

TRAINED IMMUNITY AND HOMEOSTATIC COLLAPSE

Compartment-Specific Logic of BCG in Alzheimer's Disease:
A Mechanistic Reconciliation of Two Incompatible Myeloid States

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Dr. James Truchard & Benjamin Aaron Gustafsson

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Abstract

The candidacy of *Bacillus Calmette-Guérin* (BCG) vaccination as a disease-modifying intervention in Alzheimer's disease rests on two independently developed bodies of literature that have progressed in parallel with almost no mechanistic integration. The first, originating in the work of Mihai Netea and colleagues, establishes that BCG induces durable epigenetic and metabolic reprogramming of hematopoietic progenitors and their monocyte-derived macrophage (MDM) progeny, generating a hyperresponsive state termed "trained immunity" characterized by elevated glycolytic flux, enhanced cytokine output, and augmented phagocytic capacity upon secondary stimulation. The second, originating in the work of Oleg Butovsky and colleagues and extended through the Homeostatic Collapse framework, establishes that adult central nervous system microglia occupy a distinct, TGF- β /SMAD-maintained homeostatic state defined by low inflammatory tone, restrained cytokine output, and high surveillance capacity, and that the age-dependent loss of this signature is the upstream event driving Alzheimer's disease microglial pathology. These two frameworks describe states that are not merely different but appear to be mutually incompatible at the level of the individual cell: trained immunity is a metabolically hyperactive, inflammation-amplified state, while homeostatic microglia are a metabolically restrained, inflammation-suppressed state. The question of how BCG could produce therapeutic benefit in Alzheimer's disease therefore cannot be answered by invoking both mechanisms simultaneously without resolving this apparent contradiction.

This paper undertakes that resolution. We argue that the incompatibility dissolves when the central nervous system myeloid compartment is disaggregated into its developmentally and functionally distinct populations—parenchymal microglia, perivascular macrophages, meningeal macrophages, choroid plexus macrophages, and infiltrating monocyte-derived cells—and when BCG's mechanism is reframed as a **compartment-specific division of labor** rather than as a uniform reprogramming of "myeloid cells." Under this framework, BCG is best understood as an intervention that (1) durably trains peripheral monocytes and their progeny through epigenetic reprogramming at the bone marrow progenitor level, (2) replenishes and reprograms border-associated macrophage (BAM) compartments that turn over from peripheral precursors and are accessible to trained monocyte signaling, (3) reduces the peripheral inflammatory and infectious load whose cumulative effect drives the oxidative stress underlying TGF- β /SMAD signaling failure in parenchymal microglia, and (4) achieves none of these effects through direct reprogramming of the yolk-sac-derived, self-renewing parenchymal microglial population whose homeostatic identity is maintained by niche-specific signaling that trained immunity is not equipped to reinstate. The therapeutic logic is therefore indirect for the parenchymal compartment and direct for the peripheral and border compartments, with net benefit depending on a division of labor in which trained MDMs and BAMs handle clearance functions while parenchymal microglia retain homeostatic identity because the upstream drivers of their collapse have been reduced.

We develop this argument through a systematic examination of the trained immunity literature, the homeostatic microglia literature, the developmental ontogeny of central nervous system myeloid populations, and the specific biochemical incompatibilities between the trained and homeostatic states. We identify the compartment-specific predictions that follow from the division-of-labor model and distinguish them from the predictions of alternative mechanisms. We propose a set of experimental tests—principally involving compartment-resolved transcriptomic analysis of BCG-vaccinated aged animals, longitudinal imaging of perineuronal net integrity in BCG-vaccinated humans, and functional assays distinguishing trained-state clearance from homeostatic-state surveillance—that would discriminate between the division-of-labor hypothesis and its alternatives. We argue that the existing clinical trial infrastructure for BCG in Alzheimer's disease, which relies on amyloid PET, plasma p-tau217, and cognitive composite scores as primary endpoints, is structurally incapable of testing the compartment-specific mechanism we propose, and that the most informative trials would be those that add perineuronal net imaging, gamma oscillation coupling, and compartment-resolved cerebrospinal fluid biomarkers to their endpoint panels. Finally, we address the uncomfortable structural implication of this analysis: the trial design that would actually test BCG's mechanism in Alzheimer's disease is one that no current commercial sponsor has incentive to fund, no regulatory framework currently accepts, and no existing infrastructure is equipped to execute.

1. Introduction: Two Non-Overlapping Literatures

The literature on trained immunity and the literature on homeostatic microglial biology have developed in near-total mechanistic isolation for more than a decade. The trained immunity field, originating with Netea, Quintin, and colleagues' 2011 demonstration that BCG vaccination confers protection against secondary unrelated infections through persistent reprogramming of innate immune cells, has grown into a mature experimental program spanning bone marrow progenitor biology, epigenetic memory, metabolic reprogramming, and clinical epidemiology. Its core finding—that innate immune cells can acquire durable, antigen-nonspecific memory through epigenetic and metabolic mechanisms—has reshaped the conceptual boundary between innate and adaptive immunity and has generated a specific set of therapeutic hypotheses about BCG as a multi-target intervention in conditions as diverse as cancer, type 1 diabetes, and neurodegeneration.

