessa flow cytometers were used to evaluate the expression of key microglial surface markers and confirm the successful and reproducible differentiation of microglia from iPSCs for downstream analyses. Additionally, a pH-sensitive lysosomal dye was used to assess changes in lysosomal acidity in human induced microglia from GD patients compared with those from healthy controls under different conditions.

Flow cytometry characterization of PBMCs

The frozen PBMCs were thawed, rinsed, and stained for 30 minutes with a panel of monoclonal antibodies against CD91, CD14, CD16, CD3, CD19, and CD56 cell surface antigens directly conjugated to various fluorophores. Afterwards, the cell suspensions were washed and strained for analysis on a BD FACSAria II system. The nuclear dye DRAQ7 was added five minutes before analysis to stain the nuclei of dead or membrane-compromised cells and exclude non-viable cells. The spectral overlap from autofluorescence and fluorophore emissions was corrected against non-stained, single-stained, and FMO control samples. Gating strategies were validated through back-gating and replicate analysis.

Monocytes were identified by CD91 expression and further classified into classical (CD14+/CD16-), intermediate (CD14+/CD16+), and non-classical (CD14-/CD16+) subsets. For all monocytes and each subtype, 20 cells per biological and technical replicate were sorted into 96-well plates pre-filled with lysis buffer for downstream RT-qPCR analysis.

Flow cytometry characterization and lysosomal activity assessment of hiMG

After 14 days of differentiation, the hiMG were washed, dissociated with Accutase (ThermoFischer Scientific), and stained with antibodies against the microglial surface antigens CD11b, TREM2, and CX3CR1 for 30 minutes. The cells were washed and strained for analysis on BD FACSAria III and LSR Fortessa flow cytometers. DRAQ7 was added before analysis to exclude non-viable cells.

To assess lysosomal activity, the cells were stimulated with IFNγ or TGFβ 6 hours before collection. Dissociated live cells were incubated with the lysosomal dye LysoSensor DND-189 for 5 minutes. The controls included non-stained cells, single-color compensation, and FMOs. The median fluorescence intensity (MFI) was normalized to that of non-stained control hiMG to correct for inter-batch variability.

Table 3 | Flow cytometry antibody information

Target	Fluorochrome	Marker	Dilution	Company
CD91	PE	Monocytes	1:25	BD Pharmingen
CD14	APC	Monocytes	1:25	BD Pharmingen
CD16	BV421	Monocytes	1:50	BD Pharmingen
CD3	PE-Cy7	T-cells	1:50	BD Pharmingen
CD19	BB515	B-cells	1:25	BD Pharmingen
CD56	BV605	NK-cells	1:10	BD Pharmingen
Dead	Darq7	Dead cells	1:100	BD Pharmingen
CD7	AlexaFlour700	NK-cells	1:50	BD Pharmingen
CD11b	PE-Cy5	Microglia	1:40	BD Pharmingen
TREM2	APC	Microglia	1:25	R&D Systems
CX3CR1	BV605	Microglia	1:40	R&D Systems

Flow cytometry data acquisition and analysis

One million events per sample were collected via a flow cytometer, DIVA software was used, and the data were analyzed via FlowJo software (Version 10.10.0, BD Bioscience) to estimate the overall cell population and visualize the gating strategy.

Human iPSC generation

In **Paper III**, the mRNA reprogramming approach was used to generate iPSCs from fibroblasts derived from a neuronopathic GD patient and a healthy control. This virus-free method was used to establish a disease-specific cellular model for studying the role of microglia in neuronopathic GD. iPSCs are an ideal tool for modeling rare, human-

specific, and multi-faceted diseases. By enabling the generation of patient-specific models, iPSCs more accurately recapitulate the biological complexity of human disease. These models provide a more translational approach and human-relevant context than traditional animal studies do, reducing the reliance on animal models while enhancing the investigation of disease mechanisms and the development of potential therapies.

Human fibroblast acquisition and culture

Fibroblasts from healthy donors (GM04503) and GD type II patients (GM02627) were obtained from the Coriell Institute Biobank. The cells were cultured in fibroblast medium consisting of DMEM supplemented with 10% FBS and 1% GlutaMAX (all from Thermo Fisher Scientific). The cells were expanded under standard culture conditions and seeded at a density of 5–7.5 × 10^4 cells per well in 2 mL of medium on iMatrix-511-coated 6-well plates (REPROCELL, UK).

Reprogramming of fibroblasts into iPSCs

The fibroblast culture medium was replaced with NutriStem (REPROCELL, UK), and the cells were transferred to a hypoxic incubator (5% O_2, 5% CO_2, 37°C). From days 1 to 4, the cells were transfected overnight with synthetic mRNAs encoding the reprogramming factors OCT4, SOX2, KLF4, and c-MYC, using the StemRNA™ Gen Reprogramming Kit (REPROCELL, UK). The medium was replaced each morning post-transfection to reduce toxicity. Colonies expressing the pluripotency marker TRA-1-60 were identified by live staining as early as day 8 and were manually picked between days 10 and 14 for clonal expansion and maintenance under standard iPSC culture conditions.

Karyotyping of iPSC lines

The cells at ~80% confluence were treated with 10 µg/mL colcemid (30-45 min), dissociated with Accutase, and subjected to hypotonic treatment with 0.075 M KCl (all from Thermo Fisher). The cells were fixed with Carnoy's solution (methanol:acetic acid, 3:1) and stored at 20°C. Karyotype analysis was performed at Sant Joan de Déu Hospital, Barcelona (Spain). Following reprogramming, the iPSC karyotype was found to be normal.

Culture of iPSCs

The iPSCs were cultured in Laminin521-coated (Thermo Fisher Scientific) 6-well plates (Corning) in mTeSR1 media (STEMCELL Technologies). StemPro Accutase

Cell Dissociation Reagent (Thermo Fisher Scientific) was used for cell dissociation. The cells were pelleted via centrifugation and seeded in mTeSR1 (STEMCELL Technologies) containing 10 µM Rho-associated protein kinase (ROCK) inhibitor Y-27632 (STEMCELL Technologies) to prevent dissociation-induced apoptosis.

Viral tools and transduction

In **Paper III**, lentiviral vectors were employed to deliver transcription factors under the control of a doxycycline-inducible tetracycline operator (tetO) promoter system. This system allows precise temporal regulation of transgene expression. Lentiviral constructs encoding CEBPA-SPI1 or NGN2, along with the reverse tetracycline transactivator (rtTA), were used to induce microglial and neuronal differentiation, respectively. Puromycin selection was applied to the transduced cells to ensure homogeneous expression and reproducible induction across experiments.

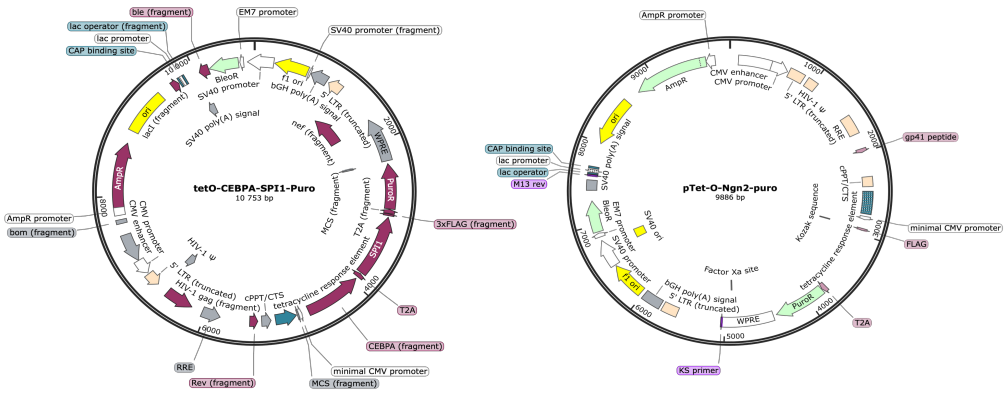


Figure 15 | Lentiviral vectors used for inducible transcription factor expression. Schematic maps of the two lentiviral constructs: The tetO-CEBPA-SPI1-Puro (Chen et al., 2021) vector contains a tetracycline-responsive element that drives the expression of the miccroglial transcription, while the tetO-Ngn2-Puro (Addgene #52047) vector for the neuronal transcription factor NGN2, along with a puromycin resistance gene for selection. These vectors were used to induce rapid and controlled differentiations in human iPSC-derived cells.

Lentiviral vector production

Lentiviral particles were produced via the third-generation packaging system in HEK293T cells by co-transfecting the plasmids PMDLg/pRRE, pMD2.G, pRSV-Rev, and lentivectors for FUW-M2rtTA, tetO-Ngn2-Puro, or tetO-CEBPA-SPI1-Puro (Addgene #20342, #52047; Chen et al., 2021). Transfections were carried out in poly-L-ornithine-coated T175 flasks using calcium phosphate precipitation. Viral supernatants were harvested on day 3, centrifuged, filtered, and concentrated by

ultracentrifugation. The virus pellets were incubated overnight in 100 µL of DMEM (Thermo Fisher Scientific), resuspended and stored in single-use aliquots at -80°C. Viral titers were confirmed via a GFP reporter assay or RT-qPCR.

Viral transduction of iPSCs

iPSCs were transduced with tetO-CEBPA-SPI1-Puro for microglial induction and tetO-NGN2-Puro for neuronal induction, each in combination with an M2-rtTA lentivirus. iPSCs were plated as single cells at 3.5 - 4 × 10^4 cells/cm^2 and transduced the next day in mTeSR1 with 8 µg/mL protamine sulfate. After overnight incubation, the medium was changed daily with mTeSR1 until the cells reached 70-80% confluency. The transduced lines were expanded for two passages, cryopreserved, and used for differentiation within five passages. For microglial induction, the cells were seeded at 2 × 10^4 cells/cm^2.

Plasmid DNA extraction

Plasmid DNA was extracted from bacteria carrying the target plasmids via the Qiagen Plasmid Maxi Kit (Qiagen). Bacterial cultures were grown in 250 mL of LB medium (Thermo Fisher Scientific) supplemented with puromycin, harvested, and lysed. Plasmid DNA was purified using a resin column, eluted, precipitated with isopropanol, and resuspended in DNase- and RNase-free water. DNA quality and concentration were assessed using a NanoDrop spectrophotometer, with a 260/280 ratio of approximately 1.8. The plasmid DNA was then diluted to 1 µg/µL for lentiviral production.

Differentiation of iPSCs into microglia and neurons

In **Paper III**, microglia and neurons were generated from iPSCs using a doxycycline-inducible transcription factor system in combination with defined small molecules. This approach enables rapid and consistent lineage specification with controlled timing of differentiation. The resulting platform was used to investigate microglial contributions to neuronopathic GD and provides a tractable system for dissecting disease mechanisms and evaluating potential therapeutic strategies.

