The Unrepaired Breach

Lysosomal Membrane Damage as the Upstream Determinant of Microglial State — How a Childhood Storage Disease, a Dementia Gene, and an Aged Human Neuron Arrive at the Same Transcriptional Programme

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The state was named for the disease it was found in. It is a readout of the organelle.

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Abstract

The disease-associated microglial signature is among the most reproduced observations in modern neurodegeneration research. It was discovered in an amyloid mouse, it was named for the diseases in which it was found, and it has since been recovered in tauopathy, in demyelination, in ageing, and in human Alzheimer's brain. Because it was discovered in an amyloid model, and because the receptor that licenses its second stage binds lipids and lipoproteins that decorate plaques, the signature has been read for most of a decade as a *response to a proteinopathy* — a phagocyte's reaction to an extracellular aggregate. This paper argues that this reading inverts the mechanism. The signature is not a readout of what the cell is eating. It is a readout of what the eating has done to the cell's lysosomes.

The argument rests on a convergence that has become unavoidable only very recently. Two back-to-back studies in one journal issue arrive at the same transcription factors from two unrelated lysosomal lesions. In a mouse model of mucopolysaccharidosis type IIIA — a childhood storage disease caused by deficiency of a single lysosomal sulfamidase, in a brain with no amyloid plaques and no tau tangles — microglia are the most profoundly affected cell type in the brain, and the MITF/TFE family of transcription factors drives a microglia-specific epigenetic and transcriptional programme that substantially overlaps the disease-associated signature of amyloid and tau models and of human Alzheimer's disease. In aged mice lacking progranulin, a lysosomal protein whose haploinsufficiency causes frontotemporal dementia, the same factor family is enriched at active enhancers of a lysosomally stressed microglial subpopulation, deletion of those factors reverses the signature, and — decisively — a panel of mechanistically diverse lysosomal perturbations drives one common transcriptional and functional signature, with lysosomal deacidification the closest phenocopy of the genetic lesion. Two unrelated causes, one programme, one regulatory family. A proteinopathy is not required.

We then supply the mechanism the state literature has never named, because the cell-biological literature that owns it has developed in near-total isolation from the neurodegeneration literature that needs it. A lysosome whose membrane is perforated is not simply a lysosome that has failed. It is a lysosome that has entered a staged, ordered, well-characterised damage-response programme: the endosomal sorting complex required for transport is recruited within minutes to small perforations, before any autophagic machinery arrives, and reseals them; if the breach is too large to reseal, cytosolic galectins bind the luminal glycans that the breach has exposed, and the organelle is routed instead to degradation by lysophagy; galectin-3 is not merely the marker of that decision but its coordinator, recruiting the ESCRT adaptor ALIX to the repair pathway and, when repair fails, handing the cell over to a transcription-factor-driven replacement programme. Repair, removal, replacement — three tiers, in order, each with its own molecular signature. The microglial state is what a cell looks like when it is running the third tier chronically.

The human evidence that this is not merely a mouse phenomenon comes from an unusual model. Neurons transdifferentiated directly from the dermal fibroblasts of 8 young donors, 12 aged donors, 16 patients with sporadic Alzheimer's disease and 5 with presenilin-1 mutations — cells that, unlike

induced pluripotent stem cell derivatives, retain the donor's ageing epigenome — show constitutive lysosomal membrane damage at baseline, marked by exactly the two markers the cell-biological literature predicts: ESCRT-III puncta and galectin-3 puncta. The burden is slight in aged cells and dramatic in aged/sporadic-Alzheimer cells. When damage is imposed experimentally and then withdrawn, repair kinetics are impaired along the same gradient. And three compounds acting by three unrelated mechanisms — promoting acidification, altering a lysosomal phospholipid, and upregulating ESCRT transcripts — each reduce intraneuronal amyloid-β deposits and inflammatory cytokine secretion. The organelle is upstream of the aggregate, not downstream of it.

Human genetics arrives at the same node from a third direction. Sporadic Alzheimer risk variants are largely confined to microglial enhancers, and allele-specific chromatin mapping across 26 late-onset loci now assigns most of them to microglia. At one of them, the risk allele reduces binding of the master myeloid transcription factor PU.1 — the same factor the storage-disease work identifies as an obligate collaborator of MITF/TFE at lysosomal-stress enhancers — lowers expression of an endocytic adaptor, impairs uptake of amyloid-β and of myelin debris, and drives the cell into cholesterol synthesis and lipid-droplet accumulation. Lipid droplets are what a myeloid cell makes when its degradative capacity is exceeded by its ingestive load, and three independent human-anchored literatures — on ageing, on an amyloid-driven acyltransferase, and on an apolipoprotein genotype — now converge on lipid-droplet-laden microglia as a dysfunctional, phagocytosis-impaired, neurotoxic state. This is not a separate story. It is the same failure of degradative throughput, read out in lipid rather than in protein.

Finally, we identify a weld that neither literature has made. Galectin-3 — the canonical cytosolic sensor of a ruptured lysosome — is also, on the evidence of a separate literature, an endogenous ligand of TREM2, the receptor that licenses the second stage of the microglial state programme, with direct binding through its carbohydrate-recognition domain, colocalisation in microglial processes by super-resolution microscopy, and signalling through the TREM2–DAP12 axis in a reporter system. If both literatures are correct, the microglion possesses a route by which lysosomal rupture is transduced directly into the receptor signalling that drives its own state transition. We grade this weld as *inferred*, we state precisely what has not been shown, and we name the experiment.

The paper closes with the finding that most sharply discriminates our reading from the conventional one. In progranulin-deficient models, ablating TREM2 — genetically or with antagonist antibodies — reduces microglial hyperactivation, exactly as the state-centred model predicts it should help. It does not rescue the lysosomal dysfunction, the lipid dysregulation, or the glucose hypometabolism; and the double-deficient animals are worse, with greater synaptic loss and higher cerebrospinal neurofilament light. Removing the state does not mend the organelle, because the organelle was never downstream of the state. The complementary human experiment has also now read out: a TREM2 agonistic antibody, tested in 381 participants with early Alzheimer's disease, achieved sustained central target engagement and a measurable pharmacodynamic response, and missed its primary clinical endpoint at every dose. Pushing the state and removing the state have both been tried; neither mends the organelle. This is the asymmetry a causal arrow predicts and a co-occurrence does not, and it is the asymmetry on which the therapeutic argument turns: the tractable target is the breach, not the response to it.

I. The State That Was Named After a Plaque

There is a recurring hazard in disease biology, and it is not error so much as inheritance. A cellular state is discovered in a particular model, in a particular disease, under a particular stimulus. The name it receives records the circumstances of its discovery. The name is then carried forward into every subsequent study, and because the name encodes a context, it quietly encodes a causal hypothesis as well. Investigators who would never assert the hypothesis in print assert it every time they use the term.

The disease-associated microglion is a case of this. It was identified by single-cell transcriptional sorting of all immune populations in the brains of wild-type and amyloid-transgenic mice, and it was reported with markers, spatial localisation, and associated pathways [1]. Immunohistochemistry showed these cells containing intracellular and phagocytic amyloid-β particles. The programme was shown to activate in two stages: an initial stage, independent of the myeloid receptor TREM2, consisting largely of the downregulation of homeostatic microglial checkpoints, followed by a second stage that requires TREM2. The authors were careful, and the paper's title says *restricting* — the state was proposed to be protective. But the observation that framed the field was a microglion with amyloid inside it, sitting beside a plaque, having changed.

