REVIEW

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The role of innate immune genes in Alzheimer's disease

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Purpose of review
The aim of this study was to provide an update on the role of the innate immune system and neuroinflammation in the pathogenesis of Alzheimer's disease, with an emphasis on microglial receptors CD33 and TREM2.

Recent findings
Genome-wide association studies (GWAS) have identified many Alzheimer's disease risk genes related to immune response and microglia including the phagocytic receptors *CD33* and *TREM2*. Recent GWAS and pathway analyses emphasize the crucial role of the innate immune system and neuroinflammation in the pathogenesis of Alzheimer's disease. Disease-associated microglia have been characterized by TREM2-dependent upregulation of phagocytic and lipid metabolism genes. Impaired microglial phagocytosis results in amyloid beta (Aβ) accumulation leading to neuroinflammation that is the primary cause of neurodegeneration. CD33 and TREM2 modulate neuroinflammation in Alzheimer's disease and have emerged as therapeutic targets in Alzheimer's disease. Progress has been made to inhibit CD33 by gene therapy, small molecules or immunotherapy, and to increase TREM2 activity by immunotherapy. Finally, mAbs against CD33 and TREM2 have entered clinical trials and may reduce neuroinflammation in Alzheimer's disease brain.

Summary
Targeting neuroinflammation via CD33 inhibition and/or TREM2 activation may have important implications for neurodegeneration in Alzheimer's disease and may be an addition to monoclonal anti-Aβ antibody treatments that remove plaques without reducing neuroinflammation.

Keywords
Alzheimer's, CD33, microglia, neuroinflammation, TREM2

INTRODUCTION

Alzheimer's disease is the leading cause of dementia among the elderly. After ageing, genetics is the strongest risk factor for Alzheimer's disease [1]. Autosomal dominant mutations in *APP* and *PSEN1/2* accelerate the rate of cognitive decline leading to early-onset dementia [2,3]. However, most Alzheimer's disease patients suffer from late-onset forms (LOAD) that represent 'sporadic AD'. Susceptibility for LOAD is likely caused by a combination of numerous genomic variants and environmental factors [4▪]. To date, apolipoprotein E (*APOE*) is the strongest genetic risk factor for LOAD. However, *APOE* accounts for only 10–20% of the LOAD risk [5], suggesting the existence of additional genetic risk factors.

Recently, in addition to *APOE*, genome-wide association studies (GWAS) have identified more than 30 genetic loci for Alzheimer's disease, many related to immune response and microglia, the resident immune cells of the brain [6,7]. Among these are the microglial receptors *CD33* [8–10] and *TREM2* [11,12]. The review provides an update on the results of current GWAS in Alzheimer's disease and summarizes recent findings related to phagocytic receptors CD33 and TREM2 and their association with Alzheimer's disease. Finally, it provides insights into

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KEY POINTS

- Genome-wide association studies implicate innate immunity genes including *CD33* and *TREM2* in Alzheimer's disease.
- Reduced neuroinflammatory response may lead to human resilience to Alzheimer's disease in the presence of amyloid beta plaques and neurofibrillary tangles.
- Microglial receptors CD33 and TREM2 inhibit and promote microglial phagocytosis and activation, respectively.
- Inhibiting CD33 and/or increasing TREM2 activity represent therapeutic strategies for Alzheimer's disease, for example by gene therapy, small molecules or immunotherapy.
- mAbs against CD33 and TREM2 have entered clinical trials and show great promise for targeted immunotherapy for Alzheimer's disease.

the therapeutic strategies to target CD33 and TREM2 for treating this devastating disorder.