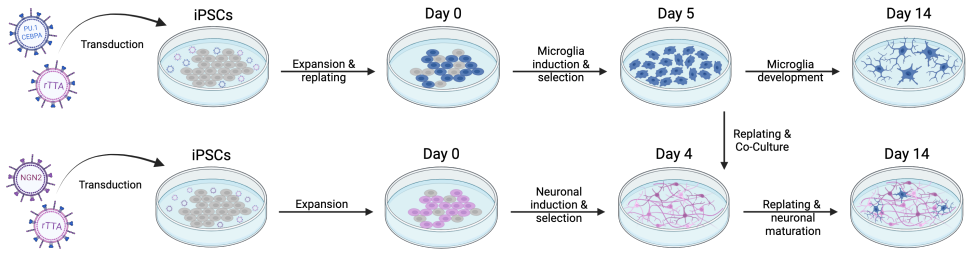


Figure 16 | Flow diagram illustrating the induced conversion of iPSCs into microglia and neurons. (A) Generation of hiMG by forced expression of the transcription factors PU.1 and CEBPA within 14 days. (B) Generation of hiNs by forced expression of the transcription factor NGN2 over 14 days. A coculture system was established by replating hiNs on day 4 and seeding the hiMG on day 5 at a 1:4 ratio. Blue, induced microglia; pink/purple, induced neurons; gray, undifferentiated iPSCs. The figure was created in BioRender. Tampe, J. (2025) https://BioRender.com/99o990n

Generation of iPSC-derived microglia

Control and GD hiMG were generated following the protocol outlined by Chen et al., with some modifications (Chen et al. 2021, Chen and Wong 2023).

Briefly, human iPSCs carrying the inducible CEBPA-SPI1 construct were seeded at a density of 2 × 10^4 cells/cm² in mTeSR1 medium supplemented with 10 µM ROCK inhibitor in Matrigel-coated 6-well plates (Corning). On day 0, the medium was replaced with mTeSR1 containing 2.5 µg/mL doxycycline. On day 1, the cells were transitioned to microglia (MG) medium (DMEM/F12/N2/NEAA, Thermo Fisher Scientific) supplemented with doxycycline (MERCK), 50 ng/mL BMP4, 50 ng/mL FGF2, 20 ng/mL activin A, and 50 µM β-mercaptoethanol (Thermo Fisher Scientific). On day 2, the medium was replaced with MG medium containing doxycycline, 50 ng/mL VEGF, 50 ng/mL SCF, 20 ng/mL FGF2, β-mercaptoethanol, and 1 µg/mL puromycin (Thermo Fisher Scientific). From days 3 to 4, the cells were cultured in MG medium supplemented with 10 ng/mL IL-34, 10 ng/mL M-CSF, 10 ng/mL TGFβ, and puromycin. For immunocytochemistry, the cells were passaged on day 5 using Accutase and replated on poly-D-lysine and fibronectin for monoculture at 4-6 × 10^4 cells/cm² or on polyethylenimine and laminin-521 (Biolamina) for coculture with human induced neurons (seeded at 20% of total hiNs). On days 6, 7, 8, 10, and 12, half-media changes were performed using MG medium supplemented with IL-34, M-CSF, and TGFβ. The media were changed every 24 hours until day 7 and every 48 hours thereafter. All growth factors and small molecules (Peprotech if not stated otherwise) were added fresh prior to each media change. The cells were used for experiments on day 14.

Generation of iPSC-derived neurons

Healthy and GD hiNs were generated following the protocol described by Zhang *et al.*, with slight modifications (Zhang et al. 2013).

In brief, on day -2, iPSCs were seeded at 3.5×10^4 cells/well in a GFR-Matrigel-coated 6-well plate in mTeSR1 medium supplemented with 10 µM Rock inhibitor. On day -1, the medium was replaced with 2 mL of mTeSR1, and the cells were transduced with 1 µL of M2-rtTA and pTetO-NGN2-Puro lentiviral vectors. On day 0, the medium was changed to mTeSR1 containing 2.5 µg/mL doxycycline to initiate neuronal transduction. Starting on day 1, the cells were maintained in neuronal medium consisting of BrainPhys (STEMCELL Technologies), N2, and B27 (both Thermo Fisher Scientific) supplemented with 2.5 µg/mL doxycycline and 1.25 µg/mL puromycin for selection. On day 3, puromycin selection was stopped to allow recovery before replating.

On day 4, the NG2-induced cells were dissociated with Accutase for 15 min, filtered through a 40 µm strainer, washed, and resuspended as single cells. The cells were plated at 1.5×10^4 cells/well in a PEI- and laminin-521-coated 24-well plate in neuronal media (BrainPhys/N2/B27) supplemented with doxycycline. From day 5 onward, neurotrophic factors (10 ng/mL NT3 and 10 ng/mL BDNF) were added to support neuronal maturation. The medium was changed every 2–3 days. The cells were used for coculture with hiMG after 14 days of neuronal induction or for GCase activity after 7 days in monoculture.

Coculture of hiMG with hiNs

To study microglia in a neural context that more closely resembles physiological conditions and promotes hiMG survival and maturity, hiMG were cocultured with hiNs. Neuronal media supplemented with the microglial-supporting factors IL-34, M-CSF, and TGFβ were used throughout the coculture system to support both cell types. On day 4, 1.5×10^4 hiNs were seeded per well in a PEI- and laminin-521-coated 24-well plate. The following day, the hiNs were washed gently with warm DPBS (+/+, Thermo Fisher Scientific), and 3×10^4 hiMG were added to each well in fresh supplemented medium. On days 6, 7, 8, 10, and 12, half of the culture media was changed carefully. The cocultures were used for immunocytochemical analysis after 14 days of both microglial and neuronal induction.

Immunocytochemistry

In **Paper III**, immunocytochemistry was used to confirm iPSC pluripotency and validate microglial differentiation. iPSCs were stained for the pluripotency markers OCT4, NANOG, and SOX2. Microglial and neuronal differentiation were assessed by immunostaining for IBA1 and MAP2, respectively. Immunostaining for the lysosomal marker LAMP2 enabled visual assessment of lysosomal morphology, including size and shape. Together, these types of staining served to verify the identity, maturation status, and integrity of the cells throughout the differentiation protocols.

Characterization of iPSC pluripotency

The pluripotency of iPSCs from GD patients and a healthy control was validated through immunofluorescence staining for OCT4, NANOG, and SOX2. The cells were fixed with 4% PFA and blocking solution containing the appropriate normal serum, followed by incubation with primary antibodies targeting the pluripotency marker mentioned above. Following incubation with a fluorescent secondary antibody and nuclear counterstaining with DAPI, fluorescence imaging was performed to confirm pluripotency.

Table 4 | Antibody information

Target	Marker	Host	Dilution	Company
OCT4	Pluripotency	Mouse	1:200	Santa Cruz
NANOG	Pluripotency	Rabbit	1:150	Abcam
SOX2	Pluripotency	Rabbit	1:250	Millipore
IBA1	Microglia	Goat	1:200	Wako
MAP2	Neuron	Chicken	1:1000	Abcam
LAMP2	Lysosome	Mouse	1:200	Abcam
anti-mouse Alexa Fluor 488	secondary		1:500	Thermo Fisher
anti-rabbit Alexa Fluor 568	secondary		1:500	Thermo Fisher
anti-goat Alexa Fluor 488	secondary		1:500	Thermo Fisher
anti-mouse Alexa Fluor 568	secondary		1:500	Thermo Fisher
anti-chicken Alexa Fluor 647	secondary		1:500	Thermo Fisher
Höchst 33342	Nucleus		1:4000	Thermo Fisher
DAPI	Nucleus		1:1000	Sigma

Microglial identity and coculture

The identity of differentiated cell types in GD and control hiMG cocultures was confirmed by immunofluorescence staining for IBA1 (microglia), MAP2 (neurons), and LAMP2 (lysosomes). The cells were fixed with paraformaldehyde and incubated with primary antibodies overnight at 4°C, followed by secondary antibody labeling and nuclear staining. Imaging was performed to qualitatively verify the presence and identity of the respective cell populations. A quantitative comparison between the GD and control cocultures was not performed, as no overt differences were observed.

Microscopy

Fluorescence imaging was conducted using an inverted microscope (LRI Olympus IX73 and IX51) and a confocal microscope (LSM 780, Zeiss. Images were acquired using cellSens Standard software (LRI Olympus, Version 2.2) or ZEN Blue (Zeiss), with consistent exposure times and acquisition settings applied across all samples.

Image processing and analysis were carried out using ImageJ (Version 1.53a), including contrast adjustment, background subtraction, and channel merging. Representative images were selected to illustrate the expression and localization of pluripotency and lineage-specific markers in both control and GD-derived iPSC cultures.

All imaging was performed under identical acquisition conditions for each experiment to ensure comparability, and the fluorescence intensity was not quantified unless otherwise stated.

RNA and DNA analysis

All the papers employed genetic tools for gene expression analysis across studies:

In **Papers I and II**, a chip-based high-throughput RT-qPCR platform (Fluidigm) was used to quantify the expression of 40 selected genes related to neuroinflammation and regeneration in peripheral monocytes and their three different subtypes. Gene expression levels were analyzed in the context of aging, sex, and stroke recovery outcomes.

In **Paper III**, RT-qPCR was used to compare gene expression in iPSCs and to assess the expression of key cytokines involved in microglial function in hiMG derived from GD patients and healthy controls. The cDNA of the iPSC lines was sequenced to confirm the GD genotype and that of a healthy control.

RNA extraction (Paper III)

RNA was extracted from cultured hiMG on day 14 of microglial induction using the Qiagen RNeasy Micro Kit (Qiagen) according to the manufacturer's instructions. The cells were lysed and homogenized before ethanol was added to facilitate RNA binding to the silica membrane in the column. After two wash steps, RNA was eluted in DNase- and RNase-free water. RNA quality and quantity were assessed via the Qubit RNA High Sensitivity Assay Kit (Qiagen), with a 260/280 absorbance ratio of approximately 2.0. Total RNA was obtained from approximately 50,000 hiMG for downstream gene expression analysis.

RT-qPCR gene expression analysis

For RT-qPCR, extracted RNA was diluted in nuclease-free water to a concentration of 1 µg in a total volume of 15 µL. To synthesize cDNA, 4 µL of qScript RM (5x) and 1 µL of qScript RT enzyme (qScript cDNA Synthesis Kit, Quantabio) were added. For the no-RT controls, water was added instead of the reverse transcriptase enzyme. Reverse transcription was carried out at 25 °C for 5 min and 42 °C for 30 min, followed by enzyme inactivation at 85 °C for 5 min. The resulting cDNA was diluted (1:3) and mixed with TaqMan assay probes (1:7; Table 5) and Universal TaqMan Master Mix (Thermo Fisher Scientific) for amplification. RT-qPCRs were prepared with 1 µL of cDNA, 4 µL of probe, and 5 µL of Master Mix and run for 40 amplification cycles. Thermal cycling was performed with initial denaturation at 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 sec and 60 °C for 1 min. Plate preparation was automated using a BRAVO pipetting robot (Agilent).

