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How to Get from Here to There: Macrophage Recruitment in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is pathologically defined by presence of intracellular neurofibrillary tangles and extracellular amyloid plaques comprised of amyloid- β (A β) peptides. Despite local recruitment of brain microglia to sites of amyloid deposition, these mononuclear phagocytes ultimately fail at restricting β -amyloid plaque formation. On the other hand, it is becoming increasingly clear that professional phagocytes from the periphery possess A β clearance aptitude. Yet, in order to harness this beneficial innate immune response, effective strategies must be developed to coax monocytes/macrophages from the periphery into the brain. It has previously been suggested that A β 'immunotherapy' clears cerebral A β deposits via mononuclear phagocytes, and recent evidence suggests that targeting transforming growth factor- β -Smad 2/3 signaling and chemokine pathways such as Ccr2 impacts blood-to-brain trafficking of these cells in transgenic mouse models of AD. It has also been shown that the fractalkine receptor (Cx3cr1) pathway plays a critical role in chemo-taxis of mononuclear phagocytes toward neurons destined for death in AD model mice. In order to translate these basic science findings into AD treatments, a key challenge will be to develop a new generation of pharmacotherapeutics that safely and effectively promote recruitment of peripheral amyloid phagocytes into the AD brain.

Keywords

Mononuclear phagocyte; neurodegeneration; amyloid; Abeta; monocyte

MACROPHAGES IN ALZHEIMER'S DISEASE: PAST TO PRESENT

Many studies, the first dating back to the late 1980s, consistently reported on the intricate spatial association between brain macrophages (microglial cells) and senile plaques (A β deposits) in brains of AD patients [1-3]. These initial observations sparked the first of many debates concerning the role of microglia in AD pathology. While many initially regarded microgliosis in AD as an epiphenomenon, according to some, the presence of microglia in close vicinity of senile plaques supported the notion that these cells were actively

phagocytosing and clearing amyloid [3]. Yet, others challenged that microglial cells were actually the source of amyloid fibrils, and provided a nidus for A β plaques [4-7].

It was around the time of the early 1990s that the late neuropathologist Henryk Wisniewski offered a tantalizing explanation for beneficial *vs.* detrimental roles of brain macrophages. He proposed dichotomous functions of brain-resident microglia and blood-borne macrophages in the context of AD pathology. Wisniewski was relying on immunoelectron microscopy and reported that, in the rare comorbidity of stroke with AD, β -amyloid fibrils could be identified in lysosomal compartments of what he believed to be brain-infiltrating macrophages [8]. By contrast, Wisniewski did not observe amyloid fibrils in lysosomes of microglia associated with classical amyloid plaques [7]. Interestingly, Wisniewski's findings were later confirmed by Akiyama and McGeer, who also noted that A β deposits were apparently cleared in macrophage-dense brain regions of AD patients with comorbid stroke [9]. These early experiments raised the possibility that infiltrating peripheral myeloid cells were more efficient at phagocytosing and removing A β deposits than brain-resident microglia. However, the qualitative nature of morphological assessments made in these studies left many open questions. For instance, do microglia and macrophages have differing A β phagocytic capabilities? Further, could blood-derived macrophages restrict cerebral amyloidosis? Why were hematogenous macrophages excluded from the AD brain in the absence of stroke comorbidity? Even over twenty years later, with the advent of transgenic animal models of AD and the availability of modern cellular and molecular biology techniques, the answers to these questions have only begun to be revealed.

Part of the difficulty with addressing A β phagocytosis and clearance aptitude of brain macrophages is that these cells behave differently *in vivo vs. in vitro*. While Wisniewski's work showed that brain-resident microglia were incapable of clearing amyloid, numerous *in vitro* studies have shown that microglia do phagocytose A β . Yet, it deserves noting that brain-resident microglia have limited ability to degrade the peptide [10,11], and are significantly less efficient A β degraders than cultured macrophages. This lack of microglial A β degradation efficiency was later attributed to low hydrolytic activity of endosomal and lysosomal enzymes [12]. Interestingly, more recent *in vitro* and *in vivo* studies have shown that activation of microglia with cytokines such as interleukin (IL)-6, macrophage-colony stimulating factor or interferon- γ can increase their A β phagocytosis capability [13-17].