Genome-wide association studies implicate the immune system in Alzheimer's disease

GWAS led to the identification of many Alzheimer's disease risk genes related to the immune response and microglia, including *CD33* [8–10], *INPP5D*, *CLU*, *CR1*, *SPI1*, *ABCA7*, *EPHA1* and the *MS4As* [9,10,13,14]. Subsequently, most GWAS-defined genes were replicated, and new Alzheimer's disease candidate genes involved in immune response and inflammation were identified, such as *HLA-DRB5–DRB1*, *INPP5D* and *MEF2C* [15]. Furthermore, efforts employing whole exome/genome sequencing have elucidated Alzheimer's disease associated variants in *TREM2* [11,12]. Additional risk genes have been identified as containing rare variants, including *PLCG2* and *ABI3* that are expressed in microglia [16]. Genetic studies of sporadic Alzheimer's disease also uncovered genes that regulate early endosome function, trafficking and maturation, including *BIN1*, *CD2AP*, *PICALM* and *SORL1* [9,10,17–19].

Most recently, a very large GWAS of Alzheimer's disease and Alzheimer's disease -by-proxy (based on parental diagnoses) identified 29 risk loci [20]. This meta-analysis also confirmed that *CD33* (originally identified by our group as the first veritable innate immune gene associated with Alzheimer's disease [8]) and *TREM2* are significantly associated with Alzheimer's disease, implying a genuine genetic association with Alzheimer's disease risk. In-silico functional analysis showed that associated genes are strongly expressed in immune-related tissues and cell types (spleen, liver and microglia), emphasizing the crucial role of the innate immune system and neuroinflammation in the pathogenesis of Alzheimer's disease [20]. Moreover, genetic meta-analysis of clinically diagnosed LOAD confirmed 20 previous risk loci including the immune-mediated disease haplotype HLA-DR15 and identified five new loci. Pathway analysis implicated immunity, lipid metabolism, tau-binding proteins and APP metabolism [21].

In summary, recent GWAS and post-GWAS bioinformatic analyses implicate microglia, phagocytic clearance of cellular debris and the immune response as key players in Alzheimer's disease pathogenesis [22]. Although microglia can uptake and clear amyloid beta (Aβ), they can also secrete pro-inflammatory cytokines leading to neuroinflammation [23]. A deeper understanding of molecular mechanisms that control microglial activation and impact neuroinflammation could advance therapies for Alzheimer's disease.

The role of microglia and neuroinflammation in Alzheimer's disease pathogenesis

Neuroinflammation is as an innate immunological response of the central nervous system that is characterized by the activation of microglia and astrocytes, which play a central role in Alzheimer's disease pathogenesis [24]. Studies of human brains resilient to Alzheimer's disease pathology showed that these brains exhibit high Aβ plaque burden and tangles but reduced neuroinflammation, increased neuronal survival and preserved cognition, suggesting that a suppressed neuroinflammatory response may lead to resilience to Alzheimer's disease [25,26]. As increasing evidence shows that neuroinflammation that occurs in response to plaques and tangles is the primary cause of neurodegeneration, it is most critical to stop neuroinflammation [26].

In the healthy brain, microglia have a unique homeostatic molecular signature (M0) [27,28]. Recent studies showed characteristic expression changes in microglia around plaques, labelling them as disease-associated microglia (DAM) [29], microglial neurodegenerative phenotype (MGnD) [30] or amyloid-response microglia (ARM) [31]. DAM microglia have been characterized by decreased expression of homeostatic genes and TREM2-dependent upregulation of phagocytic and lipid metabolism genes [29]. Most recently, RNA-seq performed on the hippocampus revealed a unique gene expression module that is responsive to Aβ but not TAU pathology and is highly enriched for Alzheimer's disease risk genes, including *APOE*, *INPP5D*, *CD33* and *PLCG2* in mouse models of Alzheimer's disease [32].

Furthermore, a single-nucleus RNA-sequencing (snRNA-seq) study of human microglia from Alzheimer's disease brains revealed a subset of DAM genes upregulated around Aβ plaques, but did not detect the full set of DAM genes [33]. However, a new study suggested that snRNA-seq is not suitable for detection of microglial activation genes in human control brain due to the depletion of approximately 20% of DAM genes in nuclei compared with whole cells [34]. Futures studies will show whether isolating larger numbers of nuclei will allow detection of the full panel of DAM transcripts in human Alzheimer's disease brain by using snRNA-Seq.