Table 5 | List of Taqman probes used for RT-qPCR

Gene	Function	ThermoFischer #
TNFα	Pro-inflammatory	Hs00988069_m1
IL-1β	Pro-inflammatory	Hs99999192_m1
TGFβ	Anti-inflammatory, immune modulator	Hs00354519_m1
IL-6	Anti-inflammatory	Hs00998133_m1
OCT4	Pluripotency	Hs00742896_s1
NANOG	Pluripotency	Hs02387400_g1
SOX2	Pluripotency	Hs01053049_s1
HPRT1	Housekeeping	Hs03929096_g1
ACTβ	Housekeeping	Hs00998133_m1

Table 6 | List of Taqman probes for Fluidigm

Gene	Function	ThermoFischer #
CD36	Pro-inflammatory	Hs01060665_g1
CD169	Pro-inflammatory	Hs00167549_m1
CD86	Pro-inflammatory	Hs00968979_m1
CD64	Pro-inflammatory	Hs02718934_s1
CD40	Pro-inflammatory	Hs01128674_g1
CD62	Pro-inflammatory	Hs00922012_g1
S100A9	Pro-inflammatory	Hs00704702_s1
S100A8	Pro-inflammatory	Hs00167304_m1
TREM1	Pro-inflammatory	Hs01015064_m1
TNFα	Pro-inflammatory	Hs00988069_m1
IL-1β	Pro-inflammatory	Hs99999192_m1
TLR8	Pro-inflammatory	Hs00267207_m1
CLEC7A	Pro-inflammatory	Hs00609515_m1
ANXA1	Anti-inflammatory	Hs01065279_m1
TSPO	Anti-inflammatory	Hs01634996_s1
CX3CR1	Anti-inflammatory	Hs01076282_g1
TGFβ	Anti-inflammatory, immune modulator	Hs00354519_m1
CD31	Anti-inflammatory	Hs01120071_m1
STAT6	Anti-inflammatory	Hs00374176_m1
CD33	Anti-inflammatory	Hs00941829_m1
SIGLEC10	Anti-inflammatory	Hs00927900_m1
IL-10	Anti-inflammatory	Hs00417598_m1
CD91	Immune modulator	Hs00196191_m1
TGM2	Immune modulator	Hs01567022_m1
CD38	Immune modulator	Hs00233856_m1
CD32	Immune modulator	Hs01902549_s1
MARCO	Phagocytotic activity, scavenger receptor	Hs00365842_m1
CCR2	Monocyte infiltration	Hs04420632_g1
CD11c	Monocyte adhesion	Hs04194297_g1
CD11b	Monocyte adhesion and migration	Hs01547656_m1
BDNF	Neuroplasticity	Hs00961622_m1
IFITM2	interferon response and antiviral immunity	Hs01555410_m1
SLC24A4	cellular calcium ion homeostasis	HS00170103_m1
CD206	Anti-inflammatory	Hs00198937_m1
TSP1	Anti-inflammatory	Hs00957562_m1
IGF1	Immune modulator	Hs05019740_g1
INHBA	Immune modulator	Hs00610058_m1
CCL19	Pro-inflammatory	Hs01549532_g1
MMP9	Pro-inflammatory	Hs00542236_m1
CCL17	Anti-inflammatory	Hs00598625_m1
ACTβ	Housekeeping	Hs00998133_m1
B2M	Housekeeping	Hs01096678_m1
GAPDH	Housekeeping	Hs00152972_m1
CD19	Control (neg.)	Hs00174128_m1
CD3	Control (neg.)	Hs00218624_m1
CD56	Control (neg.)	Hs01041471_m1
CD7	Control (neg.)	Hs00559362_m1
Xeno	Control (pos.)	Cells-to-C Control Kit

Targeted multiplex gene expression profiling using Fluidigm

Gene expression was analyzed with Fluidigm technology to assess 40 genes related to neuroinflammation and regeneration. Three housekeeping genes were included for normalization, 4 genes served as negative controls, and an additional technical control was included to ensure assay robustness and correct cell population profiling (Table 6). The cDNA was synthesized via the use of all 47 TaqMan probes and Xeno primers (TaqMan Cells-to-Ct Control Kit, Thermo Fisher Scientific). The Taq-SSIII reaction mix (CellsDirect One-Step qRT-PCR Kit, Ambion) was used for reverse transcription. Negative, positive, no-RT, and linearity controls were included. Reverse transcription was carried out at 50°C for 1 hour, followed by enzyme inactivation at 95°C for 2 minutes. Pre-amplification was performed with 18 cycles of 95°C for 15 seconds and 60°C for 4 minutes. This step was essential for enhancing the detection sensitivity for low-abundance transcripts in monocyte and macrophage subtypes. The resulting cDNA was either used immediately for RT-PCR or stored at -80°C for later use.

For the Fluidigm run, TaqMan Gene Expression Master Mix (Thermo Fisher Scientific) and GE Sample Loading Reagent (Fluidigm) were mixed at a 10:1 ratio and dispensed into a 96-well plate. The cDNA was diluted 1:5 and added to the pre-mixed sample plate wells. The integrated fluid circuit (IFC) chip was primed under pressure to remove air bubbles and debris before loading 4 µL of each sample or assay mixture. Gene expression was quantified using a 50-70-25-50-96 cycle protocol. The data were analyzed via BioMark HD Data Analysis software (Fluidigm), with a quality threshold of 0.65, linear baseline correction, and automatic global cycle threshold (Ct) detection (Tampe et al. 2024). The data were compiled and analyzed using the software R (https://github.com/JTampe/Monocytes).

Genotypic confirmation of GD mutation

The genomic DNA of iPSCs from GD patients and healthy donors was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen). The cells were lysed, and the DNA was bound to a silica column, washed, and eluted in DNase- and RNase-free water. DNA quality and concentration were assessed using a NanoDrop spectrophotometer with a 260/280 absorbance ratio of approximately 1.8. Automated Sanger sequencing (Eurofins Genomics, TubeSeq) confirmed the presence of the GD-associated mutation in the patient-derived cell line.

Functional assays

In **Paper III**, a GCase enzymatic activity assay was used to confirm the decreased enzyme activity in GD-iPSC-derived cells, which is a hallmark of Gaucher disease.

GCase enzymatic activity assay

To assess GCase activity, 1.5 million cells were resuspended in extraction buffer, lysed by sonication, and centrifuged. The protein concentration of the supernatant was quantified using a Bradford assay and normalized to 1 mg/mL. For the GCase activity assay, 20 µL of the lysate was mixed with assay buffer and incubated at 37°C for 1 hour. The reaction was terminated by the addition of glycine-NaOH solution, and fluorescence was measured at an excitation wavelength of 365 nm and emission wavelength of 460 nm. GCase activity was quantified on the basis of the fluorescence intensity of 4-methylumbelliferone (4-MU) and expressed as nmol of 4-MU released per hour per mg of protein via the following equation:

$$\text{Enzymatic activity} = 0.283817 * \frac{X}{Y} \text{ nmol/h/mg}$$

where X is the sample fluorescence value, which is converted to ng/mL 4-MU equivalents, and Y is the total protein concentration in mg/mL.

Statistics and computational methods

Across all the papers, appropriate statistical tests were applied based on data distribution and experimental design. The samples were block randomized and blinded when applicable. Group comparisons were conducted via unpaired t-tests for normally distributed data or Wilcoxon rank-sum tests for non-parametric data, and the results are presented as the means ± SEMs. Associations between continuous variables, such as age or clinical scores, and normally distributed gene expression or cell frequencies were evaluated using linear regression and Pearson correlation analysis. Outliers were systematically identified as values more than 3 times the standard deviation and excluded. All the statistical analyses were performed in GraphPad Prism (Version 10.2.3) or with R software (https://github.com/JTampe/Monocytes), and a p-value < 0.05 was considered significant.

Data availability

All raw data used and needed to reproduce the results presented in all studies are publicly available in supplementary files or on GitHub (https://github.com/JTampe/Monocytes for **Papers I and II**).

Use of language models

This thesis has in part been corrected and restructured with the assistance of the generative AI model ChatGPT. I have processed the generated text (and images) and take full responsibility for the content.

Graphics and design

Adobe InDesign (2025) was used to compile the figures. The experimental study flows were created with BioRender.com. The histograms were created via FlowJo Layouts (10.10.0). SnapGene (Dotmatics) was used to align and visualize the Sanger sequencing data. All the bar plots were generated via GraphPad Prism. The heatmaps were generated using the matrix visualization and analysis software Morpheus (https://software.broadinstitute.org/morpheus) and R (R Core Team (2021). All Pearson correlation regressions were generated using R.

Acknowledgments

As I reach the end of this thesis, I find myself reflecting not only on the scientific journey but also on the personal transformation that accompanied it. These past years have been marked by intense learning, unexpected challenges, and moments of both doubt and determination.

Starting this PhD, I stepped into a field that was largely unfamiliar, neuroimmunology, armed with curiosity and a strong foundation in neuroscience and biochemistry. What followed was a steep learning curve, especially as I navigated the complexities of immunology, clinical collaboration, and translational research. The pandemic added another layer of difficulty, halting sample collection and isolating me from the collaborative environments that are so vital in science. Yet, it was in this isolation that I learned to be resourceful, to set up protocols, and to initiate collaborations that would later become central to my work.

Beyond the bench, I found another kind of purpose. I became involved in the union, helped push for re-opening the beloved gym, and contributed to initiatives around sustainability and equity. These experiences reminded me that science doesn't happen in isolation; it is shaped by the institutions we build and the principles we're willing to defend.

Along the way, I've had the chance to learn from many people: those who guided me, those who challenged me, and those who simply stood beside me. They helped me develop a stronger sense of scientific ownership, sharpen my thinking, and build the resilience needed to navigate this journey.

Just as macrophages are shaped by their surroundings, I've grown through the environments that have supported and challenged me during this journey.

For this, I owe a great deal of gratitude:

To my main supervisor, **Zaza** – I am deeply grateful for the opportunity to work on such a special, translational project. Being chosen to bring life to a long-standing idea, which may have seemed ambitious at first, was both an honor and a challenge. Your critique

has made me into a stronger scientist, with greater awareness and resilience. Thank you for your trust and for pushing me to grow beyond what I thought possible.

To my co-supervisors: **Emanuela (aka Monni)** – Thank you for your expertise, warmth, and sharing your passion for bold colors and great food. **Henrik** – I thoroughly enjoyed our collaborative meetings and truly appreciated your feedback, especially when it came to the molecular aspects. **Arne** – Thank you for guiding us through the hospital culture and making this amazing project possible.

To the mentors who have had a lasting impact: **Isaac** – Your unwavering support for the microglia idea, your constructive feedback, and trust in me, even from afar, meant so much. Like an rtTA lenti virus, you truly induce great things in others. **Olle** – for teaching critical thinking and reminding us that *"There are no positive or negative results, just conclusive or inconclusive results."* Your passion for science is contagious. **Tomas** – Whether as an opponent, mentor, or advocate for mental and physical well-being at the BMC, your presence has been invaluable. Like microglia, you keep the medical faculty alive through both challenge and care.

To the people who stood beside me in the lab: **Linda** – My first office mate and companion on Day 1. Your empathy makes you a truly exceptional *in vivo* scientist, and your insistence on safety and respect is admirable. **Yu-Ping** – From student to teacher, it was an honor to both mentor and learn from you. Teaching you blood and stem cells, and later learning organoid protocols from you, was a beautiful full-circle moment. Thank you for your kindness and for showing your support at my concerts! **Sara** – for "be creative", showing me ambition, self-preservation, and embracing the magic of the lab. **Raquel** – for taking over the cell lab. **Sophiko** – for teaching me and letting me take blood from you. You're an incredible doctor. **Johan** – for your impressive determination and support. **Jenny** – for your honesty, realness, and for speaking up for others. Finally, **Natalia, Marita, Mazin,** and the many students who brought life to the lab even though they stayed too short.