A β 'IMMUNOTHERAPY' AND THE ROLE OF MONONUCLEAR PHAGOCYTES IN AMYLOID PLAQUE CLEARANCE

Although the studies by Wisniewski and others highlighted the potential of mononuclear phagocytes to clear amyloid, they did not address a fundamental question: could the immune system be harnessed to militate against AD? Seminal work by Dale Schenk in the late 1990s [18] brought the field significantly closer to definitively answering this question. By actively immunizing AD model mice with A β ₁₋₄₂ peptide plus adjuvant, Schenk and colleagues were able to both prevent cerebral amyloid accumulation and to clear existing amyloid plaques. These findings not only gave rise to the field of A β 'immunotherapy', but they also laid the foundation for the Elan/Wyeth 'active' A β vaccine early developmental clinical trials. Unfortunately, the phase IIa A β vaccine trial (AN-1792) was halted in 2002, when approximately 6% of vaccinated AD patients developed aseptic meningoencephalitis, thought to have arisen from brain-infiltrating A β -specific autoaggressive T-cells [19-21]. Shortly after the initial pre-clinical AD mouse model vaccination studies, A β immunotherapy was advanced to passive transfer of antibodies raised against the A β ₁₋₄₂ peptide to transgenic AD model mice. Similar to the original active A β vaccine, 'passive' immunization effectively attenuated AD-like pathology in transgenic mice [22]. Following suspension of AN-1792, passive A β immunotherapy became particularly attractive, because

it circumvents active A β immune response driven by T-cells. A number of other groups have continued to advance A β immunotherapy by employing A β peptide fragments that lack cytotoxic T-cell epitopes or by altering vaccine parameters including adjuvants, carrier proteins, and route of administration for active immunization and by exploring other immunotherapy strategies including DNA-based vaccines [20, 23-30].

Interestingly, while active and passive A β immunization approaches are distinct, they seem to share the same mechanism of action: antibody-mediated A β phagocytosis. Specifically, passive transfer of A β antibodies stimulates microglia to phagocytose and clear amyloid present *ex vivo* in AD brain sections *via* engagement of IgG-recognizing microglial Fc receptors [22]. Furthermore, in an elegant study by Bacskai and colleagues that relied on *in vivo* multiphoton microscopic analysis of passively immunized AD transgenic mice, those authors showed A β antibody-mediated disruption of amyloid plaques in real-time [31, 32] that may be mediated by amyloid phagocytes. Despite restriction of cerebral amyloid after A β immunization, there appear to be negative side-effects to this immunotherapeutic strategy. Notably, AD model mice either actively or passively vaccinated with A β antibodies develop cerebrovascular microhemorrhage [33, 34]. Additionally, A β immunotherapy has been linked to exacerbated cerebral amyloid angiopathy (CAA), which is believed to result from antibody-mediated amyloid clearance from the brain parenchyma into the vasculature [33-36]. Accordingly, while resident mononuclear phagocytes (microglia) seem to clear antibody-opsonized A β deposits, this mechanism does not operate in isolation, and may occur with unwanted side-effects. Future A β vaccination strategies will need to be designed to increase amyloid clearance, for example by targeting brain-intrinsic amyloid phagocytes, without coming at the cost of potentially unsafe side-effects such as cerebral microhemorrhage or CAA.

It is important to note that, even if the safety issues associated with A β vaccination can be overcome, it is not yet clear whether therapeutic strategies that rely on removing cerebral A β in general will ultimately be effective at restoring cognitive function in AD patients. Clearly, there are numerous mitigating factors to consider, including the possibility that any A β -lowering approach is simply 'too little, too late' if administered as an active disease treatment, and that the damage that has already occurred leading up to a clinical diagnosis of AD is irreversible. A logical endpoint of this reasoning is that A β -directed therapeutics would need to be administered early – likely before a diagnosis of AD is given – and that we should focus on prevention rather than treatment of active disease. This caveat is reinforced by a report on the long-term effects of active A β immunotherapy following suspension of the AN-1792 trial. Those authors found that despite reduction in A β , the biological target of the drug, there was no notable improvement in cognition in patients that received the A β vaccine [37]. It should be stressed that these findings have broad implications for any A β -lowering strategy, including targeting blood-borne mononuclear cells as described in this review.