ALZHEIMER'S DISEASE RISK GENES *CD33* AND *TREM2* MODULATE NEUROINFLAMMATION

Impaired phagocytic activity of microglia results in Aβ accumulation, which leads to neuroinflammation, thereby creating a self-perpetuating cycle, which further enhances the inflammatory response in the brain. Microglial phagocytosis is a complex process that consists of recognition, engulfment, digestion and response [35]. Recent studies show that established Alzheimer's disease risk genes control the functions of microglial phagocytosis [36▪]. For the recognition step, phagocytic receptors such as CD33, TREM2 and CR1 play an important role in recognizing 'find-me' signals. The response step encompasses a transcriptional programme of clearance, that is DAM genes involved in lysosomal, phagocytic and lipid metabolism pathways, such as *APOE*, *CTSD*, *LPL*, *TYROBP* and *TREM2* [36▪]. We next discuss in detail the roles of CD33 and TREM2 and how they control microglial phagocytosis and neuroinflammation.

Molecular genetics of CD33 and its impact on neuroinflammation

CD33 (Siglec-3) is a member of the sialic acid-binding immunoglobulin-like lectins (Siglecs) and is expressed on the surface of myeloid progenitor cells, granulocytes, monocytes, macrophages and microglia [37]. CD33 binds to α2–3 and α2-6 linked sialic acids, attached to glycan chains on cell surface, and can mediate *cis* or *trans*-cellular interactions [38]. CD33 contains the V-type immunoglobulin-like (V-Ig) domain, which is the binding site of sialic acids, an extracellular C2-Ig domain and cytosolic immunoreceptor tyrosine-based inhibitory motif (ITIM) and ITIM-like sequence. The ITIM domain is responsible for inhibitory signal transduction in cells [39]. CD33 has been implicated in cell adhesion, endocytosis, immune cell growth [40] and inhibition of cytokine release by monocytes [41]. CD33 was also reported to negatively regulate Tlr4 signalling [42]. Finally, C1q binding to CD33 led to activation of CD33/LAIR-1 inhibitory motifs [43].

Our group first reported *CD33* as a novel LOAD candidate gene as a result of a large family-based genome-wide association study; the single nucleotide polymorphism (SNP) rs3826656 has been associated with LOAD in the NIMH family sample [8]. Case–control GWAS identified a SNP located upstream of *CD33*, rs3865444, as associated with LOAD risk [9,10]. Higher CD33 expression levels in the brain were associated with greater cognitive decline [44] and increased Alzheimer's disease pathology [45]. We previously showed that CD33 exhibits increased expression in microglial cells in Alzheimer's disease brain. The minor allele of the *CD33* SNP rs3865444, which confers protection against Alzheimer's disease, was associated with reductions in levels of full-length CD33 and insoluble Aβ42 in Alzheimer's disease brain [46] (see Fig. 1). We also showed CD33 inhibited microglial uptake and clearance of Aβ42, and that plaque burden was reduced in *APP/PS1;CD33*$^{-/-}$ mice [46].

In contrast, the risk allele of rs3865444 was associated with decreased Aβ42 uptake and increased expression of full-length CD33 and TREM2 in monocytes [47,48] and monocyte-derived microglia-like cells [49]. The protective allele of *CD33* SNP rs12459419 (coinherited with rs3865444) was associated with skipping of *CD33* exon 2 [50,51], leading to the *CD33-ΔV-Ig* isoform [52]. Exon 2 encodes the sialic acid-binding domain (V-Ig) that is required for CD33-mediated inhibition of Aβ uptake in microglia [46]. The protective allele of rs3865444 was also associated with increased levels of a *CD33* splice variant that retains intron 1, resulting in the *R1-CD33* isoform [53].