Within a year the same group generalised the claim, proposing that these cells constitute a dedicated sensory apparatus for detecting damage within the central nervous system, responding to what they termed neurodegeneration-associated molecular patterns, with TREM2 signalling as the sensing pathway [2]. This was a considerable advance, because it detached the state from amyloid specifically and reframed it as a general damage response. But it left the sensory question in a particular form. A *molecular pattern* is something in the extracellular space that a receptor binds. The framework asks what the cell detects outside itself.

The alternative — that the state is instructed from inside, by the condition of an organelle, and that what the cell ingests matters only insofar as it damages that organelle — was not available as a serious competitor, for a reason worth stating plainly. The competing hypothesis requires evidence from a disease in which microglia adopt the signature with no proteinopathy present at all. Until very recently, that evidence did not exist in a form anyone could cite. This paper exists because it now does.

The human data have in the meantime grown considerably more refined, and in a direction that makes the question more pressing rather than less. Single-nucleus profiling of 194,000 microglia across 443 human subjects resolved twelve transcriptional states, including Alzheimer-dysregulated homeostatic, inflammatory, and — a category worth marking — *lipid-processing* states, with 1,542 differentially expressed genes partitioned by both state and disease stage [3]. The same work inferred upstream regulators and gene-regulatory networks from integrated

epigenomic and motif information, and then did something the field had not previously managed: it tested the inference causally, showing that ectopic expression of predicted homeostatic-state activators induces homeostatic features in human microglia-like cells, while inhibiting activators of inflammation blocks inflammatory progression. Microglial state, in human cells, is a transcription-factor-driven property that can be pushed in either direction.

If state is transcription-factor-driven, then the question of what instructs the state becomes the question of what activates the transcription factors. That is a mechanistically tractable question, and it has now been answered twice, from two unrelated lesions, with the same answer.

II. What the Signature Is, and What It Was Assumed to Mean

Before proceeding it is worth being exact about what the signature consists of, because a good deal of loose argument in this area depends on treating it as a single monolithic thing.

Operationally, the programme is defined by the coordinate downregulation of a set of homeostatic genes and upregulation of a set of genes heavily enriched for lysosomal, phagocytic and lipid-handling functions. This composition has been remarked on since the original description, and it has generally been read as teleologically appropriate: a cell that is going to eat a plaque needs more phagocytic and more degradative machinery, so it makes more. The gene list is taken as evidence of *function being scaled up to meet demand*.

But a gene list enriched for lysosomal genes is equally consistent with a quite different reading: that the cell is transcriptionally responding to lysosomal insufficiency. The lysosomal compartment has been known since 2009 to be under coordinate transcriptional control by a specific factor family. A conserved motif in the promoters of most lysosomal genes is bound by the transcription factor EB, and under conditions of aberrant lysosomal storage this factor translocates from cytoplasm to nucleus and activates its targets, driving lysosomal biogenesis and increasing the degradation of complex substrates [4]. Coordinate upregulation of lysosomal genes is, in other words, the canonical signature of a cell that does not have enough functioning lysosome — the transcriptional equivalent of a compensatory response to organelle failure.

These two readings of the same gene list make opposite predictions about causation, and they have coexisted in the literature for years largely unexamined, because in the amyloid models where the signature was characterised the two are confounded: the microglion is eating amyloid *and* its lysosomes are under strain, and no experiment separates them.

There is one further element of the conventional reading that deserves scrutiny. TREM2 licenses the second stage of the programme, and TREM2 hypomorphs raise Alzheimer risk. What is less often emphasised is what TREM2-deficient cells actually look like inside. Microglia from

patients carrying TREM2 risk variants, and TREM2-deficient mice with amyloid pathology, have *abundant autophagic vesicles* — an accumulation of undegraded autophagic cargo — traced to defective mTOR signalling with consequences for ATP levels and biosynthesis [5]. The phenotype could be offset by supplying an alternative energy substrate, which restored microglial clustering around plaques and decreased plaque-adjacent neuronal dystrophy. The receptor whose loss raises Alzheimer risk turns out, on close inspection, to be a receptor whose loss produces a cell that cannot clear what it has internalised. The genetics of the state programme was pointing at the degradative compartment from the beginning.

III. A Storage Disease Makes the Signature Without a Proteinopathy

The experiment that separates the confound is one that nobody designed for this purpose. It is a study of a childhood lysosomal storage disease.

Mucopolysaccharidosis type IIIA is caused by deficiency of *N*-sulfoglucosamine sulfohydrolase, a single lysosomal hydrolase in the heparan sulfate degradation pathway. The resulting brain accumulates undegraded glycosaminoglycan in lysosomes. It does not accumulate amyloid plaques. It does not accumulate neurofibrillary tangles. The lesion is lysosomal, it is genetic, it is singular, and it is upstream of nothing else in the Alzheimer cascade.

Systematic imaging, transcriptomic and epigenetic analysis of the brains of sulfamidase-deficient mice established, first, that microglia are the most profoundly impacted cell type in the brain [6]. This is not trivial and it is not obvious. The enzyme is expressed in every cell type, and germline deficiency has the potential for cell-autonomous consequences everywhere it is expressed. Electron microscopy of frontal cortex nonetheless showed markedly enlarged, electron-lucent vacuoles — greater than one micrometre — concentrated in cells with the ultrastructural features of microglia, with secondary intraluminal lamellar material; cells with neuronal, astrocytic or oligodendrocytic features showed either smaller vacuolar structures or none. A generalised genetic lesion in lysosomal catabolism produces a disproportionately microglial phenotype. The microglion is, among brain cells, the one whose function most closely approximates *running its lysosomes continuously at capacity*, and it is therefore the cell that a marginal reduction in lysosomal throughput will decompensate first.

The second finding is the one that matters for the causal arrow. Motif analysis of regions gaining the active-enhancer mark across disease progression identified the MITF/TFE family of transcriptional regulators as the second most enriched motif at every time point examined, present in 41 per cent, 31 per cent and 28 per cent of induced regions respectively; in the active regulatory landscape of wild-type microglia the same motif ranks seventh and occurs in 6.2 per cent of

regions [6]. This family — which includes the transcription factor EB discussed above, together with MITF and TFE3 — is the lysosomal stress-response family. Its motif does not become the dominant feature of a cell's induced enhancer landscape unless the cell is transducing lysosomal stress.

Critically, the family does not act alone. Enhancer activation depended on collaborative interaction with signal-dependent and lineage-determining factors: AP-1/ATF motifs became enriched at the later time points, while PU.1 and C/EBP motifs remained prominent throughout, consistent with their established role in establishing the microglia- and macrophage-specific enhancer landscape. The output is therefore *microglia-specific* not because the stress is microglia-specific but because a general lysosomal-stress transcription factor is reading a myeloid enhancer landscape. The same stress in another cell type, collaborating with another set of lineage-determining factors, would produce a different programme. This is the mechanistic explanation for why lysosomal disease produces a *microglial* signature.

The third finding closes the loop to neurodegeneration. Genes upregulated in sulfamidase-deficient microglia were intersected with those upregulated in two independent mouse models: amyloid-responsive microglia from amyloid-transgenic animals, which carry the disease-associated phenotype and are linked to TREM2-dependent protective responses to amyloid; and tau/APOE4-responsive microglia from animals co-expressing human P301S tau and APOE4, which are associated with severe neurodegeneration. A substantial fraction of the storage-disease signature was shared with both [6]. Features of the transcriptional and epigenetic alterations were also recovered in microglia from mouse models of age-related neurodegeneration and in human Alzheimer's disease patients.