BONE MARROW IRRADIATION CHIMERAS AS MODELS OF MONONUCLEAR PHAGOCYTE ENGRAFTMENT IN ALZHEIMER'S MODEL MICE

Irradiation chimeras represent a practical, commonly used approach to study infiltration of bone marrow-derived cells into the healthy and diseased CNS. In this method, recipient animals are exposed to a 'lethal' dose of irradiation (effectively killing all bone marrow cells) and transplanted, via tail vein injection, with bone marrow expressing a tracer such as green fluorescent protein (GFP). The biggest advantage this method affords is ease of distinctly identifying bone marrow-derived mononuclear phagocytes from resident CNS

microglia, as activated microglia and macrophages share many of the same cell surface receptors and signaling proteins. Initial studies touted this technique, as otherwise unmanipulated, healthy animals demonstrated physiologic renewal of perivascular and meningeal macrophages by GFP⁺ bone marrow-derived cells, but rarely showed GFP⁺ 'resident' parenchymal microglia [38-40]. However, quantitatively minor renewal of parenchymal microglia by peripherally-derived, GFP-expressing donor bone marrow cells in the non-diseased CNS was later observed [41]. Using bone-marrow chimeras, it was Josef Priller who clearly demonstrated enhanced CNS engraftment of mononuclear cells of bone marrow origin in three separate acute models of brain injury [41]. This work was exciting and thought-provoking as it showed a 2- to 50-fold increase (depending on the injury paradigm) in GFP⁺ bone-marrow derived cells in the CNS of lesioned mice compared to controls. Succeeding studies utilized bone-marrow chimeras to investigate the possibility of mononuclear cell infiltration in mouse models of chronic neurodegenerative diseases including experimental autoimmune encephalomyelitis (EAE), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), prion-associated disease and AD [42-47].

Two independent studies from the laboratories of Mathias Jücker and Serge Rivest confirmed infiltration of peripherally-derived mononuclear cells in the CNS of AD model mice [46, 47]. While both studies showed a clear spatial association between bone marrow-derived cells and amyloid plaques, electron microscopy analysis by Stalder *et al.* of GFP-labeled amoeboid cells with macrophage-like ultra-structural features did not disclose evidence of amyloid phagocytosis. By contrast, the work of Simard and coworkers convincingly showed presence of amyloid deposits in GFP⁺ infiltrating mononuclear phagocytes. Moreover, in the latter study, the authors crossed a doubly-transgenic mouse model of AD with CD11b-tyrosine kinase (TK^{mut30}) transgenic mice in which proliferating CD11b⁺ cells can be specifically ablated by administration of the anti-viral drug ganciclovir. In this next-generation model system, the authors demonstrated the importance of proliferating major histocompatibility class II⁺ peripheral mononuclear phagocytes in restricting β -amyloid plaques. More recent results have shown a significant reduction in A β deposit accumulation in another double transgenic mouse model of AD transplanted with wild-type bone marrow [48]. Reduction in cerebral A β load was further improved by grafting AD transgenic animals with mononuclear cells deficient in the E prostaglandin receptor subtype 2 (EP2). Increased efficiency of A β clearance was attributed to the higher phagocytic capability of EP2 knockout mononuclear cells [48, 49].

By contrast, reduction in mononuclear cell infiltration in AD transgenic mice grafted with Ccr2 deficient bone marrow accelerated disease progression, as recently reported by Joseph El Khoury and Josef Priller at the 2010 Keystone Symposium 'Alzheimer's disease beyond A β ' [50]. The beneficial effect of reconstituting the hematopoietic compartment of AD transgenic mice with wild-type bone marrow has recently been demonstrated [51]. In this study, engraftment of wild-type bone marrow mitigated cerebral amyloid load. This beneficial effect correlated with decreased expression of the canonical pro-inflammatory cytokines tumor necrosis factor- α and IL-1 β , and with increased abundance of the cardinal anti-inflammatory molecules IL-4 and IL-10. Further, transplantation of mutant *presenilin-1* (PS1) bone marrow into Tg2576 'Swedish' amyloid precursor protein (APP) mutant mice exacerbated cerebral amyloidosis in the authors' model system. Finally, it was just recently reported that irradiation may not be necessary to promote brain infiltration of adoptively transferred CD11b⁺ cells bearing A β -directed therapeutic genes into brains of AD model mice [52].