To the Weekend Worriers: **James** – Living together, we incubated many of the ideas that became the microglia project. I thank you for sharing your many insights and perspectives. **Oskar** – for sharing the office, all the dinners, trips, scientific support, and most of all, your friendship. **Franky** – for "coffins", incredible food, gym, and the most entertaining Thursday morning presentations. You've brought laughter and crazy colours to science.

To the rest of B10: **Jonas** – for your calm and remote desktop expertise; **Ella**, for our shared struggles; **Henrico** – for your constant positivity; **Laura** – for your directness; **Constanza** – for the breaks, the conversations, and being on point; **Giggi** – for always being down for gym, coffee, or a beer; **Iran** – for your honesty, shared passion for food (especially BBQs), and after works; **Nika** – for hugs, political advice, and your hard work for MDR; **Darcy** – for being a power woman, an inspiration and speaking up;

Tania – for being part of the unofficial night shift at B10 and the FACS core, as well as trying out daring ideas; **Agnes** – for keeping it real; **Nadja** – for all the happy vibes; **Qianren** – for guidance and shared B2+ worries; **Magda** – for your dry humor and perseverance; **Andreas** – for the gossip; **Edo S.** – for the deep talks. **Tanja** – for telling me everything will be alright; **Emma**, **Magdalena, Anna H. & Servi** for the beginning. Thank you all for Thursday's fikas, BBQs, feedback, and spontaneous help.

*To the **Stem Cell Centre core facilities**, especially the Bioinformatics and FACS core:* **Stefan** – for your patient support with R scripts. **Shamit** – for your humor and open door. **Anna F. –** for teaching me everything about the Flow cytometer and staying calm.

*To the **Stem Cell Centre & Research School**:* especially **Christine**, **Jenny**, and **Pernilla** – your courses offered essential skills and meaningful connections. Thank you for the **PDP** and **MentLife**. And a special thanks to **Sofia**, my mentor and powerful guide from the industry – your advice helped me navigate male-dominated spaces with confidence. Thank you to **Per** – for guidance, **Stefan Hansson –** for your support (and Sicilian travel tips!), and **Olga** – for your transparency and fairness. I also gratefully acknowledge the financial support of **Region Skåne**, **VR**, and the **Hardebo Foundation**. Thanks to **ChatGPT and Copilot** – for always being there at 2 a.m.

To the A10 support crew: **Carolina** – your love for nature, animals, art, and extreme sports made you the perfect partner for recharging. Thank you for hikes, climbing (especially Assassin's Creed style in Lecco!), highlining, and sharing your space. **Alina** – danke für deine unglaubliche Hilfsbereitschaft und gute Gespräche, sowohl wissenschaftlich als auch privat. Also, thanks to **Robert**, **Sub**, **Katarina**, and **Steffen** for all the fika and kindness.

To the B12 / Gym allys: My 3 power women and inspiring me to do great things in this crazy world: **Anna** – You were always there to talk and support. Thank you for always having a shoulder to lean on; **Johanna** – Best roommate for SCC retreats, unmatched energy, and a killer trainer. **Anna R.** – for showing me resilience, fighting for what's right, and also having too much energy! **Mo** – for your kindness and steady presence. **Sara** – for all the "I mean WTF?!" moments. **Jonas & Mikael** – for getting me into 750 and pushing through during the COVID times. **Sandro** – for all the concerts, 3D printing, Mario cart & beer pong tournaments, body doubling, and a great friendship. **Julia**, **Leto**, **Simone, Elena**, **Rasmus**, **Roger**, **Niklas, Oscar, Sally, Roxette**, and all who braved the gym and sauna – your conversations made it unforgettable.

To my Norsemen: **Robi** –for never judging, your strength, and always being there for me on good and bad days. I wish more people would navigate the world with as much kindness as you do! **Marija** – for being unapologetically you, and one of my closest friends since the very beginning of this journey. I love that we can laugh and worry so much together! **Isak** – for the spontaneous music, haircuts, many (very needed) hugs, and food – especially the ramen. Thank you both for always having your home and

hearts open for me (#3rd-wheeling hard). **Eliška** – for the many *Jeli* moments, yoga sessions, CPH outings, my first Swedish camping trip, gaming, and burping together. **Tiago** – for bringing *Benefinica* into my life, the gaming, and the rage quits. **Fabio** – for *picanha*, countless dinners, trips, parties, music, deep discussions, lore, and for being there since the very start. **Evelyn –** danke, fürs Helfen wenn der Sorter einen verrückt macht, die Gesellschaft an zu späten Abenden, und geniales veganes essen! **Albert –** for the amazing Pizza's and other foods we bonded over – thank you all for enriching my world. To all the extended be creative gang: **Kat**, **Davide**, **Maria**, **Alex**, **Marta**, **Lavanya, Chang, Edo B.**, **Jessica**, **Victoria**, **Valeriia, Nic**, **Mert**, **Carletto**, **Edo**, **Margareta**, **Alrik**, **Liva**, **Giorgios**, **Luis**, **Rosali**, **Steven**, **Alex & Barbara** – and all the others whose laughter, company, and solidarity accompanied me.

*To **AMC Bleckhornen*** – When the PhD became too much, you provided the much-needed balance to all the science. Music has always been an escape for me, and being part of this amazing *corpset* truly gave me that outlet. **David** – I found a true friend in you – thank you for all the spontaneous trainings at Victoriastaden, the section groggs, or simply catching some fresh air together. **Hugo**, **Karin & Tove** – I loved being part of the **Styrelsen** with you! We're all so different, yet we share a commitment to transparency and equal opportunity. Keep fighting for that, wherever life takes you. **Stina** & **Nanna** – for amazing moments, great music, and for being fierce, fearless forces (*fff*) of nature. **Simon** & **Frida** – for always being so welcoming and helpful. **Ludvig**, our dearest leader, of the best section there is! *"Var är pocket?"* still echoes fondly in my ears. **Rogge, Emma, Siggi, Marre, Eric, Freddy**, **Ferdi**, **Marina** & **Florian** – I'll miss our balcony chats, lunches, and those spontaneous conversations that made every day lighter. I truly hope we'll continue making music together – whether at Christmas concerts, Doktorspromovering, or Karneval. *Fjong!*

*To the whole **FUNKtional Group:*** **Martin, Ferdi, Simon P., Hanbang, Simon S., Albin, Edo, Romain,** and **Hanna** – for letting me be part of your jams, concerts, and great atmosphere.

To the many roommates: **Jonas** – my 1st Swedish friend! For welcoming me into your home, your trust, and great discussions. You are a great friend, and I hope the future holds more beers, camping trips, or jt balcony hangouts. **Halley** – your energy is unmatched! Keep on shining light into this crazy world! **Lotte** – for the many conversations, perspectives, board games, and watching glassblowing! **Piet** – for the 30 min before you got food and I was allowed to cuddle you. Miauuuuu! **Lukas** – for the Mett; **Elias** – für unseren NaturKlub den wir mit 7 Jahren gegründet haben, unerwartete Begegnungen, und schon ewige Freundschaft. **Theresa** – for not giving up on academia – yet, the many shared beers on the balcony, and enduring me during this last crazy stretch of the PhD. **Max** – for the beer club and passion for fire. **Kay** – for crazy instant coffee & workout routines.

Für die Mönsche, wo ich i de Schwiiz han müesse zruggloh: **Laura** – dass du immer mini direkti, furchtlosi Fründin gsi bisch. Du frögsch alles, nimmst kei Blatt vor dä Mund, und ich vermiss League of Legends-Abig und kajak toure mit dir; **Eric** – dass du immer für mich da bisch – ganz gliich, öb zum tiefsinnige Diskutiere oder zum zäme nüt mache. Ich schätz es ungemein, dass i eu beidi jederzeit ahlüüte cha, ganz gliich, wie's mir grad gaht – und dass mir, wenn mir eus gseh, eifach chönd zäme sii, ganz locker und unkompliziert. **Max** – du warst wahrscheinlich der Erste, der mir klargemacht hat, was für ein fettes Ding ich mir da aufgehalst habe: *"4 Jahre in f**** Schweden!"** – Und du hattest sowas von recht. **Aro & Sabi –** for all the crazy trips, chicken nights, infinity pool moments or sending just Meme's around the world! **Roy** – für die Pfingstrips und guten Rat. **Challenge OK '15** – and all the wonderful energy and passion of the amazing humans I met through the challenge community: **Leonie**, **Eti**, **Fabio**, **Cosima**, **Eva** & **Cookie** – *HARDY PART forever!* **Bibi** – für ois beidne Liebi für Studenteverein und ois charmant-nervige Ufrege über schwedischi Klischees. Mit dir ha-n-i immer chönne lache, uufrege und Plän schmiede – merci viu mau! **Andi & Henni** – für die Gespräche; **Nici** – du bisch ä richtigs Einhorn!

To the people furthest away: The San Diego gang. **Steffania**, **Xavi, Pablo, Stjepan, and Patrick** – for the great science discussions, escapes, climbing, shared food, and evenings! Thank you! **Peter** – for the weird humor, meeting up in the most random places on earth, and being my brother from another mother!

An die, mit denen ich groß geworden bin: **Luki –** ich glaube, wir beide waren die ersten Versuchskaninchen in der Freundesgruppe unserer Eltern… Ich bin sehr froh, dass ich damit nicht allein war! **Xenia (Xeni-Beni)**, **Jessi & Max** – dass ihr mich schon so lange ertragt, dass es immer genauso ist wie früher, ganz egal, wie viel Zeit vergangen ist. Und danke dafür, dass ihr einfach tolle Menschen seid! Ein riesiges Dankeschön auch an meine „**Herde**" – besonders **Hanna**, **Franzi**, **Marcel**, **Robin** und **Carlie**! Ich bin so dankbar, dass wir es trotz aller Distanzen immer wieder schaffen, uns zu sehen. Ihr habt mich schon als verrückten Teenager unterstützt – und nie losgelassen. **Seb & Caro** – dass ihr immer am Start seid – ob auf dem Boot, auf der Piste oder bei awkward FaceTime-Calls!

To **Thomas,** thank you for being so patient throughout this entire journey and for more than 15 years of friendship. You are my anchor and safe harbour. Thank you for making even the hardest days feel lighter, for believing in me, and for reminding me to take breaks. Thank you for all the feedback – whether on English, how to navigate tough situations, or just how to handle life. You've helped make me a better person.

Danke an **Roxanna,** für deine enorme Stärke, Liebe, tiefgründigen Diskussionen und dafür, dass du immer ein offenes Ohr für mich hattest. **Werner** – für deinen Humor und Lebensfreude. **Clara** – dafür, dass du genauso stark bist wie deine Mum, für dein Vertrauen und deine Begeisterung für die Wissenschaft.

