Cytosolic Fc receptor TRIM21 inhibits seeded tau aggregation

William A. McEwan[a,1], Benjamin Falcon[a], Marina Vaysburd[a], Dean Clift[a], Adrian L. Oblak[b], Bernardino Ghetti[b], Michel Goedert[a,1], and Leo C. James[a,1]

[a]Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom; and [b]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202

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Alzheimer's disease (AD) and other neurodegenerative disorders are associated with the cytoplasmic aggregation of microtubule-associated protein tau. Recent evidence supports transcellular transfer of tau misfolding (seeding) as the mechanism of spread within an affected brain, a process reminiscent of viral infection. However, whereas microbial pathogens can be recognized as non-self by immune receptors, misfolded protein assemblies evade detection, as they are host-derived. Here, we show that when misfolded tau assemblies enter the cell, they can be detected and neutralized via a danger response mediated by tau-associated antibodies and the cytosolic Fc receptor tripartite motif protein 21 (TRIM21). We developed fluorescent, morphology-based seeding assays that allow the formation of pathological tau aggregates to be measured in situ within 24 h in the presence of picomolar concentrations of tau seeds. We found that anti-tau antibodies accompany tau seeds into the cell, where they recruit TRIM21 shortly after entry. After binding, TRIM21 neutralizes tau seeds through the activity of the proteasome and the AAA ATPase p97/VCP in a similar manner to infectious viruses. These results establish that intracellular antiviral immunity can be redirected against host-origin endopathogens involved in neurodegeneration.

neurodegeneration | tau | intracellular immunity | antibodies | immunoreceptors

The cell's ability to identify intracellular viruses and bacteria relies on the detection of pathogen-associated molecular patterns (PAMPs) by specialized host receptors. Although highly effective at detecting microbial pathogens, this strategy is poorly equipped to identify host-derived pathogenic species such as aggregated proteins. As an alternative to PAMP detection, recent work has demonstrated that mammalian cells can use host-derived serum proteins, which are normally excluded from the cell interior, to target invading viruses and bacteria in the cytosol. For instance, nonenveloped viruses and bacteria carry antibodies with them into the cytoplasm during infection. These translocated antibodies are then sensed by the cytoplasmically expressed antibody receptor TRIM21 (tripartite motif protein 21), which binds with subnanomolar affinity to the antibody Fc domain (1–4). After binding to antibody, TRIM21 triggers a potent neutralization response that inhibits viral infection. Neutralization of infection is accompanied by degradation of viral components, which requires the activity of the proteasome and the molecular unfoldase, valosin-containing protein (VCP) or p97 (1, 5). Detection of viruses and bacteria by TRIM21 does not rely on microbial PAMPs, as model substrates such as antibody-coated latex beads can be bound and detected by TRIM21 (1, 3). We therefore hypothesized that the intracellular innate immune system could be repurposed to recognize and degrade host-derived pathogenic proteins.

Microtubule-associated protein tau occurs in an assembled and hyperphosphorylated state in the cytoplasm of neurons and glial cells in Alzheimer's disease (AD), progressive supranuclear palsy, chronic traumatic encephalopathy, and other neurodegenerative disorders (6). Tau assemblies applied to the extracellular spaces can seed further aggregation: injection of tau assemblies into the brains of tau transgenic mice promotes the aggregation of intracellular tau (7), and ectopic addition of recombinant tau assemblies promotes the aggregation of intracellular pools in cultured cells (8, 9). Consistent with transcellular propagation of misfolding, tau pathology in AD spreads between connected regions of the brain in a stereotyped manner (10). Seeded propagation of misfolded tau is therefore proposed to underlie many common neurodegenerative disorders. Akin to viral infection, this model of seeded tau propagation relies on the physical transfer of tau assemblies between cells, and is therefore potentially susceptible to interception by antibody. The broad tissue expression of TRIM21 and its ultrahigh affinity for Fc imply that intracellular antibodies will be bound by TRIM21 whenever they occur. Numerous intracellular entities may therefore be targeted by TRIM21, including DNA and RNA viruses (1, 11, 12), cytosolic bacteria (3), and soluble host proteins, including an Fc fragment (5) and a cis-tau conformer (13). However, it is not known whether self-propagating protein assemblies are susceptible to neutralization by TRIM21.

Here we report the development of highly sensitive tau seeding assays, which measure the ability of extracellular tau assemblies to promote the conversion of intracellular, full-length tau from a soluble to aggregated state after their entry to the cell. We demonstrate that tau seeds that enter the cell with attached antibody are rapidly bound and inactivated by TRIM21. Our results demonstrate that given an appropriate danger signal, here antibody, the

Significance

The mammalian cell cytoplasm contains numerous proteins with direct antimicrobial activity. Although these have been extensively studied in the context of viral and bacterial infection, it is unknown whether pathogenic self-propagating proteins, proposed to underlie common neurodegenerative diseases, can be targeted in a similar manner. We studied the ability of tripartite motif protein 21 (TRIM21), a newly identified intracellular antibody receptor, to intercept assemblies of misfolded tau, a cytoplasmic protein that aggregates in patients with Alzheimer's disease. We developed tau "seeding" assays in human cells and found that TRIM21 could intercept and potently neutralize antibody-labeled tau assemblies. These findings demonstrate that the intracellular immune system can act against self-propagating misfolded proteins, with implications for ongoing attempts to develop antibody-based therapies for neurodegenerative disorders.

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[1]To whom correspondence may be addressed. Email: wmcewan@mrc-lmb.cam.ac.uk, mg@mrc-lmb.cam.ac.uk, or lcj@mrc-lmb.cam.ac.uk.

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intracellular immune system is capable of detecting and inactivating self-propagating protein assemblies.