While radiation chimerism has been widely used to address key questions related to immunity in the normal and diseased CNS, a number of technical concerns have been raised regarding this experimental paradigm [39, 47]. Indeed, lethal irradiation can cause vascular

alterations and induce cytokine expression, both of which can artificially influence CNS infiltration of peripheral mononuclear cells. Moreover, lethal irradiation and bone marrow reconstitution may lead to a non-physiologic increase in circulating pools of monocyte progenitors with enhanced capacity to migrate into the CNS. Consequently, it is possible that artifacts associated with radiation chimerism lead to enhanced monocyte CNS infiltration and therefore overestimate the contribution of peripherally-derived cells to the microglial cell population in the healthy or disease CNS. Nonetheless, most – if not all – experimental models have drawbacks. Head-sparing irradiation chimeras or parabiosis, techniques that have been used by others to physically connect circulatory systems of mice and thereby circumvent irradiation chimeras, are also flawed [53-55]. In fact, both of these alternative techniques to irradiation-reconstitution likely underestimate the contribution of bone marrow-derived cells to the CNS microglial cell pool. Further, Mildner and colleagues were unable to detect transplanted GFP⁺ brain perivascular macrophages (a well-established resident macrophage subset) after head-sparing irradiation and bone marrow reconstitution, and the parabiosis technique employed by Ajami and coworkers has been noted to underestimate constitutive infiltration of mononuclear cells into lymphoid tissues [56]. Hence, even when considering technical limitations, irradiation chimeras have taught us that, at least under certain circumstances, bone marrow-derived cells can enter the CNS of AD model mice and remodel cerebral amyloid. The use of alternative strategies for investigating the role of peripheral macrophages will undoubtedly lead to clarification of the role of these cells in A β clearance and AD pathology. Accordingly, it was recently demonstrated that depletion of perivascular macrophages by liposome-encapsulated clodronate exacerbated CAA as evidenced by increased deposition of cerebral A β peptides in leptomeningeal and cortical blood vessels in a mouse model of AD. Those authors demonstrated the converse effect on CAA when increasing turnover of perivascular macrophages following chitin administration [57], again indicative of a direct relationship between peripheral macrophages and cerebral amyloid.

TARGETING PERIPHERAL MACROPHAGES IN ALZHEIMER'S MICE BY BLOCKING THE TRANSFORMING GROWTH FACTOR- β PATHWAY

The remarkable demonstration by Serge Rivest that infiltrating peripheral mononuclear phagocytes may restrict cerebral amyloid pathology begged the important question of whether these cells could be targeted to infiltrate into the brain *en masse* and to militate against AD-like pathology. An approach from our group has shed light on this problem. We genetically interrupted transforming growth factor-beta (TGF- β - a pleiotropic polypeptide cytokine master regulator of immune cell activation) and downstream Smad 2/3 signaling on peripheral macrophages (as opposed to brain-resident microglia) by engineering a CD11c promoter-driven dominant-negative TGF- β type II receptor transgene in C57BL/6 mice (CD11c-DNR mice) [56, 58]. We subsequently crossed CD11c-DNR mice with the Tg2576 AD mouse model, and evaluated behavioral impairment and AD-like pathology. Strikingly, bitransgenic animals showed up to 90% attenuation of brain parenchymal and cerebrovascular amyloid deposits. Moreover, bigenic animals exhibited partial amelioration of cognitive impairment and reduced astrogliosis. These therapeutic effects in a mouse model were associated with increased infiltration of A β -containing peripheral mononuclear phagocytes in and around cerebral vessels and amyloid plaques. Further, A β immunoreactivity could be observed within the cytoplasm of these cells, suggesting a productive A β phagocytosis/clearance response. Notably, this beneficial anti-amyloid effect did not come at the cost of increased brain inflammation, as these peripheral mononuclear cell immigrants displayed a CD45⁺CD11b⁺Ly-6C⁻ cell surface phenotype (known to mark 'anti-inflammatory' macrophages [59, 60]) and were present in brains of bigenic mice together with increased levels of the prototypical anti-inflammatory cytokine, IL-10 [61-63].

It seems that removing the immunosuppressive TGF- β signal to hematogenous mononuclear phagocytes not only allows these cells to gain access into the brain, but also maximizes their phagocytic potential. As *ex vivo* validation of the latter, we found ~3-fold increased phagocytosis of A β by CD11c-DNR *vs.* wild-type macrophages. In addition, we observed similar effects *in vitro* with pharmacologic inhibitors of TGF- β -Smad 2/3 signaling [61] (manuscript in preparation). These results suggest that inhibition of TGF- β -Smad 2/3 signaling is essential for both peripheral mononuclear phagocyte recruitment to brains of AD model mice and for phagocytic amyloid removal.