Zu guter Letzt an meine großartige Familie: Am Ende gehen die wichtigsten Danksagungen an die Menschen, die mich mein ganzes Leben begleitet haben – leider auch nicht immer in Person. **Opa Karl-Heinz** – als Tüftler und Visionär hast du sowohl mich als auch meinen Bruder stets zu neuen, verrückten Ideen gebracht – und sie mit uns umgesetzt: ob FischerTechnik oder umgebauter Rasenmäher… Du hast mich von Anfang an dazu inspiriert, Dinge auszuprobieren und nach Lösungen zu suchen, anstatt die Welt einfach so hinzunehmen, wie sie ist. Aber am wichtigsten war, du hast mir beigebracht, anderen mit Freundlichkeit und ohne Vorurteile zu begegnen. **Oma Trude** – du hast mir mit deinen raffinierten Zügen beim Räuber-Rommé gezeigt, dass es oft versteckte Lösungen gibt, die nicht gleich sichtbar sind. Du hast mir beigebracht, wie wertvoll ein anderer Blickwinkel sein kann. **Oma Renate** – meine Verbündete, wenn alles gegen mich stand. Du hast immer zu mir gehalten und mich daran erinnert, dass ich genug bin – und dass es völlig okay ist, gegen den Strom zu schwimmen, aus Überzeugung für das, was richtig ist. **Opa Heinz** – wir hatten leider nur wenig Zeit zusammen, aber ich bin mir sicher, dass ich mein analytisches Denken deinen Genen zu verdanken habe. **Tante Annelie** – du warst mein Licht in der dunkelsten Zeit meines PhDs. Ich vermisse dein Lachen – und dich – noch viel mehr. **Dad** – es ist kein Geheimnis, dass der Apfel nicht weit vom Stamm gefallen ist. Deine Leidenschaft und Energie für die Wissenschaft inspirieren mich bis heute – auch wenn unsere Diskussionen über das akademische System oft kritisch waren. Du hast mir Verantwortung und Ehrlichkeit mitgegeben und mich immer dazu ermutigt, für das zu kämpfen, was man wirklich will. **Mum** – du warst immer meine Balance – auf kreative, spirituelle oder empathische Art. Mit tausenden Museen, Kirchen und Bildern hast du mir die schönen, sanften, ruhigen, aber auch dramatischen Seiten der Welt gezeigt. Du hast mir beigebracht, für mich selbst einzustehen, innezuhalten und die Schönheit des Moments und der Details zu erkennen. Du stellst immer andere vor dich – und so auch mich. Gemeinsam mit Papa habt ihr mir meine Ausbildung ermöglicht, die mir nun mein ganzes Leben eröffnet. DANKE! **Johnny** – *la gente está muy loca* – und oh ja, sind die Leute verrückt… Dank dir, den vielen Telefonaten, Reisen und deiner ständigen Präsenz bin ich nicht verrückt geworden. Keine Worte können beschreiben, wie dankbar ich bin, dich als Bruder zu haben. Egal ob Problem, Freude oder völliges Chaos – ich kann alles mit dir teilen, und du nimmst dir immer Zeit für mich. Danke, dass ihr immer für mich da seid!

This journey has not been linear, and it certainly hasn't been easy. But it has been transformative. I leave this chapter not only with new scientific insights but with a clearer understanding of the kind of scientist – and person – I want to be: collaborative, curious, and committed to making science more reproducible, transparent, and inclusive.

References

Abdu, H. and Seyoum, G. (2022). *"Sex Differences in Stroke Risk Factors, Clinical Profiles, and in-Hospital Outcomes among Stroke Patients Admitted to the Medical Ward of Dessie Comprehensive Specialized Hospital, Northeast Ethiopia."* Degener Neurol Neuromuscul Dis 12: p. 133-144.

Abildgaard, J., et al. (2020). *"Increased Systemic Inflammation and Altered Distribution of T-Cell Subsets in Postmenopausal Women."* PLoS One 15(6): p. e0235174.

Abud, E. M., et al. (2017). *"Ipsc-Derived Human Microglia-Like Cells to Study Neurological Diseases."* Neuron 94(2): p. 278-293 e279.

Aflaki, E., et al. (2016). *"Efferocytosis Is Impaired in Gaucher Macrophages."* Haematologica 102(4): p. 656-665.

Aflaki, E., et al. (2016). *"Lysosomal Storage and Impaired Autophagy Lead to Inflammasome Activation in Gaucher Macrophages."* Aging Cell 15(1): p. 77-88.

Aflaki, E., et al. (2014). *"Macrophage Models of Gaucher Disease for Evaluating Disease Pathogenesis and Candidate Drugs."* Science Translational Medicine 6(240).

Alaei, M. R., et al. (2019). *"Gaucher Disease: New Expanded Classification Emphasizing Neurological Features."* Iran J Child Neurol 13(1): p. 7-24.

Arnold, I. C., et al. (2016). *"Cd11c(+) Monocyte/Macrophages Promote Chronic Helicobacter Hepaticus-Induced Intestinal Inflammation through the Production of Il-23."* Mucosal Immunol 9(2): p. 352-363.

Auffray, C., et al. (2009). *"Blood Monocytes: Development, Heterogeneity, and Relationship with Dendritic Cells."* Annu Rev Immunol 27: p. 669-692.

Austermann, J., et al. (2022). *"The Good and the Bad: Monocytes' and Macrophages' Diverse Functions in Inflammation."* Cells 11(12).

Barrowman, M. A., et al. (2019). *"How Unmeasured Confounding in a Competing Risks Setting Can Affect Treatment Effect Estimates in Observational Studies."* BMC Medical Research Methodology 19(1): p. 166.

Benetó, N., et al. (2020). *"Neuronal and Astrocytic Differentiation from Sanfilippo C Syndrome Ipscs for Disease Modeling and Drug Development."* Journal of Clinical Medicine 9(3).

Blumenfeld, H. (2010). "*Neuroanatomy through Clinical Cases"*, Sinauer Associates.

Boddupalli, C. S., et al. (2022). *"Neuroinflammation in Neuronopathic Gaucher Disease: Role of Microglia and Nk Cells, Biomarkers, and Response to Substrate Reduction Therapy."* Elife 11.

Boehme, A. K., et al. (2017). *"Stroke Risk Factors, Genetics, and Prevention."* Circ Res 120(3): p. 472-495.

Brunialti, E., et al. (2021). *"Inhibition of Microglial B-Glucocerebrosidase Hampers the Microglia-Mediated Antioxidant and Protective Response in Neurons."* Journal of Neuroinflammation 18(1).

Bushnell, C. D., et al. (2014). *"Sex Differences in Quality of Life after Ischemic Stroke."* Neurology 82(11): p. 922-931.

Canals, I., et al. (2023). *"Astrocyte Dysfunction and Neuronal Network Hyperactivity in a Crispr Engineered Pluripotent Stem Cell Model of Frontotemporal Dementia."* Brain Communications 5(3): p. fcad158.

Cao, X., et al. (2019). *"Differentiation and Functional Comparison of Monocytes and Macrophages from Hipscs with Peripheral Blood Derivatives."* Stem Cell Reports 12(6): p. 1282-1297.

Cao, Y., et al. (2024). *"Why Is a Small Sample Size Not Enough?"* The Oncologist 29(9): p. 761-763.

Carmona-Mora, P., et al. (2023). *"Monocyte, Neutrophil, and Whole Blood Transcriptome Dynamics Following Ischemic Stroke."* BMC Medicine 21(1): p. 65.

Chen, S., et al. (2023). *"Macrophages in Immunoregulation and Therapeutics."* Signal Transduct Target Ther 8(1): p. 207.

Chen, S. W., et al. (2021). *"Efficient Conversion of Human Induced Pluripotent Stem Cells into Microglia by Defined Transcription Factors."* Stem Cell Reports 16(5): p. 1363-1380.

Chen, S. W. and Wong, Y. H. (2023). *"Directed Differentiation of Human Ipscs into Microglia-Like Cells Using Defined Transcription Factors."* Methods Mol Biol 2683: p. 53-68.

Chin, M. Y., et al. (2022). *"Phenotypic Screening Using High-Content Imaging to Identify Lysosomal Ph Modulators in a Neuronal Cell Model."* Acs Chemical Neuroscience 13(10): p. 1505-1516.

Chiu, S. and Bharat, A. (2016). *"Role of Monocytes and Macrophages in Regulating Immune Response Following Lung Transplantation."* Curr Opin Organ Transplant 21(3): p. 239-245.

Cho, S., et al. (2005). *"The Class B Scavenger Receptor Cd36 Mediates Free Radical Production and Tissue Injury in Cerebral Ischemia."* J Neurosci 25(10): p. 2504-2512.

Choquet, K., et al. (2025). *"The Regulation and Function of Post-Transcriptional Rna Splicing."* Nature Reviews Genetics 26(6): p. 378-394.

Chou, A., et al. (2018). *"Persistent Infiltration and Impaired Response of Peripherally-Derived Monocytes after Traumatic Brain Injury in the Aged Brain."* International Journal of Molecular Sciences 19(6): p. 1616.

Colonna, M. and Butovsky, O. (2017). *"Microglia Function in the Central Nervous System During Health and Neurodegeneration."* Annu Rev Immunol 35: p. 441-468.

Conradi, N. G., et al. (1984). *"Neuropathology of the Norrbottnian Type of Gaucher Disease. Morphological and Biochemical Studies."* Acta Neuropathol 65(2): p. 99-109.

Conti, E., et al. (2023). *"Tspo Modulates Oligomeric Amyloid-Beta-Induced Monocyte Chemotaxis: Relevance for Neuroinflammation in Alzheimer's Disease."* J Alzheimers Dis 95(2): p. 549-559.

Cramer, S. C. and Riley, J. D. (2008). *"Neuroplasticity and Brain Repair after Stroke."* Curr Opin Neurol 21(1): p. 76-82.

Croese, T., et al. (2021). *"Immune Cell Compartmentalization for Brain Surveillance and Protection."* Nat Immunol 22(9): p. 1083-1092.

Cronk, J. C., et al. (2018). *"Peripherally Derived Macrophages Can Engraft the Brain Independent of Irradiation and Maintain an Identity Distinct from Microglia."* J Exp Med 215(6): p. 1627-1647.

Cuadros, M. A., et al. (2022). *"Microglia and Microglia-Like Cells: Similar but Different."* Front Cell Neurosci 16: p. 816439.

Cuschieri, J., et al. (2023). *"The Injured Monocyte: The Link to Chronic Critical Illness and Mortality Following Injury."* Journal of Trauma and Acute Care Surgery.

D'Ambrosio, A. L., et al. (2004). *"A Modified Transorbital Baboon Model of Reperfused Stroke."* Methods Enzymol 386: p. 60-73.

D'Amore, S., et al. (2021). *"In-Depth Phenotyping for Clinical Stratification of Gaucher Disease."* Orphanet Journal of Rare Diseases 16(1).

D'Anna, L., et al. (2023). *"Time Course of Neuroinflammation after Human Stroke - a Pilot Study Using Co-Registered Pet and Mri."* BMC Neurol 23(1): p. 193.

Dancause, N. and Nudo, R. J. (2011). *"Shaping Plasticity to Enhance Recovery after Injury."* Prog Brain Res 192: p. 273-295.

De Francesco, D., et al. (2020). *"Monocyte and T Cell Immune Phenotypic Profiles Associated with Age Advancement Differ between People with Hiv, Lifestyle-Comparable Controls and Blood Donors."* Front Immunol 11: p. 581616.

DeLaughter, D. M. (2018). *"The Use of the Fluidigm C1 for Rna Expression Analyses of Single Cells."* Current Protocols in Molecular Biology 122(1).

Deng, Z., et al. (2024). *"Tgf-Beta Signaling in Health, Disease, and Therapeutics."* Signal Transduct Target Ther 9(1): p. 61.