The homeostatic microglia field, originating with Butovsky and colleagues' 2014 identification of a unique adult microglial transcriptional signature and extending through the work surveyed in the Homeostatic Collapse thesis, has grown into an equally mature program spanning niche signaling biology, developmental ontogeny, disease-associated microglial states, and the neuropathology of cognitive resilience. Its core finding—that adult parenchymal microglia occupy a discrete, TGF- β /SMAD-maintained homeostatic state whose loss is the upstream event in Alzheimer's

disease microglial pathology—has reshaped the conceptual framework of neuroinflammation and generated a specific set of therapeutic hypotheses about homeostatic restoration as the appropriate therapeutic target class.

These two programs have almost never spoken to each other. The trained immunity literature rarely engages with the question of whether trained states are compatible with tissue-specific homeostatic identities; it implicitly treats "trained" as uniformly beneficial. The homeostatic microglia literature rarely engages with the question of whether peripheral immune interventions could reach and reshape central nervous system myeloid populations; it implicitly treats the parenchymal microglial compartment as the unit of analysis. When BCG has been proposed as a candidate intervention in Alzheimer's disease, as in the epidemiological studies showing 26–47% risk reduction associated with BCG vaccination and in the systematic reviews that have followed, the mechanistic account has tended to invoke both frameworks simultaneously—"BCG trains myeloid cells and restores homeostatic microglia"—without confronting the fact that these are, as states of the individual cell, contradictory propositions.

The incompatibility is not superficial. Trained monocytes and homeostatic microglia differ at the level of their core biochemistry. Trained cells exhibit aerobic glycolysis driven by mTOR-HIF1 α signaling, elevated glutaminolysis feeding the TCA cycle, enhanced mevalonate pathway activity producing cholesterol and isoprenoid intermediates, H3K4me3 and H3K27ac marks at inflammatory gene promoters, and a functional phenotype characterized by amplified cytokine output upon secondary stimulation. Homeostatic microglia exhibit oxidative phosphorylation-dominant metabolism, restrained cytokine output, high expression of purinergic receptors (P2ry12) that couple them to neuronal ATP gradients, transcriptional maintenance by Sall1 and Tmem119, and a functional phenotype characterized by fine-process surveillance with minimal inflammatory output. These are not points on a continuum; they are distinct cellular states with different metabolic substrates, different epigenetic landscapes, and different functional outputs. The proposition that a single intervention could simultaneously produce both states in the same cell is *prima facie* implausible and requires mechanistic justification that the existing BCG-Alzheimer's literature has not provided.

This paper undertakes that mechanistic justification by abandoning the proposition. We argue that BCG does not produce both states in the same cell; rather, it produces different states in different myeloid compartments, and the therapeutic logic in Alzheimer's disease depends on the resulting division of labor. The trained state is the appropriate and achievable outcome for peripheral monocyte-derived macrophages and for the border-associated macrophage compartments that turn over from peripheral precursors. The homeostatic state is the appropriate and—we will argue—preserved outcome for parenchymal microglia, but BCG's contribution to this preservation is indirect, operating through reduction of the upstream drivers of homeostatic collapse rather than

through direct reprogramming of the parenchymal cells themselves. Understanding this division of labor is the prerequisite for any rigorous account of BCG's mechanism in Alzheimer's disease, and it has consequences for trial design, patient selection, and endpoint choice that the current infrastructure has not absorbed.

2. Trained Immunity: The Peripheral Mechanism

The foundational experiments of the trained immunity program established that BCG vaccination produces persistent functional changes in peripheral blood mononuclear cells that cannot be explained by classical adaptive immune memory. In the 2012 demonstration by Kleinnijenhuis, Quintin, and Netea, BCG-vaccinated individuals showed enhanced cytokine responses to unrelated pathogens including *Candida albicans* and *Staphylococcus aureus* for periods of months to years after vaccination, and this enhancement was mediated by monocytes rather than by T or B cells. The persistence of the effect in a cell population with a circulating half-life of three days implied that the memory was being stored at the level of bone marrow progenitors rather than in the peripheral pool itself.

Subsequent work established the bone marrow mechanism explicitly. Mitroulis, Ruppova, and colleagues demonstrated in 2018 that BCG vaccination reaches hematopoietic stem and progenitor cells (HSPCs), expanding the myeloid progenitor compartment and imprinting epigenetic changes that are propagated to downstream monocyte progeny. The epigenetic signature is characterized by increased H3K4me3 deposition at the promoters of genes encoding glycolytic enzymes, mTOR pathway components, and inflammatory response genes including TNF, IL-6, and IL-1 β . These marks are stable across multiple rounds of monocyte differentiation, such that circulating monocytes produced months or years after BCG administration emerge from the bone marrow already carrying the trained phenotype.

The metabolic signature of trained cells is distinctive. Cheng, Quintin, and colleagues established in 2014 that BCG-trained monocytes exhibit a shift toward aerobic glycolysis—the Warburg-like metabolism characteristic of activated immune cells—mediated by mTOR-HIF1 α signaling. The metabolic shift is not merely correlated with the trained phenotype but is mechanistically required for it: pharmacological inhibition of mTOR, HIF1 α , or glycolysis abolishes the enhanced cytokine response, and the metabolic intermediates of the mevalonate pathway (which produces cholesterol and isoprenoids) serve as the specific link between metabolic flux and epigenetic marking through the fumarate-mediated inhibition of KDM5 histone demethylases. The result is a self-reinforcing state in which glycolytic flux produces metabolic intermediates that maintain the epigenetic marks that specify the glycolytic program—a bistable attractor distinct from the baseline monocyte state and distinct from the terminally differentiated states of tissue macrophages.