A recent study showed that rs201074739 in *CD33* exon 3 results in premature termination codon and loss of cell surface CD33 [54]. A new *CD33* isoform was found in carriers of the 4-bp insertion/deletion (indel) rs201074739; it encodes a secreted CD33 protein. Genetic analysis showed that the 4-bp indel was not associated with Alzheimer's disease risk [55▪]. Considering that the protective allele of rs12459419 increases levels of CD33-ΔV-Ig, the authors hypothesize that CD33-ΔV-Ig induces microglial activation through mechanisms similar to TREM2 (gain-of-function isoform) [55▪]. However, another study showed that CD33-ΔV-Ig is diverted to intracellular peroxisomes (loss-of-function isoform) [56,57▪]. As the protective allele of rs3865444 is associated with reductions in full-length CD33 and Alzheimer's disease risk, targeting functional CD33 may attenuate

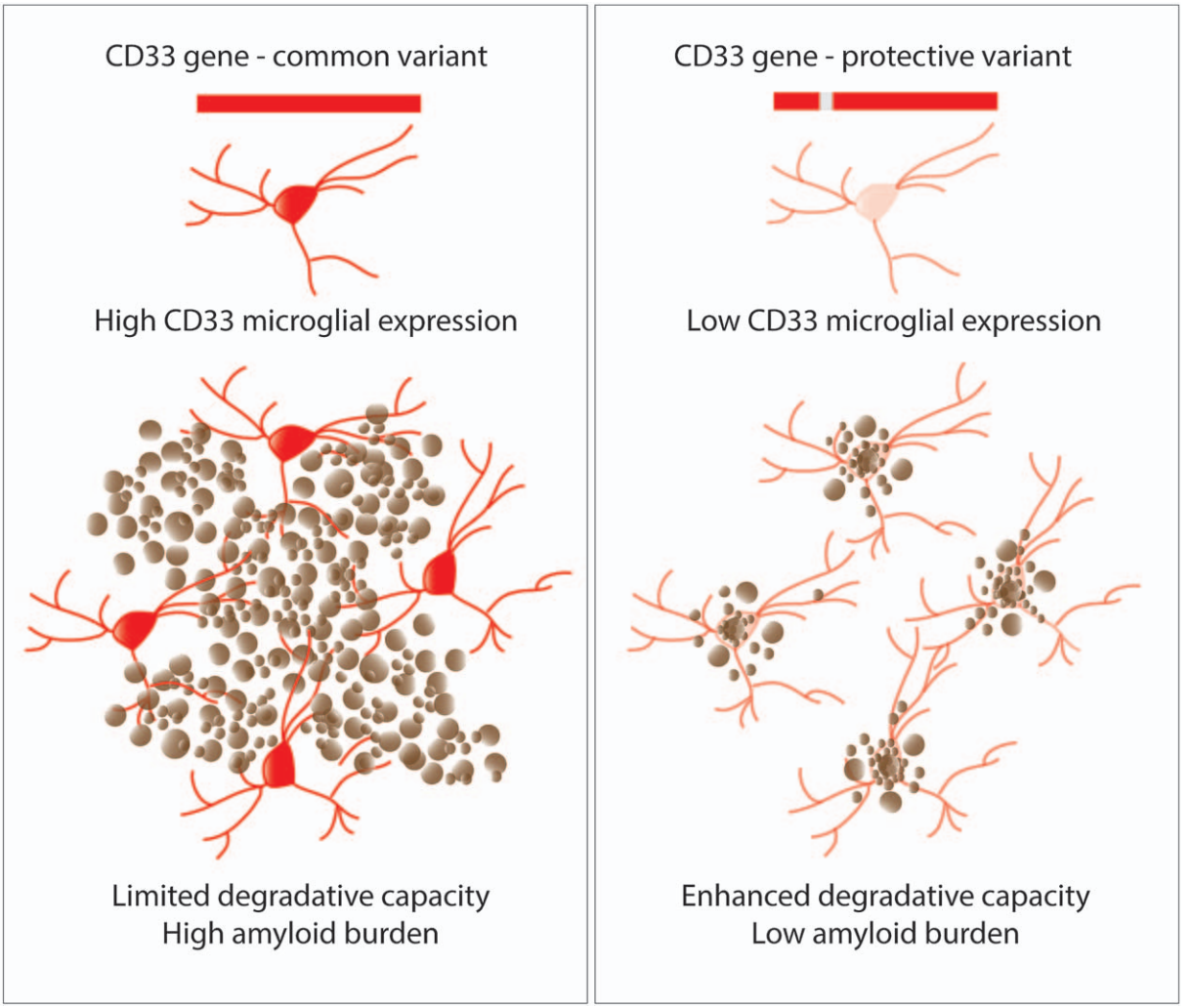

FIGURE 1. The protective allele of the *CD33* SNP rs3865444 is associated with reductions in both CD33 expression and insoluble Aβ42 levels in Alzheimer's disease brain. The minor allele of the *CD33* SNP rs3865444, which confers protection against Alzheimer's disease, leads to reductions in both CD33 expression and amyloid burden in human Alzheimer's disease brain (right). Microglial cells expressing high levels of CD33 are limited in their capacity to uptake and clear Aβ; the result is high levels of Aβ that accumulate in the brain (left).