A childhood disease of one lysosomal enzyme, with no amyloid and no tau anywhere in the brain, produces a microglial transcriptional and epigenetic state substantially overlapping the state that amyloid produces, that tau produces, that ageing produces, and that is found in human Alzheimer's disease. The most economical explanation is that none of these stimuli instructs the state directly. They instruct it through a shared intermediate, and the storage disease identifies which intermediate that is, because the storage disease has nothing else.

One caution must be entered here, and we enter it because the temptation to overstate is considerable. This study examined one genetic lysosomal lesion. It did not itself apply a panel of chemically distinct lysosomal insults and demonstrate their convergence. That experiment exists, and it is a different paper.

IV. A Dementia Gene Makes It Too, Through the Same Factors

Published back-to-back with the storage-disease work, in the same issue of the same journal, is a study that supplies exactly the missing arm [7].

Its entry point is progranulin. Haploinsufficiency of the progranulin gene causes frontotemporal lobar degeneration; progranulin is a lysosomal protein; and the loss-of-function phenotype is lysosomal. Multi-omic profiling of myeloid cells from the brains of aged progranulin-null mice identified a microglial subpopulation defined by expression of GPNMB, displaying what the authors describe as hallmarks of lysosomal stress: altered lysosomal protein expression, lipofuscinosis, and metabolic and lipid dysregulation.

Epigenetic profiling of these cells revealed enrichment of MITF/TFE transcription factor motifs at active enhancers — the same family, in an unrelated genetic lesion, in a different disease. And deletion of those factors reversed the progranulin-specific myeloid transcriptional signature. This is the causal test the storage-disease paper could not perform on its own: the factors are not correlates of the state, they are required for it.

The finding that most directly serves the present argument is stated by the authors in one sentence: diverse lysosomal perturbations drove a common transcriptional and functional signature, with conditions that induce lysosomal deacidification closely phenocopying progranulin deficiency [7]. Distinct insults, applied to the same organelle, converge on one programme. The programme is therefore not a readout of the insult. It is a readout of the organelle's condition.

The paper's final result adds a dimension that is easy to miss and important to keep. GPNMB induction in progranulin-deficient myeloid cells was shown to be *compensatory*: it promoted lysosomal acidification, and loss of myeloid GPNMB exacerbated neurotoxicity. The marker that defines the stressed state is, in part, the cell's attempt to fix the thing that is stressing it. This is the correct general posture toward the disease-associated signature. It is not a pathological programme that has been switched on by disease; it is a homeostatic programme running against a load it cannot clear, and its markers are a mixture of failure and of compensation for failure. Treating the whole signature as a therapeutic target is, on this reading, an error of the same species as treating a fever.

Taking sections III and IV together: two unrelated genetic lysosomal lesions, in two diseases, in two laboratories, converge on one transcription factor family; that family is required for the signature; and a panel of mechanistically diverse lysosomal perturbations reproduces the same signature. Against this, the hypothesis that the disease-associated state is a specific response to a specific proteinopathy is no longer the most parsimonious available reading.

V. The Grammar of a Damaged Lysosome

To convert this from a transcriptional correlation into a mechanism, we require the cell biology of lysosomal damage itself. This literature is mature, precise and almost entirely absent from the neurodegeneration discussion it should be informing. We set it out here in the order in which the cell executes it, because the order is the mechanism.

Detection. A lysosome whose limiting membrane is perforated exposes its luminal face to the cytosol. That luminal face is densely glycosylated, and the cytosol contains a family of soluble β-galactoside-binding lectins, the galectins, which under normal conditions never encounter those glycans. On perforation they bind, and they do so rapidly and with sufficient local concentration to be visible as discrete puncta. This is the basis of the galectin puncta assay, validated as a highly sensitive readout of lysosomal membrane permeabilisation, with galectin-1 and galectin-3 the most suitable members by virtue of widespread expression and rapid translocation [8]. Two features of that validation matter here. Galectin staining marks *individual* leaky lysosomes early, allowing the question of whether membrane permeabilisation is a primary or a secondary event to be settled rather than assumed. And cells can survive limited permeabilisation — the breach is not automatically lethal, which is what makes the downstream decision meaningful. The assay works in paraffin-embedded tissue, which means it is applicable to human post-mortem material.

Repair. The first response is not degradation. The endosomal sorting complex required for transport, long known for membrane budding and fission on endolysosomes, is recruited to acutely injured endolysosomes within minutes, through a pathway requiring calcium and specific activating factors, and — this is the key point — *independently of lysophagy* [9]. Live-cell imaging showed the machinery responding to small perforations and enabling compartments to recover from limited damage. Silica crystals, a physiologically relevant particulate insult, trigger the same recruitment. An independent study established the temporal order explicitly: components of ESCRT-I, -II and -III are recruited on lysosomal membrane injury *before* galectin-3 and before the lysophagy machinery arrives, with ESCRT-III recruitment particularly prominent and dependent on the ESCRT-I component TSG101 and the adaptor ALIX; interfering with that recruitment abolished repair and converted otherwise reversible lysosomal damage into a lethal event [10].

That last clause is the load-bearing one for everything that follows. *Damage that is repairable is not the same event as damage that is not.* Whether a perforated lysosome is a transient perturbation or a terminal one is decided by the competence of the repair machinery, not by the size of the insult alone. A cell with degraded repair capacity converts survivable insults into unsurvivable ones.

Removal. When the breach exceeds what resealing can handle, the organelle is routed to destruction. Damaged lysosomes are selectively sequestered by autophagy — demonstrated with monosodium urate, with silica, and with the lysosomotropic dipeptide ester L-leucyl-L-leucine methyl ester — with the autophagic machinery recruited only to the damaged organelles and not to their intact neighbours [11]. In an autophagy-dependent manner, low pH and degradative capacity are recovered; when autophagy is lost, lysosomal biogenesis itself is inhibited and tissue injury worsens in vivo.

Coordination, and the third tier. The element that unites these is galectin-3, and the demonstration is worth stating in full because it recasts galectin-3 from a marker into a mechanism.

Galectin-3 unifies and coordinates the ESCRT and autophagic responses to lysosomal damage [12]. Galectin-3, and specifically its capacity to recognise damage-exposed glycans, is *required* for efficient recruitment of the ESCRT adaptor ALIX; both galectin-3 and ALIX are required for restoration of lysosomal function; and galectin-3 promotes the interaction between ALIX and the downstream ESCRT-III effector CHMP4. At later times after injury, galectin-3 controls the autophagic response instead. And when that too fails — as in galectin-3 knockout cells — a lysosomal *replacement* programme takes over, driven by transcription factor EB.

The architecture is therefore three-tiered and ordered: repair the membrane; if that fails, remove the organelle; if that fails, transcribe new ones. The third tier is a MITF/TFE-family transcriptional programme. Which is to say: *the transcriptional signature identified in sections III and IV as the driver of the disease-associated microglial state is the third tier of the lysosomal damage response, running chronically.* A cell in which repair and removal are keeping pace does not need to run it. A cell in which they are not, does, continuously, and that continuous running is what we have been calling a disease-associated state.