The functional consequences of the trained state are substantial. Trained monocytes show enhanced phagocytic capacity, enhanced killing of engulfed pathogens, enhanced antigen presentation, and enhanced cytokine output upon exposure to pattern recognition receptor ligands. Critically for the Alzheimer's disease question, trained cells also show altered responses to non-infectious stimuli including oxidized lipids, crystalline cholesterol, and amyloid- β oligomers. The mevalonate pathway activity that sustains the epigenetic memory also enables more efficient handling of lipid cargo, and the augmented NLRP3 inflammasome priming in trained cells produces stronger IL-1 β responses to both infectious and sterile stimuli. Whether this augmented response is beneficial or harmful in the context of amyloid pathology is an empirical question that depends on whether the enhanced clearance outweighs the enhanced inflammatory output—a question the trained immunity literature has not resolved and that the homeostatic collapse framework reframes in important ways.

The therapeutic logic for BCG in Alzheimer's disease, as articulated in the epidemiological and trial-design literature, has typically rested on three claims: that trained monocytes clear amyloid more effectively, that trained myeloid cells produce the antimicrobial peptide LL-37 which chaperones amyloid monomers against aggregation, and that the systemic anti-inflammatory effects of trained immunity reduce the peripheral inflammatory tone that drives central nervous system pathology. Each of these claims has experimental support in isolation, but none of them directly addresses the question that the homeostatic collapse framework forces: does BCG restore, or could it restore, the TGF- β /SMAD-maintained homeostatic identity of parenchymal microglia? The answer, as we will now develop, is almost certainly no, and understanding why is the key to a proper account of BCG's actual mechanism.

3. The Homeostatic Microglial State: A Different Kind of Cell

The Butovsky signature, as developed in Section 2 of the Homeostatic Collapse thesis, specifies a transcriptional and functional identity that is the product of continuous TGF- β signaling from the surrounding parenchymal niche. The signature includes P2ry12, Tmem119, Sall1, Hexb, Fcrls, Olfml3, and Cx3cr1 at the transcript level, and it is functionally characterized by restrained cytokine output, high surveillance capacity, rapid process extension toward neuronal injury sites, and a metabolic profile dominated by oxidative phosphorylation rather than glycolysis. The critical insight from the Butovsky program is that this state is not the resting condition of a cell that can become activated and return; it is an energetically expensive, niche-dependent phenotype whose maintenance requires continuous signaling input and whose loss is properly understood as a collapse rather than as an activation.

The biochemical properties of homeostatic microglia are, in essentially every respect, the opposite of those that characterize trained monocytes. Where trained cells exhibit aerobic glycolysis,

homeostatic microglia rely on oxidative phosphorylation. Where trained cells amplify cytokine output upon stimulation, homeostatic microglia restrain cytokine output even in response to substantial perturbations. Where trained cells are metabolically primed for rapid response, homeostatic microglia are metabolically tuned for sustained low-level surveillance. Where trained cells carry H3K4me3 marks at inflammatory gene promoters that amplify transcription upon re-stimulation, homeostatic microglia carry transcriptional programs maintained by Sall1 and TGF- β -SMAD signaling that actively suppress the inflammatory response. Where trained cells turn over from bone marrow progenitors and can be replaced by new trained monocytes emerging from reprogrammed HSPCs, homeostatic microglia are yolk-sac-derived, self-renewing, and largely independent of peripheral bone marrow contributions throughout adult life except under conditions of severe injury or experimental ablation.

This last point is the one that the trained immunity literature has most systematically failed to integrate. Parenchymal microglia are not replaced by monocytes under normal conditions. The developmental origin of microglia in the yolk sac, established through fate-mapping studies by Ginhoux and colleagues and extended by the Prinz and Kierdorf programs, means that adult parenchymal microglia are descendants of primitive myeloid progenitors that colonized the brain during embryonic development and have self-renewed locally ever since. Under normal physiological conditions, peripheral monocytes contribute negligibly to the parenchymal microglial pool, and the contributions that do occur—in settings of severe neuroinflammation, experimental microglial ablation, or advanced disease with BBB breakdown—produce cells that are transcriptionally and functionally distinguishable from yolk-sac-derived parenchymal microglia even after they have acquired microglia-like morphological features.

The implication for BCG's mechanism is unavoidable. A peripheral intervention that reprograms bone marrow progenitors cannot reach parenchymal microglia directly, because those cells do not derive from bone marrow progenitors and are not replaced by their progeny. The proposition that BCG restores homeostatic microglial identity through peripheral monocyte reprogramming is therefore mechanistically untenable. If BCG affects parenchymal microglia at all, it must do so indirectly—through effects on the upstream drivers of homeostatic collapse, not through direct cell replacement or direct epigenetic reprogramming of the parenchymal pool.

4. The Incompatibility of States: Why a Single Cell Cannot Be Both

The mechanistic incompatibility between the trained and homeostatic states deserves explicit development because the failure to engage with it is the central weakness of the existing BCG-Alzheimer's literature. The two states differ at four mechanistic levels, and at each level the differences are not merely quantitative but categorical.

Metabolic substrate. Trained immunity is defined by the glycolytic shift: HIF1 α -mediated upregulation of glucose transporters, hexokinase, phosphofructokinase, and lactate dehydrogenase, with consequent accumulation of glycolytic intermediates that feed the pentose phosphate pathway and the mevalonate pathway. Homeostatic microglia are characterized by the opposite metabolic profile—OXPHOS-dominant respiration, efficient ATP production from substrate oxidation, and minimal lactate output. A single cell cannot simultaneously execute both programs because they make incompatible demands on mitochondrial substrate allocation and redox balance. Cells can transition between the states, but they cannot occupy both at once.