BLOCKING CCR2 INHIBITS BRAIN MACROPHAGE RECRUITMENT IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Chemokines are a family of small (8-10 kDa) chemotactic cytokines produced by both immune and non-immune cells. These proteins exert their biological effects by binding to cognate G protein-coupled seven-transmembrane receptors on target cells [64]. Chemokines are classified into three subfamilies based on the pattern of primary sequence conserved cysteine residues [65]. The α subfamily of chemokines, termed CXC, is defined on the basis of two amino-terminal cysteines separated by one amino acid, represented with an 'X'. The β subfamily contains adjacent cysteine residues, and is thus termed CC [65]. Additionally, fractalkine, the only member of the CXXXC subfamily, contains three amino acids between the first two cysteine residues. Several reports have indicated that chemokines induce recruitment of leukocytes to sites of acute and chronic inflammation [66]. For instance, absence of *Ccr2* reduced numbers of peritoneal monocytes in a model of acute inflammation and restricted atherosclerotic lesion size associated with decreased abundance of pro-inflammatory monocytes [65]. Moreover, mice deficient in *Ccr2* or its cognate ligand *Ccl2* had reduced monocyte recruitment into the CNS in the EAE mouse model of multiple sclerosis [67].

Recent evidence suggests that chemokines are also critical for mononuclear phagocyte trafficking in the context of AD. Specifically, Joseph El Khoury and colleagues bred the Tg2576 AD mouse model with *Ccr2* null mice and analyzed offspring for AD-like pathology. Interestingly, his group reported that *Ccr2* deficiency significantly impaired trafficking of mononuclear cells to A β plaques as quantified by CD11b⁺ cell abundance. Perhaps more significantly, diminished recruitment of mononuclear phagocytes was associated with increased mortality and higher A β plaque load [68]. These data indicate that *Ccr2* plays a non-redundant role in restricting cerebral amyloidosis by promoting accumulation of monocytes/microglia that are capable of phagocytosing A β deposits. Yet, it should be noted that the role of brain-resident microglia in clearing A β in AD patients and in mouse models of the disease is a controversial area. Recent work from the group of Mathias Jücker has prompted the iconoclastic conclusion that brain-resident microglia do not play a significant role in the clearance of A β . These researchers utilized a CD11b-TK/ganciclovir suicide gene approach to kill microglia for two-to-four weeks in two different AD mouse models (APP/PS1 and APP23). After selective ablation of microglia in APP/PS1 transgenic mice, those authors did not detect differences between treated and untreated animals on total A β burden, plaque morphology, or distribution of cerebral A β deposits [69]. While, at face value, it is difficult to reconcile these results with work from our group and from El Khoury *et al.*, it remains possible that ablation of brain macrophages for only two-to-four weeks is not long enough to observe altered A β plaque dynamics in transgenic AD model mice. Unfortunately, due to toxicity associated with administration of ganciclovir to the CD11bTK transgenic mice, Grathwohl and colleagues were not able to ablate cerebral macrophages for longer than four weeks, temporally limiting the conclusions that could be drawn from their study.