Two further details from this work deserve to be carried forward. First, the staged response was detected in model systems of lysosomal damage inflicted by *proteopathic tau* — establishing that a protein aggregate relevant to neurodegeneration is, among other things, a lysosome-damaging agent that engages this exact machinery. Second, the same machinery is parasitised by intracellular pathogens, which is why it is as well characterised as it is; the neurodegeneration field has been slow to claim a literature built largely by microbiologists.

VI. The Same Breach, Read in the Human Neuron

The foregoing is mouse and cell line. The human evidence comes from a model system designed to solve a specific problem: induced pluripotent stem cell derivatives are epigenetically rejuvenated by reprogramming and therefore discard the very variable — donor age — that is the dominant risk factor for the disease.

Neurons transdifferentiated directly from human dermal fibroblasts retain ageing hallmarks. They were generated from 8 healthy young donors (mean age 25.6 years), 12 aged donors (70.3 years), 16 aged patients with sporadic Alzheimer's disease (70.4 years), and subsequently 5 middle-aged patients carrying presenilin-1 mutations (47.2 years) [13]. The Alzheimer-derived neurons accumulated proteotoxic deposits including phosphorylated tau and amyloid-β. Quantitative proteomics identified ageing- and disease-linked deficits in proteostasis and organelle homeostasis, most notably in endosomal-lysosomal components.

The lysosomal phenotype was then assayed with precisely the two markers section V predicts. Under basal conditions, with no insult applied, young neurons showed no appreciable damaged

lysosomes; aged neurons showed a slight increase in the number and intensity of ESCRT-III CHMP2B puncta and of galectin-3 puncta; and aged/sporadic-Alzheimer neurons showed a dramatic increase in both. This is *constitutive* lysosomal membrane damage — present at baseline, escalating monotonically along the ageing-to-disease gradient, and detected with the ESCRT marker of the repair tier and the galectin marker of the removal decision simultaneously. No apoptotic death accompanied it, confirming that these are cells living with a chronic, sublethal breach rather than cells in the act of dying.

Repair kinetics were then measured directly. Damage was imposed with the lysosomotropic agent L-leucyl-L-leucine methyl ester for thirty minutes, washed out, and the recovery followed for up to eight hours using the ESCRT-0 component HGS as the probe — chosen because, unlike CHMP2B, its baseline distribution was comparable across all groups, an unusually careful control. Repair was impaired in aged and aged/Alzheimer neurons. The defect is not only that these cells sustain more damage; it is that they are worse at mending it. Per section V, that combination is precisely the one that converts survivable insults into unsurvivable ones.

Two consequences were then established, and they are the ones that make this paper load-bearing rather than merely descriptive.

The first is inflammatory. Chronic sublethal lysosomal damage increased neuronal secretion of interleukin-1β, interleukin-6, interferon-γ and CCL2, with cytokine secretion correlating positively with ageing, disease status and lysosomal damage [13]. Treatment with a lysosome-targeting small molecule that promotes acidification and damage resilience reduced secretion of interleukin-6, interleukin-15 and CCL2. A neuron with a damaged lysosome is an inflammatory secretory source in its own right, before any glial cell has been recruited. This supplies the missing arrow from the neuronal lesion to the microglial state: a breached neuronal lysosome instructs the local immune environment directly.

The second is amyloidogenic, and the direction of the arrow is the whole point. Increasing lysosomal damage — with the lysosomotropic agent, or with a calcium chelator — *increased* intraneuronal amyloid-β42. Three mechanistically distinct compounds that improve lysosomal function — one promoting acidification, one increasing the lysosomal phospholipid bis(monoacylglycero)phosphate, and one upregulating ESCRT transcripts — each reduced amyloid-β42 deposits, by roughly 20 to 46 per cent. Three unrelated mechanisms of improving one organelle, three reductions in the aggregate. The organelle is upstream of the aggregate.

This last point converges with an independent line in neuronal cell biology that reached the same conclusion by a different route. In five amyloid mouse models, autolysosomal acidification declines in neurons well before extracellular amyloid deposition, associated with markedly lowered vacuolar ATPase activity, with amyloid-β and its precursor fragment building up selectively within enlarged, de-acidified autolysosomes [14]. In more compromised neurons these vesicles pack into

perikaryal rosettes; lysosomal membrane permeabilisation, cathepsin release and lysosomal cell death follow, *accompanied by microglial invasion*; and the affected neurons were quantified as the principal source of senile plaques. The deacidified, breached neuronal lysosome is both the site of amyloid accumulation and the recruiting signal for the microglion. Note also that lysosomal deacidification is the same perturbation that most closely phenocopied progranulin deficiency in the microglial work of section IV. The same lesion, in two cell types, with two different readouts.

VII. What the Microglion Eats Is a Lysosomal Insult

We can now return to amyloid and give it its proper place, which is neither primary nor irrelevant.

The mechanism by which fibrillar amyloid activates microglia was established early and has been stable since. The NALP3 inflammasome acts as a sensor of amyloid-β, but not by binding it at the cell surface. The process requires phagocytosis of amyloid, followed by lysosomal damage and the release of cathepsin B into the cytosol [15]. The interleukin-1β pathway downstream was shown to be essential for microglial synthesis of proinflammatory and neurotoxic factors, and the inflammasome, caspase-1 and interleukin-1β were each critical for the recruitment of microglia to exogenous amyloid in the brain.

Read in 2008, this was a paper about how a plaque activates an inflammasome. Read against sections III to VI, it says something considerably more general: *amyloid acts on the microglion as a particulate lysosome-damaging agent*, in the same mechanistic class as the silica crystals and urate crystals used to characterise the damage-response machinery [9 11]. Its specificity as an Alzheimer molecule is not what makes it a microglial stimulus. What makes it a microglial stimulus is that it is an indigestible particulate that perforates the compartment into which it is taken.

This resolves what has otherwise been an awkward feature of the disease-associated signature: its promiscuity. The same programme appears in amyloidosis, in tauopathy, in demyelination, in ageing, in storage disease. On a proteinopathy-response model, that promiscuity requires the receptor system to recognise an implausibly diverse set of ligands. On a lysosomal-stress model, it requires only that these materials have one property in common — they are hard to degrade, and the attempt to degrade them stresses the compartment. Myelin debris, fibrillar amyloid, aggregated tau, undegradable glycosaminoglycan and the accumulated lipofuscin of age share nothing chemically. They share exactly that.

It is also worth noting that the aggregate literature supplies an independent confirmation from the opposite direction: proteopathic tau was demonstrated to inflict lysosomal damage engaging the galectin-3/ESCRT machinery directly [12]. Both of the canonical Alzheimer proteinopathies are lysosome-damaging agents, and both were shown to be so by investigators who were not studying Alzheimer's disease.

The most striking demonstration that the *soluble/particulate* distinction is the relevant one comes from outside neuroscience. An endocytic pathway mediated by the scavenger receptor CD36 was shown to convert soluble endogenous ligands — oxidised low-density lipoprotein, amyloid-β and amylin among them — into crystals or fibrils *inside* the macrophage, and the resulting particulates caused lysosomal disruption and NLRP3 activation [25]. Macrophages lacking CD36 failed to produce interleukin-1β in response to those ligands. The implication for the present argument is sharp: a cell can manufacture its own lysosome-damaging particulate from soluble material it has taken up. The insult need not arrive as an aggregate. It need only become one in the wrong compartment.