Epigenetic landscape. The trained state is specified by H3K4me3 and H3K27ac marks at inflammatory gene promoters, producing a primed transcriptional state in which subsequent stimulation generates amplified cytokine responses. The homeostatic microglial state is specified by a different chromatin architecture, including Sall1-dependent transcriptional maintenance and TGF- β -SMAD-dependent suppression of inflammatory programs. The epigenetic marks of trained immunity and those of homeostatic microglia are, where they have been compared, located at different loci and produce different transcriptional outputs. A cell cannot carry both sets of marks simultaneously at the same genomic locations, and the proposition that a cell could carry the trained marks at inflammatory loci while maintaining homeostatic marks at other loci is untested and would represent a distinct third state rather than a simultaneous occupation of the first two.

Functional output upon stimulation. Trained cells amplify cytokine output in response to secondary stimuli. Homeostatic microglia restrain cytokine output in response to stimuli. These are not different dosings of the same response; they are functionally opposite behaviors. The Butovsky framework specifies that Cx3cr1-fractalkine signaling from neurons actively suppresses microglial cytokine output under basal conditions, and that loss of this suppression is part of what characterizes the post-homeostatic state. Installing the trained phenotype in a cell that had been homeostatic would, by definition, disable the suppressive signaling that defines homeostasis. These cannot coexist.

Developmental and turnover biology. Trained peripheral monocytes turn over from bone marrow progenitors on timescales of days to weeks, with the "memory" stored at the progenitor level to ensure continuous replenishment of trained cells. Parenchymal microglia self-renew locally with minimal turnover and minimal bone marrow contribution over years to decades. A therapeutic strategy that depends on continuous replenishment from trained progenitors cannot reach a population that does not replenish from those progenitors, and a cell that has been parenchymally resident for years does not suddenly acquire the epigenetic marks of a cell that emerged from a recently reprogrammed HSPC.

The implication of these four incompatibilities, taken together, is that BCG cannot produce a cell that is simultaneously trained and homeostatic. Any therapeutic benefit must therefore operate

through a compartment-specific logic in which different myeloid populations adopt different states and the net effect depends on how these compartment-specific effects combine. The question is not whether BCG reprograms myeloid cells—it manifestly does—but which cells it reprograms, what state it installs in them, and how that state interacts with the compartments it does not directly reach.

5. The Compartment Framework: Disaggregating "Myeloid Cells"

The Prinz and Kierdorf work on central nervous system myeloid populations established that the category "microglia" has been applied with insufficient precision across the neurodegeneration literature. The CNS contains at least five distinct myeloid populations that differ in developmental origin, transcriptional identity, functional role, turnover dynamics, and relationship to peripheral immunity. Any rigorous account of a peripheral immunomodulatory intervention must specify which of these compartments is being targeted and what effect is expected in each.

Parenchymal microglia are yolk-sac-derived, self-renewing, and maintained by niche-specific TGF- β signaling from surrounding neural and vascular cells. They are the Butovsky-signature cells that the homeostatic collapse framework treats as the upstream variable in Alzheimer's disease pathology. Peripheral monocytes contribute negligibly to this pool under normal conditions, and the pool is largely inaccessible to peripheral immune interventions that operate through bone marrow reprogramming.

Perivascular macrophages reside in the Virchow-Robin spaces between the vascular basement membrane and the glia limitans. They turn over from bone marrow precursors on timescales of months to years, which is substantially slower than peripheral monocytes but substantially faster than parenchymal microglia. They express distinct markers including CD206, CD163, and Lyve1, and they play specialized roles in cerebrovascular amyloid handling and glymphatic flow regulation. Because they derive from bone marrow precursors, they are accessible in principle to peripheral immune interventions that reprogram those precursors.

Meningeal macrophages reside in the dura mater, pia mater, and arachnoid layers and mediate immune surveillance at the CNS border. They exhibit substantial peripheral monocyte contribution under both physiological and pathological conditions, particularly in the dura, and they interface directly with meningeal lymphatic vessels that have become a focus of research in age-related cognitive decline. Their accessibility to peripheral immune interventions is high.

Choroid plexus macrophages reside within the choroid plexus stroma and at the blood-CSF barrier, where they sample cerebrospinal fluid content and interact with peripheral immune cells crossing into the CNS through this route. They are derived from peripheral precursors and are accessible to bone marrow reprogramming.

Infiltrating monocyte-derived cells are peripheral monocytes that cross a compromised blood-brain barrier during pathological states including severe neuroinflammation, ischemic injury, and advanced neurodegeneration. Their contribution to the parenchymal myeloid pool is normally small but can increase substantially when barrier function is disrupted. Once resident in the parenchyma, they can acquire microglia-like transcriptional features but remain distinguishable from yolk-sac-derived cells through lineage tracing and through persistent expression of monocyte-associated markers.

The disaggregation of "microglia" into these five compartments is not a technical refinement; it is the prerequisite for any coherent account of how a peripheral intervention could affect central nervous system myeloid function. Parenchymal microglia and peripheral-derived populations differ in essentially every property relevant to therapeutic targeting: origin, turnover, accessibility, transcriptional maintenance, and functional role. A peripheral intervention that reprograms bone marrow progenitors will reach the peripheral-derived compartments proportionally to their turnover rate from those progenitors and will not reach the parenchymal compartment at all except through indirect effects mediated by changes in peripheral signaling that ultimately alter the parenchymal niche.