ROLE OF FRACTALKINE SIGNALING IN RECRUITING MICROGLIA TO NEURONS DESTINED FOR DEATH

Expression of the fractalkine receptor (Cx3cr1) is exquisitely restricted within the CNS to microglia [70]. Using three different *in vivo* models, Richard Ransohoff's group demonstrated that Cx3cr1 deficiency dysregulates microglial responses, resulting in neuronal death. In the MPTP intoxication model of PD and in a transgenic mouse model of ALS, *Cx3cr1*^{-/-} mice demonstrated increased neuronal cell loss compared to *Cx3cr1*-sufficient littermate controls [71]. These data suggest that Cx3cr1-dependent recruitment of microglia in response to non-AD neuropathologies may be beneficial by opposing neurotoxicity. Just recently, a new perspective on the role of microglia in AD was presented by Jochen Herms and colleagues [72]. These authors demonstrated neuronal loss in AD model mice, and showed that knocking out the microglial Cx3cr1 fractalkine receptor prevented neurodegeneration. The authors utilized an elegant intravital two-photon imaging approach to analyze microglial interactions with neighboring neurons in brains of coauthor Frank LaFerla's 3x Tg-AD mice. The 3x Tg-AD mouse model overexpresses pathogenic mutant forms of *PS1* (M146V), *APP* (Swedish), and *tau* (P301L). In order to observe the interaction between neurons and microglia, the Herms group crossed 3x Tg-AD mice with two additional transgenic lines- one in which subsets of layer III and V cortical neurons express yellow fluorescent protein (YFP) [73], and another carrying a GFP knock-in in place of the endogenous murine *Cx3cr1* locus [74]. This approach not only enabled tracking of microglial Cx3cr1 expression, but also interfered with neuron-microglia crosstalk owed to ablation of endogenous murine fractalkine receptor expression. Although neurodegeneration in 3x Tg-AD mice was modest (~1.8% of neurons are lost), two-photon imaging of four-to-six month-old YFP-expressing 3x Tg-AD mice revealed neuronal loss during a two-to-four week observation period. Time-lapse images using YFP to mark neurons and GFP as a surrogate for *Cx3cr1* deficient microglia in compound genetically manipulated 3x Tg-AD mice showed that, while neurons in *Cx3cr1*-sufficient YFP-expressing mice died, YFP⁺ neurons in the *Cx3cr1*^{-/-}-3x Tg-AD mice survived. Further, *Cx3cr1*-sufficient microglia in 3x Tg-AD mice rapidly localized to neurons that were destined for elimination, while microglial mobilization to dying neurons in *Cx3cr1*^{-/-}-3x Tg-AD animals occurred with strikingly reduced kinetics [72]. These data suggest that fractalkine/Cx3cr1 signaling promotes microglial homing to neurons that are destined for death. However, a tantalizing question left unanswered in this report is whether microglia initiate the neuronal death cascade or simply serve as downstream effectors.

A NEW GENERATION OF PHARMACOTHERAPEUTICS AIMED AT RECRUITING MACROPHAGES TO THE ALZHEIMER BRAIN

The 'amyloid cascade hypothesis' proposes that dysmetabolism and deposition of A β peptides as amyloid plaques is the principal etiopathological event in AD, which sets into motion a cascade of disease-perpetrating events. For this reason, anti-amyloid strategies remain the primary AD pharmacotherapeutic focus currently in developmental clinical trials. In this review, we have illustrated the importance of harnessing mononuclear phagocytes to restrict and clear cerebral amyloid plaques. In particular, we have presented evidence that blood-borne monocytes hold potential as amyloid phagocytes. An immunotherapeutic strategy targeting these cells is particularly attractive as it avoids the issue of getting drugs past the blood-brain barrier, which has remained a major obstacle to AD pharmacotherapeutics. In principle, small-molecule drugs could be administered systemically to mobilize populations of professional phagocytes in the periphery, which could subsequently infiltrate the CNS and home to and clear amyloid deposits.

However, if blood-borne mononuclear phagocytes are to be targeted as an AD therapeutic modality, then strategies for 1) increasing brain recruitment and 2) augmenting amyloid clearance potential of these cells need to be developed. Regarding the latter goal, we are currently pursuing strategies to suppress TGF- β -Smad 2/3 signaling. It remains to be determined whether targeting specific blood-borne mononuclear cell subsets such as anti-inflammatory Ly-6C⁺ monocytes with small-molecule compounds (e.g., inhibitors of TGF- β -Smad 2/3 signaling) will ultimately prove to be safe and effective. Perhaps a general targeting approach not focused on any one cellular subset may ultimately prove most successful. Although there are limited data supporting or refuting this possibility, it stands to reason that endorsing brain infiltration of pro-inflammatory mononuclear cells subsets could exacerbate neuroinflammation and bystander injury in the context of AD neuropathology. Nonetheless, continued investigation is warranted into pharmacotherapeutics aimed at TGF- β and other immunomodulatory pathways to harness peripheral macrophages as a therapeutic modality for AD.

CONCLUDING REMARKS

In this review, we have focused on strategies to promote recruitment of peripheral mononuclear phagocytes as an important immunotherapeutic approach for AD (summarized in Table 1). Seminal observations from the late Henryk Wisniewski and colleagues over 20 years ago led to the first evidence that peripheral macrophages, but not brain-resident microglia, possess A β clearance aptitude. These findings were a watershed in the field, as they called our attention to hematogenous macrophages as functionally distinct from microglia. Since Wisniewski's time, we have gained a greater understanding of the mechanisms governing recruitment of these cells to the brain in the context of AD pathology. For example, work of Rivest, Priller, El Khoury and their colleagues has prompted general acceptance of the concept that irradiation 'sensitizes' the CNS to accept bone marrow grafts [41, 50]. We are now beginning to understand the molecular mechanisms governing blood-borne macrophage recruitment to the brain. In this regard, work from our group has shown that blocking TGF- β -Smad 2/3 signaling on peripheral macrophages coaxes these cells out of the circulation and into the brain, where they appear to clear parenchymal and vascular A β deposits [61]. El Khoury's group has demonstrated the obligate need for Ccr2 signaling in order to enable brain recruitment of peripheral mononuclear phagocytes [62], and most recently, it was shown that fractalkine receptor (Cx3cr1) signaling is required for 'homing' of mononuclear phagocytes to neurons marked for death in a mouse model of AD [72].