Alzheimer's disease pathology. Thus, inhibiting CD33 activity represents a potential therapy for Alzheimer's disease, for example, by gene therapy, small molecules or immunotherapy.

Crosstalk between the microglial receptors CD33 and TREM2

TREM2 expression was increased by *CD33* Alzheimer's disease risk allele rs3865444^{C} and decreased by CD33 immunosuppression in monocytes [47]. We showed that TREM2 acts downstream of CD33 in modulating cognition, Aβ pathology, neurodegeneration and microglial cell response to Aβ plaques in the *5xFAD* mouse model of Alzheimer's disease [58▪]. RNA-seq profiling of microglia revealed that genes related to phagocytosis and microglial activation are upregulated in *5xFAD;CD33*$^{-/-}$ and downregulated in *5xFAD;TREM2*$^{-/-}$ mice. Differential gene expression in *5xFAD;CD33*$^{-/-}$ microglia depended on the presence of *TREM2*, suggesting TREM2 acts downstream of CD33. Crosstalk between CD33 and TREM2 includes regulation of the IL-1β/IL-1RN axis [58▪]. Collectively, these findings suggest that inhibiting CD33 and/or increasing TREM2 activity could represent novel therapies for Alzheimer's disease.

Although CD33 and TREM2 are cell membrane receptors that bind different ligands (e.g. sialic acids for CD33 and anionic lipids for TREM2), both functionally interact with DAP12, either directly (TREM2) or via common intracellular signalling molecules (CD33). Thus, DAP12 and interacting