6. The Division-of-Labor Model

The compartment framework makes possible a coherent account of BCG's mechanism in Alzheimer's disease that neither invokes the incompatible proposition of simultaneous trained-and-homeostatic states nor abandons the claim that BCG has meaningful effects on central nervous system pathology. We term this account the **division-of-labor model**, and it has five components.

First, BCG produces durable trained immunity in peripheral monocytes and their progeny through bone marrow progenitor reprogramming. This effect is well-established and is the foundational mechanism of trained immunity in its entirety. Trained MDMs exhibit enhanced clearance capacity, enhanced cytokine responsiveness to secondary stimuli, and altered handling of lipid and amyloid cargo.

Second, the trained state reaches border-associated macrophage compartments to the extent that those compartments turn over from the reprogrammed peripheral precursor pool. Perivascular macrophages, with their months-to-years turnover rate, are progressively replaced by trained-state cells after BCG vaccination, on a timescale that matches the months-to-years persistence of the trained phenotype at the progenitor level. Meningeal and choroid plexus macrophages, with their higher turnover rates and greater peripheral contribution, are more rapidly replaced by trained cells. The BAM compartments therefore acquire the trained state in a time-dependent manner that

depends on their specific turnover dynamics.

Third, BCG reduces the peripheral inflammatory and infectious load whose cumulative effect drives the oxidative stress underlying TGF- β /SMAD signaling failure in parenchymal microglia. The epidemiological effects of BCG on cardiovascular disease, on infectious burden including HSV-1 reactivation, and on systemic inflammatory markers are well-documented. Each of these effects reduces a specific upstream driver of the oxidative load that the von Bernhardt framework identifies as the proximate cause of TGF- β /SMAD failure. BCG does not directly supply TGF- β signaling to parenchymal microglia, but it reduces the rate at which that signaling fails.

Fourth, BCG achieves none of these effects through direct reprogramming of the yolk-sac-derived parenchymal microglial population. This population is inaccessible to peripheral interventions, and the therapeutic logic does not require that it be reached directly. The goal, under the homeostatic collapse framework, is to preserve the homeostatic identity of parenchymal microglia by reducing the drivers of collapse, not to install a trained state in those cells.

Fifth, the net effect of BCG on Alzheimer's disease pathology depends on whether the division of labor produces superadditive benefit or whether the trained state in peripheral and border compartments produces harms that offset the benefits from upstream load reduction. This is the critical empirical question that the existing literature has not addressed, and it is the question that determines whether BCG is a genuinely promising intervention or merely a more interesting variant of the anti-inflammatory approaches that have repeatedly failed in Alzheimer's disease.

The division-of-labor model has several attractive features. It reconciles the trained immunity and homeostatic microglia literatures without invoking incompatible propositions. It generates specific predictions about which compartments should show evidence of BCG effects and which should not. It explains why the epidemiological effect sizes for BCG in Alzheimer's disease are larger than those for anti-amyloid interventions (because BCG operates on upstream drivers before collapse has become self-sustaining) while also predicting that BCG will be disappointing in late-disease trials (because by that point the parenchymal compartment has already collapsed and the upstream load reduction comes too late). It situates BCG within a broader framework of homeostatic preservation rather than treating it as an isolated therapeutic curiosity.

But the division-of-labor model also has a substantial vulnerability. It depends on the claim that trained monocytes and BAMs can perform useful clearance and immunological functions without producing inflammatory output that damages parenchymal microglia or accelerates their collapse. This claim is not obviously true, and it has not been tested. The trained state is, by definition, a state of amplified inflammatory output. If trained MDMs infiltrating the CNS in settings of mild BBB compromise—a condition that is increasingly recognized as a feature of midlife and aging—produce cytokine outputs that drive parenchymal microglia further toward collapse rather than helping to

preserve their homeostatic identity, then BCG could be actively harmful in populations where BBB integrity is already compromised. This is a specific risk that the division-of-labor model acknowledges but cannot resolve without direct experimental test.

7. Mechanistic Predictions and Falsifiable Claims

The value of any mechanistic model lies in the specific predictions it generates that could distinguish it from alternatives. The division-of-labor model produces several predictions that are not shared with simpler accounts of BCG's mechanism.

Prediction 1: Compartment-resolved transcriptomics should show trained signatures in BAMs but not in parenchymal microglia. Single-cell RNA sequencing of CNS myeloid populations from BCG-vaccinated aged animals should reveal H3K4me3-associated trained signatures in perivascular, meningeal, and choroid plexus macrophage populations, with the magnitude of the effect proportional to the turnover rate of each compartment. Parenchymal microglia should show no direct trained signature but should show preserved homeostatic markers (P2ry12, Tmem119, Sall1) relative to unvaccinated controls. If parenchymal microglia in BCG-vaccinated animals show trained marks, the division-of-labor model is falsified in favor of an alternative in which BCG directly reprograms the parenchymal compartment through mechanisms the model has not identified.

Prediction 2: Temporal dynamics should match compartment turnover rates. The onset of BCG effects in each compartment should track the turnover rate of that compartment from peripheral precursors. Meningeal and choroid plexus effects should appear within weeks; perivascular effects should appear over months; parenchymal effects (if present at all) should appear over years and should be mediated by reductions in upstream drivers rather than by direct cellular reprogramming. If parenchymal effects appear early and rapidly, the division-of-labor model is falsified.