Dimitrijevic, O. B., et al. (2007). *"Absence of the Chemokine Receptor Ccr2 Protects against Cerebral Ischemia/Reperfusion Injury in Mice."* Stroke 38(4): p. 1345-1353.

Doyle, K. P., et al. (2015). *"B-Lymphocyte-Mediated Delayed Cognitive Impairment Following Stroke."* J Neurosci 35(5): p. 2133-2145.

Dupuis, L., et al. (2022). *"Phagocytosis of Erythrocytes from Gaucher Patients Induces Phenotypic Modifications in Macrophages, Driving Them toward Gaucher Cells."* Int J Mol Sci 23(14).

Ermine, C. M., et al. (2021). *"The Ischemic Penumbra: From Concept to Reality."* Int J Stroke 16(5): p. 497-509.

Ernst, M., et al. (2018). *"Impact of Ischemic Lesion Location on the Mrs Score in Patients with Ischemic Stroke: A Voxel-Based Approach."* American Journal of Neuroradiology 39(11): p. 1989.

Evans, T. A., et al. (2014). *"High-Resolution Intravital Imaging Reveals That Blood-Derived Macrophages but Not Resident Microglia Facilitate Secondary Axonal Dieback in Traumatic Spinal Cord Injury."* Experimental Neurology 254: p. 109-120.

Feigin, V. L., et al. (2025). *"World Stroke Organization: Global Stroke Fact Sheet 2025."* Int J Stroke 20(2): p. 132-144.

Feigin, V. L., et al. (2014). *"Global and Regional Burden of Stroke During 1990-2010: Findings from the Global Burden of Disease Study 2010."* Lancet 383(9913): p. 245-254.

Feigin, V. L., et al. (2007). *"Burden of Stroke in Maori and Pacific Peoples of New Zealand."* International Journal of Stroke 2(3): p. 208-210.

Feng, L., et al. (2023). *"Identification of Anhydrodebromoaplysiatoxin as a Dichotomic Autophagy Inhibitor."* Marine Drugs 21(1): p. 46.

Filiano, A. J., et al. (2010). *"Transglutaminase 2 Protects against Ischemic Stroke."* Neurobiol Dis 39(3): p. 334-343.

Filippenkov, I. B., et al. (2023). *"Differential Gene Expression in the Contralateral Hemisphere of the Rat Brain after Focal Ischemia."* Sci Rep 13(1): p. 573.

García-Bonilla, L., et al. (2018). *"Endogenous Protection from Ischemic Brain Injury by Preconditioned Monocytes."*

Garland, E. F., et al. (2022). *"Microglia and Astrocyte Function and Communication: What Do We Know in Humans?"* Front Neurosci 16: p. 824888.

Ge, R., et al. (2017). *"Choroid Plexus-Cerebrospinal Fluid Route for Monocyte-Derived Macrophages after Stroke."* J Neuroinflammation 14(1): p. 153.

Ghatnekar, O., et al. (2014). *"Costs for Stroke in Sweden 2009 and Developments since 1997."* Int J Technol Assess Health Care 30(2): p. 203-209.

Ghatnekar, O., et al. (2004). *"Cost of Stroke in Sweden: An Incidence Estimate."* Int J Technol Assess Health Care 20(3): p. 375-380.

Giannoni, E., et al. (2011). *"Estradiol and Progesterone Strongly Inhibit the Innate Immune Response of Mononuclear Cells in Newborns."* Infect Immun 79(7): p. 2690-2698.

Gomez Perdiguero, E., et al. (2015). *"Tissue-Resident Macrophages Originate from Yolk-Sac-Derived Erythro-Myeloid Progenitors."* Nature 518(7540): p. 547-551.

Grage-Griebenow, E., et al. (2001). *"Heterogeneity of Human Peripheral Blood Monocyte Subsets."* J Leukoc Biol 69(1): p. 11-20.

Gurdon, J. B. (1962). *"The Developmental Capacity of Nuclei Taken from Intestinal Epithelium Cells of Feeding Tadpoles."* J Embryol Exp Morphol 10: p. 622-640.

Haeusler, K. G., et al. (2008). *"Cellular Immunodepression Preceding Infectious Complications after Acute Ischemic Stroke in Humans."* Cerebrovasc Dis 25(1-2): p. 50-58.

Heil, F., et al. (2004). *"Species-Specific Recognition of Single-Stranded Rna Via Toll-Like Receptor 7 and 8."* Science 303(5663): p. 1526-1529.

Hertz, E., et al. (2024). *"Comparative Study of Enriched Dopaminergic Neurons from Siblings with Gaucher Disease Discordant for Parkinsonism."*

Hirokawa, K., et al. (2013). *"Slower Immune System Aging in Women Versus Men in the Japanese Population."* Immun Ageing 10(1): p. 19.

Hopf, S., et al. (2019). *"A Comprehensive Monocentric Ophthalmic Study with Gaucher Disease Type 3 Patients: Vitreoretinal Lesions, Retinal Atrophy and Characterization of Abnormal Saccades."* Orphanet Journal of Rare Diseases 14(1).

Hsieh, C. L., et al. (2014). *"Ccr2 Deficiency Impairs Macrophage Infiltration and Improves Cognitive Function after Traumatic Brain Injury."* Journal of Neurotrauma 31(20): p. 1677-1688.

Huang, Y., et al. (2018). *"Repopulated Microglia Are Solely Derived from the Proliferation of Residual Microglia after Acute Depletion."* Nat Neurosci 21(4): p. 530-540.

Huber, T. L., et al. (2004). *"Haemangioblast Commitment Is Initiated in the Primitive Streak of the Mouse Embryo."* Nature 432(7017): p. 625-630.

Hudig, D., et al. (2014). *"Properties of Human Blood Monocytes. Ii. Monocytes from Healthy Adults Are Highly Heterogeneous within and among Individuals."* Cytometry B Clin Cytom 86(2): p. 121-134.

Hughes, D. A. and Pastores, G. M. (1993). Gaucher Disease. Genereviews((R)). M. P. Adam, J. Feldman, G. M. Mirzaa et al. Seattle (WA).

Hurd, M. D., et al. (2021). *"Current Status of Ischemic Stroke Treatment: From Thrombolysis to Potential Regenerative Medicine."* Regen Ther 18: p. 408-417.

Inoue, H. and Yamanaka, S. (2011). *"The Use of Induced Pluripotent Stem Cells in Drug Development."* Clinical Pharmacology & Therapeutics 89(5): p. 655-661.

Ito, M., et al. (2018). *"Rna-Sequencing Analysis Revealed a Distinct Motor Cortex Transcriptome in Spontaneously Recovered Mice after Stroke."* Stroke 49(9): p. 2191-2199.

Jaipersad, A. S., et al. (2014). *"The Role of Monocytes in Angiogenesis and Atherosclerosis."* J Am Coll Cardiol 63(1): p. 1-11.

Jerath, N. U., et al. (2011). *"Gender Differences in Presenting Signs and Symptoms of Acute Ischemic Stroke: A Population-Based Study."* Gend Med 8(5): p. 312-319.

Jin, R., et al. (2010). *"Inflammatory Mechanisms in Ischemic Stroke: Role of Inflammatory Cells."* J Leukoc Biol 87(5): p. 779-789.

Jurga, A. M., et al. (2020). *"Overview of General and Discriminating Markers of Differential Microglia Phenotypes."* Front Cell Neurosci 14: p. 198.

Kaito, M., et al. (2013). *"Relevance of Distinct Monocyte Subsets to Clinical Course of Ischemic Stroke Patients."* PLoS One 8(8): p. e69409.

Katan, M. and Luft, A. (2018). *"Global Burden of Stroke."* Semin Neurol 38(2): p. 208-211.

Kelly-Hayes, M. (2010). *"Influence of Age and Health Behaviors on Stroke Risk: Lessons from Longitudinal Studies."* J Am Geriatr Soc 58 Suppl 2(Suppl 2): p. S325-328.

Kilpinen, H., et al. (2017). *"Common Genetic Variation Drives Molecular Heterogeneity in Human Ipscs."* Nature 546(7658): p. 370-375.

Kim, H. J., et al. (2022). *"Blood Monocyte-Derived Cd169(+) Macrophages Contribute to Antitumor Immunity against Glioblastoma."* Nat Commun 13(1): p. 6211.

Kim, S., et al. (2021). *"Chitotriosidase as a Biomarker for Gangliosidoses."* Molecular Genetics and Metabolism Reports 29: p. 100803.

Kleinig, T. J. and Vink, R. (2009). *"Suppression of Inflammation in Ischemic and Hemorrhagic Stroke: Therapeutic Options."* Curr Opin Neurol 22(3): p. 294-301.

Kogut, I., et al. (2018). *"High-Efficiency Rna-Based Reprogramming of Human Primary Fibroblasts."* Nature Communications 9(1).

Kolosowska, N., et al. (2019). *"Peripheral Administration of Il-13 Induces Anti-Inflammatory Microglial/Macrophage Responses and Provides Neuroprotection in Ischemic Stroke."* Neurotherapeutics 16(4): p. 1304-1319.

Krishnamurthi, R. V., et al. (2020). *"Global, Regional and Country-Specific Burden of Ischaemic Stroke, Intracerebral Haemorrhage and Subarachnoid Haemorrhage: A Systematic Analysis of the Global Burden of Disease Study 2017."* Neuroepidemiology 54(2): p. 171-179.

Krishnamurthi, R. V., et al. (2015). *"Stroke Prevalence, Mortality and Disability-Adjusted Life Years in Adults Aged 20-64 Years in 1990-2013: Data from the Global Burden of Disease 2013 Study."* Neuroepidemiology 45(3): p. 190-202.

Krzyszczyk, P., et al. (2018). *"The Role of Macrophages in Acute and Chronic Wound Healing and Interventions to Promote Pro-Wound Healing Phenotypes."* Front Physiol 9: p. 419.

Kumar, S., et al. (2018). *"Induced Pluripotent Stem Cells in Disease Modeling and Gene Identification."* p. 17-38.

Lambertsen, K. L., et al. (2019). *"Post-Stroke Inflammation-Target or Tool for Therapy?"* Acta Neuropathol 137(5): p. 693-714.

Laterza, C., et al. (2017). *"Monocyte Depletion Early after Stroke Promotes Neurogenesis from Endogenous Neural Stem Cells in Adult Brain."* Exp Neurol 297: p. 129-137.

Le Garff-Tavernier, M., et al. (2010). *"Human Nk Cells Display Major Phenotypic and Functional Changes over the Life Span."* Aging Cell 9(4): p. 527-535.

Li, D. L., et al. (2016). *"Doxorubicin Blocks Cardiomyocyte Autophagic Flux by Inhibiting Lysosome Acidification."* Circulation 133(17): p. 1668-1687.

Li, L., et al. (2018). *"Modeling Neurological Diseases Using Ipsc-Derived Neural Cells."* Cell and Tissue Research 371(1): p. 143-151.

Li, X., et al. (2019). *"The Prognostic Value of Serum Cytokines in Patients with Acute Ischemic Stroke."* Aging Dis 10(3): p. 544-556.

Lier, J., et al. (2021). *"Beyond Activation: Characterizing Microglial Functional Phenotypes."* Cells 10(9).