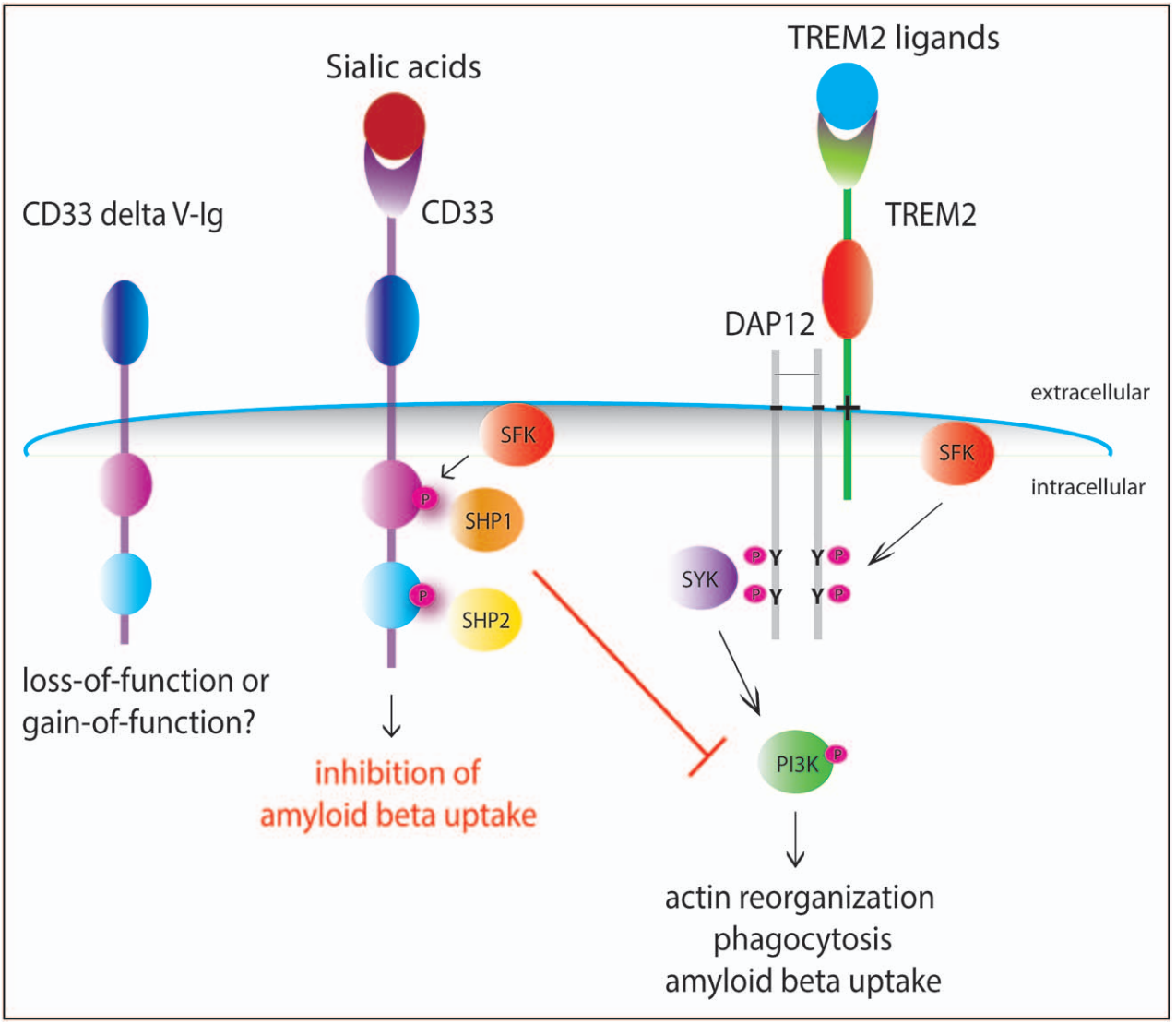

FIGURE 2. Working model of crosstalk between the microglial receptors CD33 and TREM2. Phosphorylation of full-length CD33 on ITIM domains leads to recruitment of SHP1 and SHP2 phosphatases and inhibitory signaling. Recruitment of SYK to DAP12, the adaptor of TREM2, leads to intracellular signaling that promotes phagocytosis and chemotaxis. CD33-ΔV-Ig may represent a loss-of-function or gain-of-function isoform.

signalling factors, for example INPP5D (SHIP1), SHP1/2, SYK and PI3K, are probable effectors of crosstalk between CD33 and TREM2 in microglial cells (see Fig. 2).

Microglial receptor CD33 as a drug target for Alzheimer's disease

CD33 is currently one of the most targeted Alzheimer's disease genes in the pharmaceutical industry. Alector has developed the mAb AL003 that blocks CD33 function and increases microglial activation, and is in early-phase clinical trials for Alzheimer's disease. Furthermore, CD33 is a therapeutic target for acute myeloid leukaemia (AML). Several humanized CD33-specific antibodies have been tested in AML clinical trials [59]. The risk allele rs3865444^{C} correlates with an increased efficacy of the antibody-drug conjugate gemtuzumab ozogamicin [60,61]. A recent study reported the lack of CD33-ΔV-Ig isoform on the surface of blast cells from AML patients [62], suggesting a loss-of-function role. Finally, the humanized CD33 antibody lintuzumab significantly downregulated cell-surface CD33 in monocytic cells [53]. Thus, inhibiting CD33 with humanized antibodies could represent a potential therapeutic approach for Alzheimer's disease.

Most recently, we established a gene therapy strategy to reduce CD33 expression in microglia.