VIII. The Human Genetics Arrives at the Same Node

An argument built from mouse models and cultured cells requires a human anchor that is not itself a model. Human genetics supplies one, and it has been converging on this node for some years without the convergence being named.

The cell-type assignment came first. Mapping of cell-type-specific enhancers and their promoter interactomes across major human brain cell types established that whereas psychiatric disorder variants sit predominantly in neuronal regulatory elements, sporadic Alzheimer's disease variants are *largely confined to microglial enhancers* [16]. Large-scale association work has since confirmed and extended the picture: a two-stage study across 111,326 cases and 677,663 controls identified 75 risk loci, and pathway enrichment, while confirming amyloid and tau pathways, highlighted microglial involvement [17]. The inherited component of late-onset Alzheimer's disease is disproportionately a story about what microglia do.

The functional resolution came recently, and it lands on the node. Allele-specific open-chromatin mapping in human stem-cell-derived neurons, astrocytes and microglia identified functional risk variants for 26 late-onset loci, most of them microglia-specific [18]. At one locus the mechanism was carried through to cellular phenotype in full. The risk allele of a single nucleotide polymorphism at the *PICALM* locus reduces binding of PU.1 and lowers PICALM expression, impairing microglial uptake of amyloid-β and of myelin debris. Microglia carrying the risk allele showed transcriptional enrichment of cholesterol synthesis and lipid droplet formation pathways, and genetic and pharmacological perturbation established a causal chain from reduced PICALM, through lipid droplet accumulation, to phagocytic deficit.

Two features of this deserve emphasis. First, the transcription factor whose binding the risk allele disrupts is PU.1 — the same lineage-determining factor identified in the storage-disease work as the obligate myeloid collaborator with which MITF/TFE must cooperate to activate lysosomal-stress enhancers [6]. The common human risk variant and the lysosomal stress programme

are operating on the same enhancer grammar. Second, the cellular endpoint is not inflammation. It is lipid droplet accumulation and a phagocytic deficit — that is, a *handling* phenotype.

Lipid droplets are what a myeloid cell makes when the material it has ingested exceeds what it can catabolise, and three independent literatures have now converged on them.

In ageing, lipid-droplet-accumulating microglia build up in mouse and human brain; they are defective in phagocytosis, produce high levels of reactive oxygen species and secrete proinflammatory cytokines; their transcriptional profile is distinct from previously reported microglial states; and an unbiased genome-wide screen for modifiers of droplet formation returned, among its hits, genes whose variants cause autosomal-dominant human neurodegenerative disease — including progranulin [19]. The same gene that section IV identified as a lysosomal lesion producing the MITF/TFE-driven microglial state is here identified as a modifier of lipid droplet formation. These are not two phenotypes. They are one phenotype seen through two assays.

In amyloidosis, microglia form lipid droplets upon amyloid-β exposure, and droplet load increases with proximity to plaques in human Alzheimer brain and in mouse [20]. Droplet-laden microglia show defects in amyloid phagocytosis. Lipidomics identified the underlying metabolic transition as a parallel fall in free fatty acids and rise in triacylglycerols, catalysed by the acyltransferase DGAT2, which was elevated in both mouse and human disease; pharmacological inhibition improved microglial amyloid uptake and reduced plaque load and neuronal damage. Note the direction: the droplet is not a passive marker of a busy cell, it is causally upstream of the phagocytic deficit, and relieving it restores function.

In genetics, single-nucleus sequencing of human brain identified a microglial state defined by the lipid-droplet-associated enzyme ACSL1, most abundant in Alzheimer patients homozygous for APOE4 [21]. In human microglia, fibrillar amyloid induced ACSL1, triacylglycerol synthesis and droplet accumulation in an APOE-dependent manner; and conditioned medium from droplet-containing microglia induced tau phosphorylation and neurotoxicity, again APOE-dependently. The strongest common genetic risk factor for the disease acts, in microglia, by setting how much lipid the cell strands when it eats.

The human-genetic story therefore reads as follows. Alzheimer risk variants sit in microglial enhancers; the best-resolved of them reduces a myeloid transcription factor's occupancy and impairs ingestion; the cell responds by stranding lipid; stranded lipid impairs ingestion further; and the strongest risk allele in the disease determines the magnitude of the stranding. This is a description of degradative capacity failing to meet ingestive load. It is the same lesion as sections III to VII, approached from human inheritance rather than from organelle biology, and arriving at the same organelle.

IX. The Sensor That Is Also a Ligand

We now state the connection that neither literature has made, and we state it with its grade attached, because it is the most interesting claim in this paper and the least established.

Section V established that galectin-3 is the cytosolic sensor of lysosomal membrane rupture and the coordinator of the repair-versus-removal decision. A separate literature, developed independently and without reference to that role, reports that galectin-3 is an endogenous ligand of TREM2.

That report is substantial. Galectin-3 is highly upregulated in the brains of Alzheimer patients and of amyloid mice, and is expressed specifically in microglia associated with amyloid plaques [22]. Polymorphisms in *LGALS3*, the gene encoding it, were associated with increased disease risk. Genetic deletion in amyloid mice attenuated microglia-associated immune responses, and did so *particularly those associated with TLR and TREM2/DAP12 signalling*; galectin-3 was required to fully activate microglia in response to fibrillar amyloid; deletion decreased amyloid burden and improved cognitive behaviour. Super-resolution microscopy showed close colocalisation of galectin-3 and TREM2 in microglial processes; direct interaction was demonstrated by fluorescence anisotropy involving the galectin-3 carbohydrate-recognition domain; and galectin-3 stimulated TREM2–DAP12 signalling in a reporter cell line. (A correction to this paper was published in 2023; it is a correction, not a retraction, and does not bear on the claims used here.)

Place the two literatures side by side. The protein that binds the exposed luminal glycans of a ruptured lysosome is the same protein that binds and activates the receptor that licenses the second stage of the disease-associated microglial programme. If both are correct, then the microglion possesses a route by which lysosomal rupture is transduced, via a single protein, into the receptor signalling that drives its own state transition — and, since galectin-3 is released extracellularly by activated microglia, potentially into the state transition of its neighbours.

We grade this **inferred**, and we are explicit about the gap. No study has demonstrated that the galectin-3 pool engaged by a microglion's own damaged lysosomes is the pool that reaches TREM2. The intracellular sensing role requires cytosolic galectin-3 binding a glycan face exposed inward; the receptor-ligand role requires extracellular galectin-3 binding TREM2 on the outer leaflet. These are different compartments, and a mechanism connecting them — unconventional secretion of the sensor pool, or release from dying cells — has not been shown. It is entirely possible that the two roles are independent and that their sharing of a protein is coincidence. What makes the coincidence worth reporting is that it would, if real, close the loop between the lesion and the state with a single molecule, and that the experiment to test it is straightforward.

The prediction is stated in section XIII.

X. Rival Accounts of What Sets the State

Two other candidates for the upstream determinant of microglial state are well established in the literature, and an argument for a third has an obligation to say how it stands to them. Neither is refuted here. Both, we will argue, are better understood as describing a different level of the same system.