Lindsay, M. P., et al. (2019). *"World Stroke Organization (Wso): Global Stroke Fact Sheet 2019."* Int J Stroke 14(8): p. 806-817.

Liu, L., et al. (2020). *"Interaction of Microglia and Astrocytes in the Neurovascular Unit."* Frontiers in Immunology 11.

López, M., et al. (2014). *"Thrombin Selectively Induces Transcription of Genes in Human Monocytes Involved in Inflammation and Wound Healing."* Thrombosis and Haemostasis 112(11): p. 992-1001.

Ma, Y., et al. (2021). *"The Role of Immune Cells in Post-Stroke Angiogenesis and Neuronal Remodeling: The Known and the Unknown."* Front Immunol 12: p. 1664-3224.

Macosko, E. Z., et al. (2015). *"Highly Parallel Genome-Wide Expression Profiling of Individual Cells Using Nanoliter Droplets."* Cell 161(5): p. 1202-1214.

Maecker, H. T., et al. (2012). *"Standardizing Immunophenotyping for the Human Immunology Project."* Nat Rev Immunol 12(3): p. 191-200.

Marino Lee, S., et al. (2021). *"Microglia Depletion Increase Brain Injury after Acute Ischemic Stroke in Aged Mice."* Exp Neurol 336: p. 113530.

Márquez, E. J., et al. (2020). *"Sexual-Dimorphism in Human Immune System Aging."* Nature Communications 11(1).

Mayo, L., et al. (2008). *"Dual Role of Cd38 in Microglial Activation and Activation-Induced Cell Death."* J Immunol 181(1): p. 92-103.

McNamara, E. R., et al. (2014). *"Neurogenic Bladder Dysfunction Presenting as Urinary Retention in Neuronopathic Gaucher Disease."* Jimd Reports.

McTague, A., et al. (2021). *"Genome Editing in Ipsc-Based Neural Systems: From Disease Models to Future Therapeutic Strategies."* Front Genome Ed 3: p. 630600.

Mensah, G. A., et al. (2015). *"The Global Burden of Stroke."* Neuroepidemiology 45(3): p. 143-145.

Metcalf, T. U., et al. (2017). *"Human Monocyte Subsets Are Transcriptionally and Functionally Altered in Aging in Response to Pattern Recognition Receptor Agonists."* J Immunol 199(4): p. 1405-1417.

Mindell, J. A. (2012). *"Lysosomal Acidification Mechanisms."* Annual Review of Physiology 74(1): p. 69-86.

Mondal, A., et al. (2022). *"Ppt1-Deficiency Dysregulates Lysosomal Ca++ Homeostasis Contributing to Pathogenesis in a Mouse Model of Cln1 Disease."* Journal of Inherited Metabolic Disease 45(3): p. 635-656.

Morgenstern, L. B., et al. (2013). *"Persistent Ischemic Stroke Disparities Despite Declining Incidence in Mexican Americans."* Annals of Neurology 74(6): p. 778-785.

Moskowitz, M. A., et al. (2010). *"The Science of Stroke: Mechanisms in Search of Treatments."* Neuron 67(2): p. 181-198.

Mosser, D. M., et al. (2021). *"Macrophages and the Maintenance of Homeostasis."* Cell Mol Immunol 18(3): p. 579-587.

Muffat, J., et al. (2016). *"Efficient Derivation of Microglia-Like Cells from Human Pluripotent Stem Cells."* Nat Med 22(11): p. 1358-1367.

Nayak, D., et al. (2014). *"Microglia Development and Function."* Annu Rev Immunol 32: p. 367-402.

Ornello, R., et al. (2018). *"Distribution and Temporal Trends from 1993 to 2015 of Ischemic Stroke Subtypes: A Systematic Review and Meta-Analysis."* Stroke 49(4): p. 814-819.

Oswald, I. P., et al. (1992). *"Il-10 Synergizes with Il-4 and Transforming Growth Factor-Beta to Inhibit Macrophage Cytotoxic Activity."* J Immunol 148(11): p. 3578-3582.

Pandya, H., et al. (2017). *"Differentiation of Human and Murine Induced Pluripotent Stem Cells to Microglia-Like Cells."* Nature Neuroscience 20(5): p. 753-759.

Panicker, L. M., et al. (2014). *"Gaucher Ipsc-Derived Macrophages Produce Elevated Levels of Inflammatory Mediators and Serve as a New Platform for Therapeutic Development."* The International Journal of Cell Cloning 32(9): p. 2338-2349.

Park, J., et al. (2018). *"A 3d Human Triculture System Modeling Neurodegeneration and Neuroinflammation in Alzheimer's Disease."* Nat Neurosci 21(7): p. 941-951.

Park, K. W., et al. (2022). *"Delayed Infiltration of Peripheral Monocyte Contributes to Phagocytosis and Transneuronal Degeneration in Chronic Stroke."* Stroke 53(7): p. 2377-2388.

Passlick, B., et al. (1989). *"Identification and Characterization of a Novel Monocyte Subpopulation in Human Peripheral Blood."* Blood 74(7): p. 2527-2534.

Patel, A. A., et al. (2017). *"The Fate and Lifespan of Human Monocyte Subsets in Steady State and Systemic Inflammation."* J Exp Med 214(7): p. 1913-1923.

Pedragosa, J., et al. (2020). *"Ccr2 Deficiency in Monocytes Impairs Angiogenesis and Functional Recovery after Ischemic Stroke in Mice."* J Cereb Blood Flow Metab 40(1_suppl): p. S98-S116.

Pelekanou, V., et al. (2016). *"Estrogen Anti-Inflammatory Activity on Human Monocytes Is Mediated through Cross-Talk between Estrogen Receptor Eralpha36 and Gpr30/Gper1."* J Leukoc Biol 99(2): p. 333-347.

Perretti, M. and D'Acquisto, F. (2009). *"Annexin A1 and Glucocorticoids as Effectors of the Resolution of Inflammation."* Nat Rev Immunol 9(1): p. 62-70.

Planas, A. M. (2018). *"Role of Immune Cells Migrating to the Ischemic Brain."* Stroke 49(9): p. 2261-2267.

Prabhakar, K., et al. (2019). *"Correlation of Serum Calcium Levels with Infarct Size and Severity of Stroke Using Nihss Score in Patients with Acute Ischemic Stroke."* International Journal of Research in Medical Sciences 8(1): p. 55.

Prinz, M. (2014). *"Microglia and Monocytes: Molecularly Defined."* Acta Neuropathologica 128(3): p. 317-318.

Ramirez-Santana, M. (2018). Limitations and Biases in Cohort Studies. Cohort Studies in Health Sciences. R. M. Barría. Rijeka, IntechOpen.

Rice, E., et al. (1996). *"Gaucher Disease: Studies of Phenotype, Molecular Diagnosis and Treatment."* Clinical Genetics 49(3): p. 111-118.

Ritzel, R. M., et al. (2015). *"Functional Differences between Microglia and Monocytes after Ischemic Stroke."* Journal of Neuroinflammation 12(1).

Roth, S., et al. (2018). *"Brain-Released Alarmins and Stress Response Synergize in Accelerating Atherosclerosis Progression after Stroke."* Sci Transl Med 10(432).

Rovira-Moreno, E., et al. (2023). *"The Diagnosis Communication Process in Spinal Muscular Atrophy: A Cross-Cutting View of the New Challenges Facing the Therapeutic Era."* Genet Med Open 1(1): p. 100825.

Ryczkowska, K., et al. (2023). *"Menopause and Women?S Cardiovascular Health: Is It Really an Obvious Relationship?"* Archives of Medical Science 19(2): p. 458-466.

Rymer, M. M. (2011). *"Hemorrhagic Stroke: Intracerebral Hemorrhage."* Mo Med 108(1): p. 50-54.

Sacco, R. L., et al. (2013). *"An Updated Definition of Stroke for the 21st Century: A Statement for Healthcare Professionals from the American Heart Association/American Stroke Association."* Stroke 44(7): p. 2064-2089.

Sasaki, T., et al. (2014). *"Aberrant Autolysosomal Regulation Is Linked to the Induction of Embryonic Senescence: Differential Roles of Beclin 1 and P53 in Vertebrate Spns1 Deficiency."* Plos Genetics 10(6): p. e1004409.

Schöndorf, D. C., et al. (2014). *"Ipsc-Derived Neurons from Gba1-Associated Parkinson's Disease Patients Show Autophagic Defects and Impaired Calcium Homeostasis."* Nature Communications 5: p. 4028.

Shafieesabet, A., et al. (2023). *"Plasma Bioactive Adrenomedullin Predicts Outcome after Acute Stroke in Early Rehabilitation."* Scientific Reports 13(1).

Shemesh, E., et al. (2015). *"Enzyme Replacement and Substrate Reduction Therapy for Gaucher Disease."* Cochrane Database Syst Rev 2015(3): p. CD010324.

Shi, C. and Pamer, E. G. (2011). *"Monocyte Recruitment During Infection and Inflammation."* Nat Rev Immunol 11(11): p. 762-774.

Sidransky, E. and Lopez, G. (2012). *"The Link between the Gba Gene and Parkinsonism."* The Lancet Neurology 11(11): p. 986-998.

Siebert, M., et al. (2014). *"Glucocerebrosidase Is Shaking up the Synucleinopathies."* Brain 137(5): p. 1304-1322.

Sieweke, M. H. and Allen, J. E. (2013). *"Beyond Stem Cells: Self-Renewal of Differentiated Macrophages."* Science 342(6161): p. 1242974.

Smith, A. G., et al. (1988). *"Inhibition of Pluripotential Embryonic Stem Cell Differentiation by Purified Polypeptides."* Nature 336(6200): p. 688-690.

Smith, T. M., et al. (2018). *"Stroke Survivor and Caregiver Perspectives on Post-Stroke Visual Concerns and Long-Term Consequences."* Behav Neurol 2018: p. 1463429.

So, J., et al. (2021). *"Sexual Dimorphism of Monocyte Transcriptome in Individuals with Chronic Low-Grade Inflammation."* Biol Sex Differ 12(1): p. 43.

Sochocka, M., et al. (2017). *"Inflammatory Response in the Cns: Friend or Foe?"* Mol Neurobiol 54(10): p. 8071-8089.

Somaraju, U. R. and Tadepalli, K. (2017). *"Hematopoietic Stem Cell Transplantation for Gaucher Disease."* Cochrane Database Syst Rev 10(10): p. CD006974.

Somers, A., et al. (2010). *"Generation of Transgene-Free Lung Disease-Specific Human Induced Pluripotent Stem Cells Using a Single Excisable Lentiviral Stem Cell Cassette "* The International Journal of Cell Cloning 28(10): p. 1728-1740.

Sorensen, A. G. and Ay, H. (2011). *"Transient Ischemic Attack: Definition, Diagnosis, and Risk Stratification."* Neuroimaging Clin N Am 21(2): p. 303-313, x.

Soyombo, A. A., et al. (2013). *"Analysis of Induced Pluripotent Stem Cells from a Brca1 Mutant Family."* Stem Cell Reports 1(4): p. 336-349.

Speicher, A. M., et al. (2019). *"Generating Microglia from Human Pluripotent Stem Cells: Novel in Vitro Models for the Study of Neurodegeneration."* Mol Neurodegener 14(1): p. 46.