Treatment of Alzheimer's disease mice with an adeno-associated virus (AAV) vector-based system encoding an artificial microRNA targeting *CD33* (miR^{CD33}) at an early age reduced *CD33* mRNA, brain levels of TBS-soluble Aβ40 and Aβ42, and Aβ plaque burden in *APP/PS1* mice. Early intervention with miR^{CD33} downregulated pro-inflammatory activation genes, cytokines and chemokines [63▪]. Collectively, we provided the first proof-of-concept that therapies targeting *CD33* can reduce both Aβ accumulation and neuroinflammation.

Surprisingly, a recent study showed that knock-out of *CD33* did not impact uptake of aggregated Aβ42 in primary microglial cell cultures [64]. This discrepancy related to the role of mouse CD33 in phagocytosis could be due to different genetic backgrounds of *CD33* knock-out mice. In contrast, transgenic expression of human *CD33* in microglia inhibited phagocytosis [64], confirming previous findings [46,48]. The transgenic mouse model expressing human *CD33* under control of Cre recombinase [65] and establishing novel humanized *CD33* mouse models should provide valuable models to better understand CD33 biology and test therapeutics based on targeting CD33.

An alternative approach to modulate CD33 function might be the use of selective small molecules occupying the sialic acid-binding site of CD33, such as sialic acid based ligands [66]. Liposomal nanoparticles bearing an allergen and a high-affinity glycan ligand of CD33 suppressed IgE-mediated anaphylaxis and desensitized mast cells to allergen in transgenic mice expressing human *CD33* [65]. Furthermore, the sialic acid mimetic P22 (binding to the sialic acid binding domain of CD33) presented on microparticles increased uptake of Aβ42 into microglial cells [67▪]. In summary, CD33 is a promising target for developing therapeutics for the treatment of Alzheimer's disease.

Alzheimer' disease risk gene *TREM2* modulates microglial pathology

TREM2 is an immunoreceptor expressed on myeloid cells including microglia, wherein it regulates inflammation [68,69]. Heterozygous rare variants in *TREM2* (e.g. R47H) are associated with increased risk of Alzheimer's disease [11,12]. TREM2 signals through the adaptor protein DAP12 (*TYROBP*) to suppress pro-inflammatory cytokines [70], and promote phagocytosis [71] and biosynthetic metabolism [72]. TREM2 ligands include anionic lipids [73], lipidated ApoE [74–76] and Aβ oligomers [77,78]. In collaboration with Dr Colonna, we provided evidence for increased Alzheimer's disease risk associated with several *TREM2* variants and showed that *TREM2* loss-of-function mutations (e.g. R47H and R62C) decreased binding to TREM2 ligands. To the contrary, D87N and T96K exhibited increased ligand-dependent activation [79]. Thus, further studies are required to address the effects of increasing TREM2 activation in Alzheimer's disease.

Furthermore, other studies showed that *TREM2* R47H negatively impacts binding to cell-surface TREM2 ligands [80] and Aβ oligomers [81]. By contrast, T96K appears to be a gain-of-function mutation and results in increased cellular binding [80]. Moreover, *TREM2* mutations implicated in neurodegeneration impair cell surface transport of TREM2 [80,82]. Soluble (s)TREM2 is elevated in Alzheimer's disease cerebrospinal fluid (CSF) compared with controls [83]. Increased sTREM2 in CSF is associated with reduced cognitive decline in Alzheimer's disease [84] and with slower rates of Aβ accumulation [85]. sTREM2 also protects against amyloid disease by enhancing microglial activity in *5xFAD* mice [86]. Levels of CSF sTREM2 are increased in R47H carriers, while they are significantly decreased in T96K/L211P/W191X carriers versus controls [87]. These data suggest that *TREM2* variants may impact protein expression and proper TREM2 function is important to counteract disease progression.