The first rival is the TREM2–APOE axis. An APOE-dependent molecular signature was identified in microglia across models of amyotrophic lateral sclerosis, multiple sclerosis and Alzheimer's disease, and in microglia surrounding neuritic plaques in human Alzheimer brain; the pathway mediated a switch from homeostatic to neurodegenerative phenotype after phagocytosis of apoptotic neurons; TREM2 induced APOE signalling; and targeting the axis restored the homeostatic signature in two mouse models and prevented neuronal loss in an acute model [26]. The authors describe the axis as a major regulator of microglial functional phenotype. This is a strong result and the rescue experiment is the kind of evidence a causal claim requires.

Three observations position it. First, note the trigger in that experiment: the switch followed *phagocytosis of apoptotic neurons* — that is, it followed the delivery of a large, complex, membranous cargo into the degradative compartment. The proximate stimulus in the founding TREM2–APOE experiment is an ingestion event. Second, TREM2-deficient cells accumulate undegraded autophagic cargo and are metabolically unable to complete degradation [5], which places the receptor upstream of degradative competence rather than parallel to it. Third, and decisively, the axis can be removed without correcting the organelle: in progranulin deficiency, TREM2 ablation reduced the state and left the lysosomal, lipid and metabolic phenotypes intact [23]. A regulator whose removal abolishes the output but not the lesion is a transducer, not a source. Our reading is therefore that TREM2–APOE is the principal *signalling* route by which degradative strain is converted into a state — which is a large and important role, and not the same role as being the thing that strains the cell.

The second rival is the maintenance view. Microglial identity is sustained by an extrinsic instruction: a unique molecular signature of 239 genes and 8 microRNAs distinguishes microglia from other myeloid cells, that signature requires TGF-β, and microglia are absent from the central nervous system of TGF-β1-deficient mice [27]. On this view the disease-associated state is not an activation at all but a *descent* — the lapse of a standing instruction, whose most robust transcriptional feature is the loss of the homeostatic programme rather than the gain of a coherent activation one. The two-step structure of the state supports this reading, since the first step is defined precisely by downregulation of homeostatic checkpoints and is TREM2-independent [1].

We regard this as correct and as addressing a different question. The maintenance view explains what *holds* a microglion in its homeostatic state and what the loss of that state consists of

transcriptionally. It does not specify what, in a given diseased brain, causes the instruction to lapse in some cells and not others, at some times and not others. Lysosomal stress is a candidate answer to that question, and it is compatible with the maintenance framework rather than competitive with it: a cell whose degradative compartment is failing is a cell running an escalating transcriptional programme — the third tier of section V — whose factors compete for the same enhancer landscape that the homeostatic programme occupies, with PU.1 and C/EBP as shared collaborators [6]. Displacement of one programme by another at a shared enhancer repertoire is a plausible mechanism for a descent, and it is measurable.

The honest statement of the relationship is therefore layered rather than competitive: TGF-β maintains the homeostatic programme; lysosomal stress generates the transcriptional pressure that displaces it; TREM2–APOE transduces and amplifies the resulting state. What the evidence of sections III and IV adds is that the second of these is sufficient on its own, in the absence of any proteinopathy, to produce the signature — and what the evidence of section XIV adds is that only the second, when corrected, mends anything.

XI. The State Is Written in Chromatin, Not Only in Transcript

There is a feature of the evidence in sections III and IV that has been carried along without comment and now needs drawing out, because it changes what kind of thing the microglial state is.

Both studies are epigenetic as well as transcriptional. What they report is not merely that certain genes go up but that the *enhancer landscape is remodelled* — that regions of the genome acquire the active-enhancer histone modification progressively over months, and that the motifs enriched in those newly active regions belong to the lysosomal-stress factor family collaborating with the myeloid lineage-determining factors [6] [7]. The word the first study uses is *progressive*. The proportion of induced regions carrying the MITF/TFE motif was highest at the earliest time point examined and declined thereafter, while AP-1 motifs became enriched only later — a sequence, not a snapshot.

This distinction matters for three reasons.

First, it explains the durability of the state. A transcriptional response is a response: remove the stimulus and the transcripts decay with their own half-lives. Enhancer remodelling is a different kind of object. A genomic region that has acquired an activating modification and a bound factor is primed for subsequent activation at a lower threshold. If the disease-associated state is an enhancer landscape rather than a transcriptional snapshot, it will not simply reverse when the proximate stress is relieved, and its reversal will have its own kinetics, likely much slower. This is a testable and clinically consequential claim, and it predicts that the window in which lysosome-directed intervention can restore the homeostatic landscape is earlier than the window in which it can relieve lysosomal stress.

Second, it supplies the mechanism for the displacement argument of section X. If the homeostatic programme and the stress programme draw on an overlapping set of lineage-determining factors — PU.1 and C/EBP appear on both sides — then they are not independent circuits but competitors for a finite pool of collaborating factors at a shared enhancer repertoire. Progressive redistribution of that pool toward stress-responsive regions is exactly what a gradual "descent from homeostasis" would look like at the level of chromatin. The maintenance view and the stress view describe the same remodelling from opposite ends.

Third, it connects to what is now measurable in human tissue. Human microglial state has been resolved epigenomically as well as transcriptionally, with upstream regulators and enhancer-gene links inferred from integrated data across hundreds of subjects [3]. Larger multiregion work — 3.5 million cells from 384 post-mortem samples across six regions in 111 individuals, yielding over a million candidate regulatory elements organised into 123 modules across 67 cell subtypes — reports widespread epigenome relaxation and region- and cell-type-specific epigenomic erosion during disease progression, closely associated with glial cell-state transitions and with cognitive impairment and resilience [24]. The human epigenomic substrate on which this argument would be tested exists and is public.

What it has not been asked is the specific question. None of these human atlases measures lysosomal integrity. The chromatin is described; the organelle whose condition we propose is driving the chromatin is not. The experiment that joins them is stated in section XIII, and it is, in principle, a re-analysis plus one stain.

XII. Strength of Evidence

The claims assembled above are not of uniform quality, and the argument is only as good as its willingness to say so. Grades are assigned by what was measured, in which species, and in which cell type — not by how well the claim serves the thesis. The central causal claim of this paper is graded *probable*, not *established*, and the most novel claim in it is graded *inferred*.

Claim	Grade	Basis and principal limitation
Lysosomal membrane damage triggers an ordered response: ESCRT-mediated repair first, galectin-marked lysophagy second, TFE-family transcriptional replacement third	Established	Multiple independent laboratories; live imaging with defined temporal order; loss-of-function converts reversible damage to lethal [9 10 11 12]. Cell lines, not neurons or microglia