Prediction 3: Perineuronal net integrity should be preserved in BCG-vaccinated individuals to the extent that upstream load reduction prevents parenchymal microglial collapse. The PNN readout identified in the homeostatic collapse thesis as the concrete molecular correlate of preserved homeostasis should show stronger preservation in BCG-vaccinated aged individuals than in matched unvaccinated controls, with the magnitude of preservation proportional to the reduction in peripheral inflammatory markers achieved by vaccination. If BCG reduces peripheral inflammation without preserving PNN integrity, the division-of-labor model is falsified in favor of an alternative in which peripheral effects do not translate to parenchymal protection.

Prediction 4: BCG should be more effective in prevention than in treatment. Because the model holds that BCG works by reducing the drivers of homeostatic collapse rather than by reversing established collapse, its effect size should be large in populations where collapse has not yet occurred (midlife, preclinical biomarker-positive individuals) and small in populations where collapse is established (symptomatic Alzheimer's disease, patients with substantial tau pathology). If BCG shows large effects in symptomatic populations, the model is falsified in favor of an alternative that includes direct reversal of established pathology.

Prediction 5: BCG should be ineffective or harmful in populations with compromised BBB integrity. If trained MDMs infiltrating a compromised BBB produce inflammatory output that drives parenchymal microglia further toward collapse, BCG should be ineffective or actively harmful in populations where BBB integrity is already compromised. This includes patients with significant cerebral small vessel disease, patients with uncontrolled hypertension, and patients with advanced disease. If BCG shows uniform benefit across BBB-integrity strata, the model's concern about trained monocyte infiltration is unfounded and the model should be revised.

Prediction 6: Compartment-selective interventions should produce compartment-specific benefits. If BCG works through compartment-specific mechanisms, then interventions that target only the peripheral monocyte compartment (e.g., adoptive transfer of trained monocytes) should reproduce a subset of BCG's effects corresponding to that compartment, while interventions that target only the parenchymal compartment (e.g., direct TGF- β pathway restoration in microglia) should reproduce a different subset. The ability to decompose BCG's effect into compartment-specific contributions is a strong test of the division-of-labor model.

These predictions are not currently being tested in any BCG-Alzheimer's trial. The standard endpoints—amyloid PET, plasma p-tau217, cognitive composite scores—are compatible with essentially any mechanism of action and cannot distinguish compartment-specific effects from uniform effects or direct effects from indirect effects. A trial designed to actually test the division-of-labor model would need compartment-resolved biomarkers (CSF markers that reflect BAM versus parenchymal microglial activity), longitudinal PNN imaging, and stratification by baseline BBB integrity. Such a trial does not exist and, under current regulatory and funding structures, is unlikely to be designed.

8. Implications for Trial Design and Patient Selection

The division-of-labor model has direct consequences for how BCG should be tested in Alzheimer's disease, and those consequences differ substantially from the trial designs that have been proposed or executed to date. The key implications are as follows.

Patient selection should emphasize prevention and early disease rather than established pathology. The model predicts large effects in populations where homeostatic collapse has not yet occurred and small or null effects in populations where it has. This implies that BCG trials should be conducted in midlife populations with genetic or biomarker risk (ApoE4 carriers, individuals with positive amyloid PET but preserved cognition) rather than in mild cognitive impairment or symptomatic Alzheimer's disease. The trial structure most likely to produce interpretable results is a long-duration prevention trial in biomarker-positive preclinical individuals, not a short-duration treatment trial in symptomatic patients.

Endpoints should include compartment-resolved biomarkers. Standard endpoints cannot distinguish the division-of-labor mechanism from alternatives. Trials should include biomarkers that reflect activity in specific compartments: cerebrospinal fluid markers that distinguish parenchymal microglial function from BAM function (soluble TREM2 partially serves this role but is not compartment-specific), perivascular space imaging that reflects BAM-mediated glymphatic function, and ideally perineuronal net imaging as the concrete readout of homeostatic preservation. The absence of compartment-resolved endpoints in existing trials is a structural limitation that makes it impossible to test the mechanistic claims we have developed.

Stratification by BBB integrity should be built into the trial design. The model predicts that BCG's effects depend on whether trained monocytes infiltrate the parenchyma in ways that produce beneficial clearance or harmful inflammation. This in turn depends on BBB integrity at the time of vaccination and throughout the follow-up period. Trials should stratify participants by baseline BBB markers (plasma sPDGFR β as a measure of pericyte injury, dynamic contrast-enhanced MRI of BBB permeability) and should monitor BBB integrity longitudinally. Without this stratification, a trial cannot distinguish the prediction that BCG helps when the BBB is intact from the prediction that BCG may be neutral or harmful when the BBB is compromised.

Temporal dynamics of effect onset should be tracked with sufficient granularity to distinguish compartment-specific timescales. The model predicts different timescales for different compartments, and a trial with endpoint assessments only at baseline and 24 months will miss the compartment-specific temporal structure entirely. Serial assessment every three to six months, with biomarker panels that can detect changes in specific compartments, would be required to test the temporal dynamics prediction rigorously.

Comparison against compartment-selective alternatives would be informative. Ideally, BCG trials would be conducted alongside trials of interventions that target specific compartments selectively—for example, peripheral immunomodulators that reach BAMs but not parenchymal microglia, or direct TGF- β pathway modulators (where they become available) that target parenchymal microglia without affecting peripheral compartments. The ability to decompose BCG's effects by comparison against compartment-selective interventions would provide the strongest

possible test of the division-of-labor model.