TREM2 detects damage-associated lipid patterns and sustains the microglial response in Alzheimer's disease [73]. Previous studies reported *TREM2* knock-out decreased [30,88] or did not impact [89] Aβ plaque burden during early disease. However, *TREM2* knock-out significantly increased Aβ plaque burden at late disease stages [58▪,73,90]. Moreover, *TREM2* knock-out impaired microglial activation and clustering around plaques [73,88,91], and disrupted the microglial barrier [89,92]. Conversely, overexpression of human *TREM2* reduced plaque load, upregulated phagocytosis genes and improved cognition in Alzheimer's disease mice [93]. Overexpression of human *TREM2*-R47H in *5xFAD* mice impaired microgliosis and reduced microglial activation [94].

A recent study suggested the TREM2-APOE pathway induced the transition from homeostatic to MGnD phenotype in *APP-PS1* mice [30]. However, DAM response has been characterized by TREM2-dependent upregulation of phagocytic and lipid metabolism genes, which is protective [29]. These differences might be explained by distinct roles of TREM2 at late versus early stage of Alzheimer's disease pathology [90]. We previously showed that *TREM2* knock-out downregulated phagocytic and lipid metabolism genes in *5xFAD* microglia [58▪]. Furthermore, TREM2 is required for microglial cholesterol transport and metabolism upon chronic phagocytic challenge [95]. Finally, snRNA-seq

revealed that *TREM2*-R47H and *TREM2*-R62H carriers exhibited reduced microglia reactive signature, suggesting TREM2 is required for microglial activation [96].

Remarkably, *TREM2* knock-out or *TREM2*-R47H variant promotes the seeding and spreading of neuritic plaque tau aggregates [97▪]. In a tauopathy mouse model, *TREM2* knock-out decreased pro-inflammatory microglial activation and improved neurodegeneration [98]. Overexpression of human *TREM2*-R47H in the PS19 mouse model of tauopathy mitigated brain atrophy and synapse loss, and reduced microglial reactivity, versus *TREM2* common variant [99]. These findings suggest that *TREM2* loss-of-function decreases microglia-mediated neurodegeneration in tauopathy.

Agonist TREM2 antibodies for Alzheimer's disease treatment

TREM2 is currently targeted with agonist TREM2-specific antibodies to activate receptor signalling [100]. Alector developed mAbs, AL002 and AL002c; AL002c binds to the extracellular domain of human TREM2. AL002 is a derivative of AL002c that is in clinical trials [101▪]. Acute treatment with AL002c induced microglial proliferation in both common variant and R47H *TREM2* transgenic mice. Prolonged treatment with AL002c reduced filamentous plaques, neurite dystrophy and microglia-mediated inflammation in Alzheimer's disease mice [101▪].

mAb 4D9, which has a stalk region epitope close to the cleavage site, stabilized TREM2 on the cell membrane by decreasing its shedding, and induced phospho-SYK signalling. 4D9 increased microglial uptake of Aβ peptide *in vitro*. In Alzheimer's disease mice, 4D9 reduced amyloid plaque burden, enhanced microglial TREM2 expression and promoted transition of microglia toward the DAM state, suggesting a protective function [102▪]. Both antibodies AL002 and 4D9 engaged TREM2 and represent promising candidates for Alzheimer's disease therapy.

CONCLUSION

The microglial receptors CD33 and TREM2 modulate microglial pathology and neuroinflammation, and have emerged as targets for drug development in Alzheimer's disease. CD33 opposes the effects of TREM2 signalling and makes an attractive target because it could be potentially inhibited, for example by gene therapy, small molecules or immunotherapy. TREM2 appears to be a promising therapeutic target, with several agonist antibodies activating receptor signalling. Ongoing clinical trials with mAbs targeting CD33 and TREM2 are major steps towards targeted immunotherapy for Alzheimer's disease. In summary, inhibiting CD33 and/or activating TREM2 represent valuable therapeutic strategies to enhance neuroprotective microglia and reduce neuroinflammation, which is crucial for preventing and treating Alzheimer's disease.

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Conflicts of interest

A.G. and R.E.T. have an issued patent on all forms of gene therapy and immunotherapy for neuroinflammation using CD33 as a target.

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- ▪ of special interest
- ▪▪ of outstanding interest

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