Despite significant advances toward understanding the cellular and molecular underpinnings of peripheral mononuclear phagocyte recruitment to the diseased CNS, there have also been pitfalls to data interpretation. Perhaps most significantly, the work of Ajami, Mildner, and their colleagues has cast doubt on use of irradiation bone marrow chimeras to model brain recruitment of peripheral macrophages [54, 55]. Specifically, they showed that the act of irradiating mice artificially inflates estimates of brain-penetrating monocytes, likely by damaging the blood-brain barrier, making the CNS more permissive to infiltration. A very recent study from Jücker's group calls into question whether mononuclear phagocytes are present in high enough quantity to have any effect on remodeling cerebral amyloid [69], unless further manipulated by genetic or pharmacologic means. Other questions include whether anti-amyloid properties of peripheral mononuclear phagocytes can be harnessed without incurring inflammation-induced bystander injury, and which subset(s) of these cells will provide greatest amyloid clearance potential. Nonetheless, evidence is mounting that targeted pharmacologic strategies could be used, in principle, to increase brain infiltration of peripheral mononuclear cells and to thereby restrict cerebral amyloidosis. While these exciting approaches are just in their infancy, they nonetheless hold significant promise.

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Table 1**Studies Examining the Beneficial Impact of Infiltrating Mononuclear Phagocytes on AD-Like Pathology**

Study	Model System	Observations
Wisniewski <i>et al.</i> , 1991; Akiyama and McGeer, 1996	AD patients with co-morbid stroke	CNS infiltrating peripheral mononuclear cells containing amyloid
Stalder <i>et al.</i> , 2006	APP23/GFP ⁺ bone marrow chimeric mice	Increased CNS infiltration of GFP ⁺ mononuclear cells; reduced amyloid burden
Simard <i>et al.</i> , 2006	APP/PS1/GFP ⁺ bone marrow chimeric mice	Age/AP-dependent CNS infiltration of GFP ⁺ mononuclear cells; reduced amyloid burden
Zhu <i>et al.</i> , 2009	APP/PS1/wild-type bone marrow chimeric mice	Reduced amyloid burden
Keene and Montine, 2010	APP/PS1/EP2 ^{-/-} chimeric mice	Increased cortical mononuclear cells; reduced amyloid burden
Hawkes and McLaurin, 2009	Clodronate or chitin-treated TgCRND8 mice	Clodronate reduced while chitin increased perivascular macrophage turnover; clodronate increased while chitin reduced CAA
Town <i>et al.</i> , 2008	Tg2576/CD11c-DNR bigenic mice (blockade of TGF- β signaling)	Increased CNS infiltration of CD45 ⁺ CD11b ⁺ Ly-6C ⁺ mononuclear cells; reduced amyloid burden, CAA, astrogliosis, and behavioral impairment.
El Khoury <i>et al.</i> , 2007	Tg2576/Ccr2 ^{-/-} crossed mice	Reduced CD11b ⁺ cells in proximity to plaques; increased amyloid burden
Grathwohl <i>et al.</i> , 2009	APP23 and APP/PS1/CD11b-HSVTK crossed mice	Reduced CD11b ⁺ cells in both periphery and CNS; amyloid deposits unaffected
Fuhrmann <i>et al.</i> , 2010	3x Tg-AD/Cx3cr1 ^{-/-} crossed mice	Reduced mononuclear cell density in CNS; reduced neuronal loss; amyloid burden unaffected
Lebson <i>et al.</i> , 2010	Non-irradiated APP/PS1 mice i.v. injected with CD11b ⁺ /GFP ⁺ bone marrow	CNS infiltration of injected CD11b ⁺ /GFP ⁺ bone marrow cells; if bone marrow cells were transfected with modified neprilysin, complete arrest of amyloid deposition in APP/PS1 mice