None of these trial design features is present in the existing BCG-Alzheimer's infrastructure. The existing trials are designed to detect large effects on amyloid and cognitive endpoints in symptomatic populations, which is the design most likely to produce null results if the division-of-labor model is correct. The uncomfortable implication is that the trials most likely to be conducted are the trials least likely to test the mechanism, and that a null result from such trials would not properly falsify the model but would nonetheless be used to dismiss the intervention.

9. The Peripheral-Central Interface: What We Do Not Know

The weakest element of the division-of-labor model, and the element most in need of direct experimental investigation, is the specific mechanism by which peripheral BCG effects translate to central nervous system outcomes. We have argued that this translation occurs through three routes: direct reprogramming of BAMs from peripheral precursors, reduction of peripheral inflammatory load that drives parenchymal microglial collapse, and possible infiltration of trained monocytes in settings of BBB compromise. Each of these routes is mechanistically plausible but experimentally underspecified.

The BAM reprogramming route depends on the turnover dynamics of each BAM compartment from peripheral precursors. These dynamics have been characterized in mouse models through parabiosis and bone marrow chimera experiments, but the human data are sparse, and the relationship between mouse turnover rates and human turnover rates is uncertain. The rate at which BCG-trained monocytes replace existing BAMs in aged humans is not known. If the rate is slow enough that BCG effects on BAM compartments require years to develop, the therapeutic implications are different from a scenario in which the effects develop over weeks to months.

The peripheral load reduction route depends on which specific peripheral factors drive parenchymal microglial collapse and which of those factors are meaningfully reduced by BCG. The candidates include chronic systemic inflammation (CRP, IL-6, TNF), infectious burden (HSV-1 reactivation, gut-derived LPS, subclinical bacteremia), lipid dysregulation, and oxidative stress markers. BCG has effects on several of these, but the magnitude and persistence of those effects in aged humans, and the extent to which they translate to reductions in the specific upstream drivers of TGF- β /SMAD failure, have not been quantitatively characterized. A proper account would require biomarker-based causal mediation analysis showing which peripheral changes account for which central effects.

The trained monocyte infiltration route is the most uncertain. It depends on the extent to which trained monocytes cross a partially compromised BBB in aging and early Alzheimer's disease, on

the functional state those infiltrating cells adopt once resident in the parenchyma, and on whether their infiltration is net beneficial (through enhanced clearance and immune surveillance) or net harmful (through inflammatory output that drives parenchymal microglial collapse). The existing literature on monocyte infiltration in Alzheimer's disease is sparse and has not distinguished trained from untrained infiltrating cells. This is the single largest gap in the mechanistic account we have developed, and it is the point at which the division-of-labor model is most vulnerable to falsification.

The honest summary is that the model is the best available integration of the trained immunity and homeostatic microglia literatures, but it rests on empirical claims that have not been directly tested in the specific form the model requires. Its value lies in specifying which experiments would test it and which would not, rather than in claiming that the experiments have already been done. The gap between what the model requires and what the existing literature provides is substantial, and closing that gap is the prerequisite for any confident claim about BCG's mechanism in Alzheimer's disease.

10. Structural Obstacles to Mechanistic Testing

The final element of this analysis concerns the structural conditions under which the division-of-labor model could actually be tested, and the reasons those conditions do not currently obtain. This is not a digression from the mechanistic argument but an integral part of it, because the probability that the model will be rigorously tested depends on whether the trial infrastructure required for such testing exists or can be created.

The trial that would properly test the division-of-labor model has five features: it is a long-duration prevention trial conducted in midlife biomarker-positive populations, it uses compartment-resolved biomarkers including perineuronal net imaging and CSF markers of specific myeloid compartments, it stratifies participants by BBB integrity at baseline and monitors BBB integrity longitudinally, it assesses endpoints serially at sufficient temporal granularity to detect compartment-specific dynamics, and it compares BCG against compartment-selective alternative interventions that could decompose its effects. Each of these features adds cost, duration, and complexity to the trial, and each is outside the standard template for Alzheimer's disease trials that pharmaceutical sponsors and regulatory agencies have developed over the past two decades.

The funding structures for such a trial do not currently exist. Commercial pharmaceutical sponsors will not fund trials of a century-old, off-patent, \$0.50-per-dose vaccine whose intellectual property cannot be protected. Regulatory agencies do not accept perineuronal net imaging or compartment-resolved myeloid biomarkers as primary endpoints because those endpoints have not been validated against clinical outcomes in prior trials—a chicken-and-egg problem in which the validation requires the trials that the endpoints are needed to justify. Academic and philanthropic

funding for long-duration prevention trials exists in limited quantities (the Alzheimer's Prevention Initiative, the DIAN Trials Unit) but has been directed toward genetically defined populations and anti-amyloid interventions rather than toward peripheral immunomodulators with compartment-specific mechanisms.

The result is that the trials most likely to be conducted in the current infrastructure are the trials least likely to test the mechanism we have described. Short-duration treatment trials in symptomatic populations using standard endpoints will probably produce null or modestly positive results that cannot distinguish between "BCG has no effect on Alzheimer's disease," "BCG has effects through mechanisms the trials cannot measure," and "BCG has effects in populations the trials did not enroll." A null result from such a trial would be used to dismiss BCG as an Alzheimer's disease intervention, and the division-of-labor model would be left untested rather than falsified.

The conceptual cost of this situation is not merely that a potentially useful intervention fails to be properly evaluated. The deeper cost is that the structural mismatch between the trials the infrastructure can execute and the trials the mechanism requires produces a pattern in which interventions that work through compartment-specific, prevention-oriented mechanisms are systematically disadvantaged relative to interventions that work through direct, treatment-oriented mechanisms. The anti-amyloid antibody programs fit the infrastructure because they target a specific pathology with a standard endpoint in a symptomatic population. BCG does not fit the infrastructure, not because it is mechanistically implausible but because its mechanism operates on compartments and timescales that the infrastructure is not designed to measure.