Claim	Grade	Basis and principal limitation
Galectin puncta are a valid and sensitive readout of lysosomal membrane permeabilisation, applicable to fixed human tissue	Established	Validated against multiple insults and cell types; works in paraffin sections [8]
A purely lysosomal genetic lesion, with no proteinopathy, produces a microglial state overlapping the disease-associated signature	Established (in mouse)	Ultrastructure, transcriptome and epigenome in sulfamidase-deficient mice; overlap quantified against amyloid- and tau-responsive signatures and recovered in human Alzheimer microglia [6]. Single genetic lesion; one species for the primary data
MITF/TFE family factors are required for the lysosomal-stress microglial signature, and diverse lysosomal perturbations converge on it	Established (in mouse)	Factor deletion reverses the signature; panel of perturbations converges, with deacidification the closest phenocopy [7]
Lysosomal stress is the general upstream determinant of the disease-associated microglial state across neurodegenerative conditions	**Probable**	Two unrelated lesions converging on one factor family [6 7]; mechanistic plausibility from the damage-response literature; supported by the composition of the signature itself and by TREM2-deficient autophagic accumulation [5]. Not demonstrated by direct manipulation of lysosomal integrity in microglia in vivo with state as the readout
Human ageing and Alzheimer's disease produce constitutive lysosomal membrane damage and defective ESCRT-mediated repair in neurons	Established (human cells)	Donor-derived transdifferentiated neurons retaining the ageing epigenome; ESCRT-III and galectin-3 puncta at baseline, graded young < aged < aged/sporadic-AD; impaired repair kinetics after defined injury [13]. In vitro; donor numbers modest
Improving lysosomal function lowers intraneuronal amyloid-β	Probable	Three mechanistically distinct compounds, 20–46 per cent reduction, in human donor-derived neurons; damage-increasing agents raise amyloid [13]. Converges with lowered vacuolar ATPase activity preceding deposition in five mouse models [14]. No human in vivo evidence
Fibrillar amyloid activates microglia as a particulate lysosome-damaging agent rather than by ligand specificity	Established	Requires phagocytosis, then lysosomal damage and cathepsin B release [15]; proteopathic tau independently shown to engage the same damage machinery [12]

Claim	Grade	Basis and principal limitation
Alzheimer common risk variants act predominantly through microglial regulatory elements	Established	Cell-type-resolved enhancer maps [16]; large-scale association with pathway enrichment [17]; allele-specific chromatin across 26 loci [18]
Reduced degradative throughput in microglia is expressed as lipid droplet accumulation, which is itself causal for phagocytic failure	Probable	Converging evidence from ageing [19], an amyloid-induced acyltransferase whose inhibition restores uptake [20], an APOE4-linked human state with neurotoxic secretome [21], and a risk allele driving droplets via reduced endocytic adaptor [18]. Causal ordering relative to lysosomal damage itself not established
TREM2–APOE transduces degradative strain into state rather than originating it; TGF-β maintenance and lysosomal stress operate at different levels	Probable	Founding switch experiment is triggered by a phagocytic cargo [26]; TREM2 loss produces undegraded autophagic accumulation [5]; axis removal spares the lesion [23]. Layering is an interpretation, not a measurement; no experiment orders the three levels directly
Blocking the microglial state does not correct the underlying lysosomal lesion	Established (in mouse and human cells)	TREM2 ablation or antagonist antibody reduces hyperactivation in progranulin deficiency without rescuing lysosomal dysfunction, lipid dysregulation or glucose hypometabolism; double-deficient animals worse [23]
Galectin-3 links lysosomal rupture directly to TREM2 signalling in the same or neighbouring cells	**Inferred**	Both roles independently documented [12 22], but in different compartments; no study connects the sensor pool to the ligand pool. Experiment named in section XIII
The ordered damage response operates with the same grammar in human microglia in vivo	Speculative	No direct demonstration. Human microglial state atlases establish that state is transcription-factor-driven and manipulable [3], and epigenomic erosion accompanies glial state transitions in human disease [24], but neither measures lysosomal integrity

XIII. Predictions and Falsification

A causal claim that generates no discriminating prediction is a restatement. The following are stated so that they can fail.

1. The state should be inducible in microglia by lysosomal damage alone, with no disease-relevant cargo. Apply a graded, reversible lysosomotropic insult — the dipeptide ester used throughout the damage literature is the obvious agent — to primary or human stem-cell-derived microglia, and assay the state transcriptionally and epigenomically. The prediction

is acquisition of the disease-associated signature, with MITF/TFE motif enrichment at induced enhancers, in the complete absence of amyloid, tau, myelin or apoptotic cargo. *Falsified if* a pure membrane insult produces an inflammatory response without the characteristic homeostatic-gene downregulation and lysosomal-gene induction that define the state.

2. Repair capacity, not insult magnitude, should predict state. In the same system, hold the insult constant and vary repair competence — by depleting TSG101 or ALIX, or by the ESCRT-upregulating compound shown to lower amyloid in neurons. The prediction is that state acquisition tracks repair competence, so that a cell with impaired ESCRT enters the state at an insult dose that a repair-competent cell tolerates. *Falsified if* state acquisition tracks only cargo load.

3. Lysosomal damage markers should be measurable in human post-mortem microglia and should grade with pathology. The galectin puncta assay was explicitly validated for paraffin-embedded tissue [8]. We are not aware of its systematic application to human Alzheimer brain with microglial co-staining and state markers. The prediction is that galectin-3 and ESCRT-III puncta in microglia grade with Braak stage and with state marker expression, and — the discriminating part — that puncta burden is elevated in microglia *distant from* plaques in advanced cases, not only in plaque-proximal cells. *Falsified if* damage markers are strictly confined to plaque-associated microglia, which would restore the proteinopathy-proximity model.

4. The galectin-3 weld is testable directly. Determine whether galectin-3 released by microglia experiencing lysosomal damage signals through TREM2. Induce lysosomal damage in galectin-3-competent and galectin-3-null microglia; collect conditioned medium; apply to TREM2 reporter cells and to naive microglia; assay DAP12-proximal signalling and state acquisition. The prediction is that conditioned medium from damaged galectin-3-competent cells drives TREM2-dependent signalling in naive cells, and that this is lost in galectin-3-null donors. *Falsified if* no TREM2-dependent activity appears in the medium, in which case the two roles of galectin-3 are independent and section IX should be withdrawn.

5. Lysosome-directed intervention should uncouple pathology from state in a way that state-directed intervention does not. This is the prediction that most directly discriminates the two models, and part of it has already been tested in the direction that supports ours (section XIV). The remaining half: in an amyloid or tau model, a lysosome-directed agent should reduce both the lesion and the state, whereas a state-directed agent should reduce the state without correcting the lysosomal phenotype. *Falsified if* lysosome-directed intervention leaves the state intact while lowering pathology.

6. Cell-type specificity should be reassignable. Because the argument holds that the microglial character of the signature comes from MITF/TFE reading a myeloid enhancer landscape established by PU.1 and C/EBP, forcing MITF/TFE activity in a cell with a different

lineage-determining landscape should yield a different programme from the same stress. *Falsified if* MITF/TFE activation produces the disease-associated gene set irrespective of the lineage context.

XIV. Limitations, and the Finding That Argues Hardest for This Reading

Several limitations are material and we would rather state them than have them found.

The two studies that carry most of the causal weight are mouse studies, they were published together, and they share an author. The convergence they report is therefore less independent than the phrase "two unrelated lesions" implies. What partially offsets this is that the lesions themselves are genuinely unrelated — a sulfamidase and a secreted lysosomal glycoprotein, a childhood storage disease and an adult dementia — and that the human confirmation in each case comes from separate datasets. But an independent replication in a third lysosomal lesion, by an unaffiliated group, is owed.

The human neuronal work is in vitro, the donor numbers are modest, and transdifferentiated neurons are a young model whose fidelity to cortical neurons in situ is not fully established. Its great virtue — retention of the ageing epigenome — is also the thing that makes it hardest to benchmark, because there is no comparably aged human neuronal model to benchmark it against.

The lipid droplet material carries a specific ambiguity we have not resolved. We have treated droplet accumulation as an expression of degradative throughput being exceeded, which places it downstream of the lysosomal lesion. The evidence is consistent with this, but it is also consistent with droplets arising by a parallel route — through altered lipid metabolism directly — and then damaging lysosomes secondarily. The causal ordering between droplet formation and lysosomal membrane damage in microglia has not been established, and we do not claim it.