Spychala, M. S., et al. (2017). *"Sex Differences in Neuroinflammation and Neuroprotection in Ischemic Stroke."* J Neurosci Res 95(1-2): p. 462-471.

Stirnemann, J., et al. (2017). *"A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments."* International Journal of Molecular Sciences 18(2): p. 441.

Stowe, A. M., et al. (2012). *"Ccl2 Upregulation Triggers Hypoxic Preconditioning-Induced Protection from Stroke."* J Neuroinflammation 9: p. 33.

Su, C., et al. (2019). *"Peripheral Inflammatory Biomarkers in Alzheimer's Disease and Mild Cognitive Impairment: A Systematic Review and Meta-Analysis."* Psychogeriatrics 19(4): p. 300-309.

Sun, Y., et al. (2015). *"Properties of Neurons Derived from Induced Pluripotent Stem Cells of Gaucher Disease Type 2 Patient Fibroblasts: Potential Role in Neuropathology."* Plos One 10(3): p. e0118771.

Sun, Y., et al. (2011). *"Isofagomine in Vivo Effects in a Neuronopathic Gaucher Disease Mouse."* Plos One 6(4): p. e19037.

Supakul, S., et al. (2024). *"Mutual Interaction of Neurons and Astrocytes Derived from Ipscs with App v717l Mutation Developed the Astrocytic Phenotypes of Alzheimer's Disease."* Inflammation and Regeneration 44(1).

Svennerholm, L., et al. (1982). *"Gaucher Disease of the Norrbottnian Type (Type Iii). Phenotypic Manifestations."* Prog Clin Biol Res 95: p. 67-94.

Swindell, W. R., et al. (2013). *"Robust Shifts in S100a9 Expression with Aging: A Novel Mechanism for Chronic Inflammation."* Sci Rep 3: p. 1215.

Szulwach, K. E., et al. (2015). *"Single-Cell Genetic Analysis Using Automated Microfluidics to Resolve Somatic Mosaicism."* Plos One 10(8): p. e0135007.

Takahashi, J. (2007). *"Stem Cell Therapy for Parkinson's Disease."* Expert Rev Neurother 7(6): p. 667-675.

Takahashi, K., et al. (2014). *"Induction of Pluripotency in Human Somatic Cells Via a Transient State Resembling Primitive Streak-Like Mesendoderm."* Nature Communications 5(1).

Takahashi, K. and Yamanaka, S. (2006). *"Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors."* Cell 126(4): p. 663-676.

Tampe, J. F., et al. (2024). *"Human Monocyte Subtype Expression of Neuroinflammation- and Regeneration-Related Genes Is Linked to Age and Sex."* PLoS One 19(10): p. e0300946.

Tantawy, A. A. G. (2015). *"Cytokines in Gaucher Disease: Role in the Pathogenesis of Bone and Pulmonary Disease."* Egyptian Journal of Medical Human Genetics 16(3): p. 207-213.

Taylor, D. L., et al. (2003). *"Activation of Microglial Group Iii Metabotropic Glutamate Receptors Protects Neurons against Microglial Neurotoxicity."* J Neurosci 23(6): p. 2150-2160.

Tiscornia, G., et al. (2013). *"Neuronopathic Gaucher's Disease: Induced Pluripotent Stem Cells for Disease Modelling and Testing Chaperone Activity of Small Compounds."* Hum Mol Genet 22(4): p. 633-645.

Travis, M. A. and Sheppard, D. (2014). *"Tgf-Beta Activation and Function in Immunity."* Annu Rev Immunol 32: p. 51-82.

Tsitsi, P., et al. (2022). *"Neurocognitive Profile of Adults with the Norrbottnian Type of Gaucher Disease."* JIMD Reports 63(1): p. 93-100.

Tsou, C. L., et al. (2007). *"Critical Roles for Ccr2 and Mcp-3 in Monocyte Mobilization from Bone Marrow and Recruitment to Inflammatory Sites."* J Clin Invest 117(4): p. 902-909.

Tsuyama, J., et al. (2024). *"Sustaining Microglial Reparative Function Enhances Stroke Recovery."*

Urra, X., et al. (2009). *"Monocyte Subtypes Predict Clinical Course and Prognosis in Human Stroke."* J Cereb Blood Flow Metab 29(5): p. 994-1002.

Vankriekelsvenne, E., et al. (2022). *"Transmembrane Protein 119 Is Neither a Specific nor a Reliable Marker for Microglia."* Glia 70(6): p. 1170-1190.

Varghese, M., et al. (2022). *"Monocyte Trafficking and Polarization Contribute to Sex Differences in Meta-Inflammation."* Front Endocrinol (Lausanne) 13: p. 826320.

Vegeto, E., et al. (2008). *"Estrogen Anti-Inflammatory Activity in Brain: A Therapeutic Opportunity for Menopause and Neurodegenerative Diseases."* Front Neuroendocrinol 29(4): p. 507-519.

Virani, S. S., et al. (2020). *"Heart Disease and Stroke Statistics-2020 Update: A Report from the American Heart Association."* Circulation 141(9): p. 139-596.

Vitner, E. B., et al. (2012). *"Contribution of Brain Inflammation to Neuronal Cell Death in Neuronopathic Forms of Gaucher's Disease."* Brain 135(6): p. 1724-1735.

Vogel, D. Y., et al. (2015). *"Gm-Csf Promotes Migration of Human Monocytes across the Blood Brain Barrier."* Eur J Immunol 45(6): p. 1808-1819.

Wafa, H. A., et al. (2020). *"Burden of Stroke in Europe: Thirty-Year Projections of Incidence, Prevalence, Deaths, and Disability-Adjusted Life Years."* Stroke 51(8): p. 2418-2427.

Wang, L., et al. (2018). *"Tissue and Cellular Rigidity and Mechanosensitive Signaling Activation in Alexander Disease."* Nature Communications 9(1): p. 1899.

Wang, L., et al. (2021). *"Neurovascular Unit: A Critical Role in Ischemic Stroke."* CNS Neurosci Ther 27(1): p. 7-16.

Wang, S., et al. (2018). *"S100a8/A9 in Inflammation."* Front Immunol 9: p. 1298.

Wattananit, S., et al. (2016). *"Monocyte-Derived Macrophages Contribute to Spontaneous Long-Term Functional Recovery after Stroke in Mice."* J Neurosci 36(15): p. 4182-4195.

Wei, N., et al. (2013). *"Delayed Intranasal Delivery of Hypoxic-Preconditioned Bone Marrow Mesenchymal Stem Cells Enhanced Cell Homing and Therapeutic Benefits after Ischemic Stroke in Mice."* Cell Transplant 22(6): p. 977-991.

Wernig, M., et al. (2007). *"In Vitro Reprogramming of Fibroblasts into a Pluripotent Es-Cell-Like State."* Nature 448(7151): p. 318-324.

WHO, E. a. R. (2017). *"Who Methods and Data Sources for Global Burden of Disease Estimates 2000-2015."* Department of Information, Evidence and Research WHO, Geneva.

Wilmut, I., et al. (1997). *"Viable Offspring Derived from Fetal and Adult Mammalian Cells."* Nature 385(6619): p. 810-813.

Włodarczyk, L., et al. (2022). *"Neuroimaging Techniques as Potential Tools for Assessment of Angiogenesis and Neuroplasticity Processes after Stroke and Their Clinical Implications for Rehabilitation and Stroke Recovery Prognosis."* Journal of Clinical Medicine 11(9): p. 2473.

Wong, K. L., et al. (2011). *"Gene Expression Profiling Reveals the Defining Features of the Classical, Intermediate, and Nonclassical Human Monocyte Subsets."* Blood 118(5): p. e16-31.

Woo, M. S., et al. (2016). *"Cell Surface Cd36 Protein in Monocyte/Macrophage Contributes to Phagocytosis During the Resolution Phase of Ischemic Stroke in Mice."* J Biol Chem 291(45): p. 23654-23661.

Wu, A. R., et al. (2013). *"Quantitative Assessment of Single-Cell Rna-Sequencing Methods."* Nature Chemical Biology 11(1): p. 41-46.

Xin, Y., et al. (2016). *"Use of the Fluidigm C1 Platform for Rna Sequencing of Single Mouse Pancreatic Islet Cells."* Proceedings of the National Academy of Sciences 113(12): p. 3293-3298.

Xu, X., et al. (2021). *"Annexin A1 Protects against Cerebral Ischemia-Reperfusion Injury by Modulating Microglia/Macrophage Polarization Via Fpr2/Alx-Dependent Ampk-Mtor Pathway."* J Neuroinflammation 18(1): p. 119.

Xu, Y., et al. (2011). *"Global Gene Expression Profile Progression in Gaucher Disease Mouse Models."* BMC Genomics 12(1).

Yan, L. L., et al. (2016). *"Prevention, Management, and Rehabilitation of Stroke in Low- and Middle-Income Countries."* eNeurologicalSci 2: p. 21-30.

Yao, R., et al. (2020). *"Translocator Protein 18 Kda (Tspo) Deficiency Inhibits Microglial Activation and Impairs Mitochondrial Function."* Frontiers in Pharmacology 11.

Ye, S. Y., et al. (2019). *"Microglial Vps35 Deficiency Regulates Microglial Polarization and Decreases Ischemic Stroke-Induced Damage in the Cortex."* J Neuroinflammation 16(1): p. 235.

Yoon, C. W. and Bushnell, C. D. (2023). *"Stroke in Women: A Review Focused on Epidemiology, Risk Factors, and Outcomes."* J Stroke 25(1): p. 2-15.

Yu, J., et al. (2007). *"Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells."* Science 318(5858): p. 1917-1920.

Zawada, A. M., et al. (2011). *"Supersage Evidence for Cd14++Cd16+ Monocytes as a Third Monocyte Subset."* Blood 118(12): p. e50-61.

Zhang, T. and Peterson, R. T. (2020). *"Modeling Lysosomal Storage Diseases in the Zebrafish."* Frontiers in Molecular Biosciences 7.

Zhang, Y., et al. (2013). *"Rapid Single-Step Induction of Functional Neurons from Human Pluripotent Stem Cells."* Neuron 78(5): p. 785-798.

Ziegler-Heitbrock, L. (2015). *"Blood Monocytes and Their Subsets: Established Features and Open Questions."* Front Immunol 6: p. 423.

Ziegler-Heitbrock, L., et al. (2010). *"Nomenclature of Monocytes and Dendritic Cells in Blood."* Blood 116(16): p. e74-80.

Ziegler-Heitbrock, L. and Hofer, T. P. (2013). *"Toward a Refined Definition of Monocyte Subsets."* Front Immunol 4: p. 23.

Printed by Media-Tryck, Lund 2025 NORDIC SWAN ECOLABEL 3041 0903

About the author

JULIANE TAMPÉ completed her bachelor's degree in Biochemistry and master's in Neuroscience at ETH Zürich. Driven by a strong interest in translational research and neuroimmunology, she did her PhD at Lund University under the supervision of Zaal Kokaia. Her research focused on the role of monocytes in stroke recovery, their sex-dependent gene expression patterns, and developing iPSC-derived immune cell models.

"A neuroscientist is just a neuron's way of understanding itself."

adapted from Niels Bohr & Carl Sagan

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2025:91
ISBN 978-91-8021-744-6
ISSN 1652-8220