The appropriate response to this situation is not to abandon BCG or to abandon the division-of-labor model but to acknowledge that the mechanism we have developed generates a testable set of predictions that current trial designs cannot evaluate, and that any rigorous assessment of BCG in Alzheimer's disease will require infrastructure that does not currently exist. Articulating the mechanism with sufficient precision that the required infrastructure can be specified is a useful contribution even when the infrastructure itself remains out of reach. The alternative—continuing to invoke BCG's multi-target effects without confronting the incompatibility of trained immunity and homeostatic microglial identity, or dismissing BCG on the basis of trial designs that do not test its mechanism—is intellectually dishonest and therapeutically unproductive.

11. Conclusion: What a Rigorous Account Requires

This paper has undertaken a mechanistic reconciliation of two bodies of literature that have developed in parallel without engaging each other and that, when placed in direct contact, produce an apparent contradiction at the level of the individual myeloid cell. Trained immunity, as specified by the Netea program and its extensions, produces cells that are metabolically glycolytic, epigenetically primed for amplified inflammatory output, and replenished continuously from reprogrammed bone marrow progenitors. Homeostatic microglia, as specified by the Butovsky program and its extensions, are metabolically OXPHOS-dominant, epigenetically tuned for restrained inflammatory output, and maintained by yolk-sac-derived local self-renewal independent of bone marrow contributions. These states cannot be simultaneously occupied by a single cell, and any therapeutic account of BCG in Alzheimer's disease that invokes both mechanisms must specify how they are compatible.

We have argued that the apparent contradiction dissolves under a compartment-specific framework in which BCG produces different states in different myeloid populations and the therapeutic logic depends on the resulting division of labor. Trained immunity reaches peripheral monocytes and their BAM-compartment descendants through bone marrow progenitor reprogramming. It does not reach parenchymal microglia directly. The preservation of parenchymal microglial homeostasis, if it occurs under BCG vaccination, is mediated indirectly through reduction of the upstream peripheral drivers of TGF- β /SMAD signaling failure. The net effect on Alzheimer's disease pathology depends on whether the compartment-specific contributions combine to produce superadditive benefit or whether the trained state in peripheral compartments generates inflammatory output that offsets the benefits of upstream load reduction.

The division-of-labor model generates specific, falsifiable predictions about compartment-resolved transcriptomics, temporal dynamics, perineuronal net preservation, prevention versus treatment efficacy, BBB-integrity stratification, and the decomposability of BCG's effects through compartment-selective alternative interventions. None of these predictions is currently being tested in the existing BCG-Alzheimer's trial infrastructure, which relies on endpoints and populations that are structurally incapable of distinguishing the model from its alternatives. The mechanism we have described therefore remains the best available integration of the existing literature, but it has not been directly tested in the specific form the model requires, and its confirmation or falsification will depend on trial infrastructure that does not currently exist.

The broader lesson of this analysis extends beyond BCG. The trained immunity and homeostatic microglia literatures are not the only pair of mature neuroimmunology programs that have developed without engaging each other, and the compartment-specific disaggregation we have undertaken for myeloid cells is not the only case where the category under analysis has been applied with insufficient precision to support the mechanistic claims being made about it. The general

methodological point is that therapeutic hypotheses in complex disease require mechanistic accounts that specify which cells, which compartments, which timescales, and which states are being targeted, and that accounts which elide these distinctions in favor of multi-target handwaving cannot be rigorously tested even when the underlying intervention is genuinely promising.

BCG may or may not be a useful intervention in Alzheimer's disease. The division-of-labor model we have developed suggests that it has a plausible mechanism of action under specific conditions, that those conditions include prevention-oriented application in midlife biomarker-positive populations with intact BBB function, and that the effect size in such populations could be substantial while the effect size in symptomatic populations with established pathology is likely to be small. Whether this mechanism is correct, and whether BCG can deliver the benefits it predicts, are empirical questions that the existing infrastructure is not designed to answer. Answering them would require trials that do not currently exist, endpoints that are not currently validated, and funding structures that are not currently aligned with the intervention's commercial profile. The structural obstacles to mechanistic testing are not the mechanism's fault, but they are a real constraint on what can be known about it, and any honest account of BCG in Alzheimer's disease has to acknowledge that the mechanism is currently underdetermined by the evidence and that the evidence required to determine it is not being generated.

The task ahead is to specify the experiments that would test the division-of-labor model rigorously, to advocate for the trial infrastructure that could execute those experiments, and to resist both the uncritical enthusiasm that invokes BCG as a multi-target panacea without confronting mechanistic incompatibilities and the dismissive skepticism that treats the intervention's commercial profile as grounds for inattention. The Homeostatic Collapse framework positions BCG as a prevention-class intervention with compartment-specific effects, and it predicts both that the intervention has real therapeutic potential and that the potential will not be realized under current trial-design norms. Acting on this analysis requires changing what gets measured, what gets funded, and what gets enrolled—changes that are possible in principle but unlikely in practice without a substantial restructuring of how Alzheimer's disease research is organized.

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A full reference list compiled from the trained immunity, homeostatic microglia, developmental myeloid ontogeny, and BCG clinical epidemiology literatures will accompany the final version of this paper. Key foundational references include:

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