Most importantly, the central claim is graded *probable* rather than *established* for a concrete reason: no study has directly manipulated lysosomal membrane integrity in microglia in vivo and read out the disease-associated state. Prediction 1 exists precisely because that experiment has not been done.

We close with the finding that argues hardest for this reading, and it is a negative one.

If the disease-associated state were the pathogenic event, then removing it should help. It has been tried. In progranulin-deficient models — the lysosomal lesion of section IV — TREM2 deficiency reduced microglial hyperactivation, and antagonist TREM2 antibodies reduced hyperactivation, TREM2 signalling and phagocytic activity in progranulin-deficient human stem-cell-derived microglia [23]. The state-directed intervention worked, on the state.

It did not rescue the lysosomal dysfunction. In the human cells, lysosomal dysfunction persisted. In the mice, lysosomal dysfunction, lipid dysregulation and glucose hypometabolism were all unrescued by TREM2 ablation. And the animals were worse: synaptic loss and cerebrospinal neurofilament light, a biomarker of neurodegeneration, were further elevated in the double-deficient animals. The authors concluded that TREM2-dependent hyperactivation in progranulin deficiency is not neurotoxic but neuroprotective.

This is the asymmetry that a causal arrow predicts and a co-occurrence does not. If lysosomal stress and microglial state were merely associated features of a diseased brain, one would have no strong expectation about what happens when the state is removed. If the state is downstream of the organelle, then removing the state leaves the organelle exactly as it was, deprives the cell of a compensatory programme, and makes the animal worse — which is what was observed, including in the same direction as the compensatory GPNMB result of section IV, where deleting a marker of the stressed state exacerbated neurotoxicity [7].

The therapeutic corollary follows without further argument and is worth stating baldly, because a great deal of current effort points the other way. Intervening on microglial state — suppressing it, or enhancing it — is intervening on a response. The tractable target is the breach. That target is druggable in principle, it has been hit in human donor-derived neurons by three mechanistically unrelated compounds with concordant effects on amyloid and on cytokine secretion [13], and the relevant lesion — deacidification — is the one that most closely phenocopies a human dementia gene in microglia [7].

XV. Therapeutic Corollaries

The reordering proposed here is not merely taxonomic. It relocates the target, and the relocation is testable against trials that have already read out.

The state-directed strategy has now been tried in humans, and the result is informative. A humanised agonistic antibody against TREM2 — the receptor identified as the master regulator of the disease-associated phenotype, and the natural target if the state is the thing to fix — was tested in a phase 2 randomised, double-blind, placebo-controlled trial in 381 participants with early Alzheimer's disease, at three doses, for 48 to 96 weeks [28]. The trial did not meet its primary endpoint on the Clinical Dementia Rating–Sum of Boxes, at any dose.

The reason this is informative rather than merely disappointing is that it was not a delivery failure. The antibody demonstrated sustained central target engagement and a pharmacodynamic response in the central nervous system, evidenced by reductions in cerebrospinal soluble TREM2 and increases in osteopontin. The receptor was reached, and it responded. What did not follow was

clinical benefit. A negative trial with confirmed engagement is a test of the hypothesis rather than of the molecule, and the hypothesis under test was that driving the microglial state is therapeutic.

This sits beside the preclinical result of the opposite sign from section XIV: removing TREM2 in progranulin deficiency also failed to correct the lesion, and made the animals worse [23]. Pushing the state and removing the state have now both been tried, and neither mends the organelle. That is what one expects of an intervention applied downstream of the lesion.

The lysosome-directed strategy has preclinical support from three independent directions, none of it yet in humans. In human donor-derived neurons, three compounds acting by unrelated mechanisms — promoting acidification, raising a lysosomal phospholipid, and upregulating ESCRT transcripts — each lowered intraneuronal amyloid-β by roughly 20 to 46 per cent and reduced inflammatory cytokine secretion [13]. The convergence of three mechanisms on one outcome is the part that matters; it argues that the effect belongs to the organelle rather than to any compound's off-target activity. In microglia, relieving the downstream expression of degradative overload works too: inhibiting the acyltransferase that drives lipid droplet formation improved amyloid uptake and reduced plaque load and neuronal damage in an amyloid model [20]. And the compensatory arm identified in progranulin deficiency — GPNMB induction, which promotes lysosomal acidification and whose loss exacerbates neurotoxicity [7] — suggests the cell's own preferred remedy is acidification, which is also the perturbation that most closely phenocopies the disease when it fails.

Three cautions belong with this, and we would rather state them than let the argument run further than the evidence.

The first is that "fix the lysosome" is not yet a drug class. The compounds above are tool compounds with heterogeneous mechanisms, and the organelle is central to every cell in the body; a systemic lysosomal agonist has an obvious therapeutic index problem that none of this work addresses. The second is timing. If section XI is right that the state is an enhancer landscape rather than a transcriptional response, then late intervention may relieve the stress without restoring the programme, and the relevant window may be considerably earlier than the symptomatic window in which trials are conducted. The third is that no lysosome-directed agent has been tested against a clinical endpoint in Alzheimer's disease, and the history of this field is a history of preclinical convergence failing to survive that transition.

What the argument does license is narrower and, we think, defensible: that the therapeutic attention currently directed at modulating microglial state is directed at a response, that this has now been tested at the receptor level in humans without benefit despite engagement, and that the upstream node at which several independent lines converge has not been tested at all.

XVI. Conclusion

The disease-associated microglion was named for the diseases in which it was found, and the name has been doing causal work it was never entitled to do. A childhood storage disease with no plaques and no tangles makes the same state. An adult dementia gene makes it through the same transcription factors. A panel of chemically diverse lysosomal insults makes it, with deacidification the closest phenocopy. And the transcription factor family that makes it is the family that governs lysosomal biogenesis — the third and last tier of an ordered damage response whose first tier reseals a perforated membrane and whose second tier destroys the organelle it could not reseal.

The human evidence says the first two tiers are failing. Neurons from aged donors, and far more from donors with sporadic Alzheimer's disease, carry a constitutive, sublethal, chronic lysosomal breach marked simultaneously by the machinery of repair and the sensor of removal; they mend imposed damage more slowly; they secrete inflammatory cytokines because of it; and they make less amyloid when three unrelated compounds mend the organelle. Human genetics, arriving from a different direction entirely, places the common risk variants in microglial enhancers, resolves one of them to a loss of transcription factor occupancy that impairs ingestion and strands lipid, and identifies the strongest risk allele in the disease as the one that sets how much lipid gets stranded.

What unites these is not a molecule. It is a ratio: the degradative capacity of a cell against the load it has been asked to clear. Amyloid, tau, myelin debris, glycosaminoglycan and the lipofuscin of age have nothing in common chemically, and everything in common as load. The state we have been reading as a response to a protein is a response to a shortfall.

The consequence is a reordering, not a demolition. Amyloid remains a lysosome-damaging agent, and a potent one; tau remains a lysosome-damaging agent; the microglial state remains real, reproducible and important. What changes is the direction of the arrow between them, and with it the identification of the tractable target. Removing the response leaves the lesion and costs the cell its compensation, which is what was observed when it was tried. Mending the membrane lowered the aggregate, which is what was observed when that was tried. The breach is upstream. It is where the work should go.

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