Results

TRIM21 Is Expressed in Neural Cells. Transcriptomic and immunohistochemical analyses suggest that the cytosolic Fc receptor TRIM21 is expressed at low levels in brain tissue (14, 15). To test whether this basal expression is sufficient for protective intracellular immunity, we challenged primary mouse neural cultures with adenovirus or adenovirus treated with monoclonal antibody 9C12, a model system for intracellular neutralization (2). In neurons derived from wild-type (WT) mice, 9C12 conferred a 40-fold block to infection (Fig. 1*A*). However, in neurons derived from *Trim21*$^{-/-}$ mice, a 12-fold block to infection was observed. Basal Trim21 levels can therefore provide a significant block to infection in neurons. This finding is in agreement with previous findings that Trim21 is expressed and active in primary neurons (13). *Trim21* is an IFN-stimulated gene whose expression levels determine the potency of intracellular neutralization (2). We tested the effect of type I, II, or III interferons on intracellular neutralization in primary neurons. IFN-α (type I) and IFN-β (type II), but not IFN-λ (type III), up-regulated *Trim21* expression (Fig. 1*B*). IFN-stimulated up-regulation of *Trim21* substantially potentiated neutralization, resulting in a >700-fold reduction of infection by antibody in stimulated WT cells (Fig. 1*C*). In contrast, all IFNs exerted little effect on neutralization in *Trim21*$^{-/-}$ cells. Levels of neutralization were correlated with, and almost entirely accounted for, by levels of *Trim21* mRNA (R^2 = 0.94) (Fig. 1*D*). Importantly, TRIM21 mediated comparable levels of adenovirus neutralization in human H4 neuroglioma and SHSY-5Y neuroblastoma cells, in addition to HeLa and HEK293 lines (Fig. 1*E*), demonstrating that TRIM21 is widely expressed and active in diverse tissues, including CNS and neural derived cells.

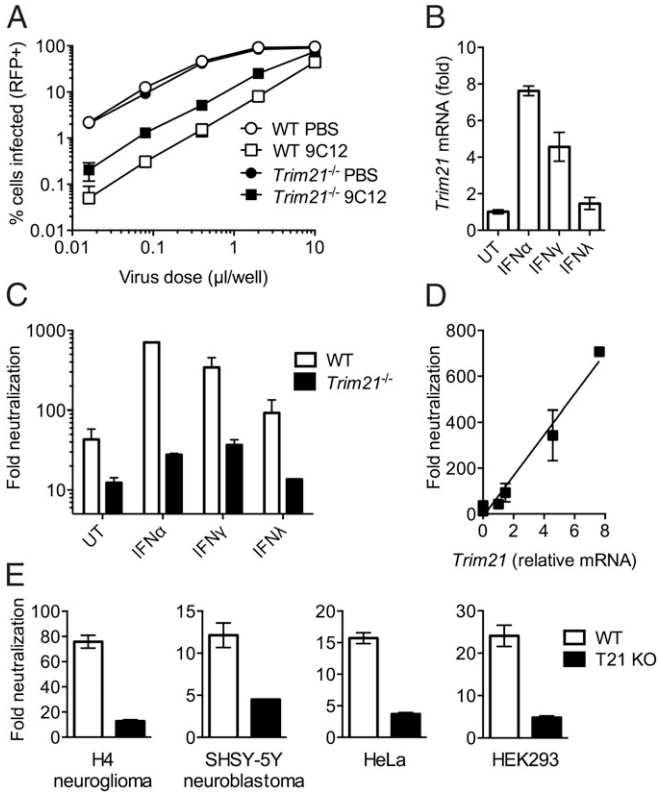

Fig. 1. Cells derived from neural tissues exert inducible intracellular neutralization via TRIM21. (*A*) Mouse primary neural cultures derived from WT or *Trim21*$^{-/-}$ C57BL/6 E18 embryos were challenged with a titration of adenovirus-RFP that was pretreated with PBS or anti-hexon antibody 9C12. (*B*) Relative levels of *Trim21* mRNA in WT neural cultures after stimulation with the indicated IFN compared with untreated (UT) cells, normalized to levels of β-actin mRNA. (*C*) Levels of neutralization in WT or *Trim21*$^{-/-}$ neural cultures treated as in *B*. Fold neutralization was calculated by dividing the level of infection with PBS-treated virus by that of 9C12-treated virus. (*D*) Fold neutralization observed after IFN stimulation is correlated with *Trim21* mRNA levels (Pearson r = 0.99; P < 0.001; R^2 = 0.94). (*E*) Fold neutralization of adenovirus by 9C12 in H4, a model of human microglia; SHSY-5Y, a human cell line with properties of neurons and HeLa and HEK293, commonly used human cell lines. TRIM21 was deleted by CRISPR/Cas9 (T21 KO).

Sensitive in Vitro Tau Seeding Assay. The study of seeded tau propagation relies on transgenic mouse models, in which disease progression is slow and the contribution of seeded vs. spontaneous aggregation is often unclear. Alternatively, in vitro assays allow these contributions to be quantified and dissected, but existing methods rely on aggregation-prone fragments of tau, have suffered from low signal-to-noise ratios, or require laborious biochemical fractionation, which is not amenable to detailed study. We therefore developed a high-content microscopy-based assay using HEK293 cells stably expressing the 0N4R isoform of human tau, bearing frontotemporal dementia-associated mutation P301S with a C-terminal venus tag. Addition of recombinant heparin-assembled P301S tau promoted the conversion of tau-venus from a disperse distribution to bright foci (Fig. 2*A*). To demonstrate that these foci are associated with tau aggregation, the sarkosyl-insoluble fraction was prepared from cell homogenates. Addition of tau assemblies resulted in a proportion of tau pelleting in the sarkosyl-insoluble fraction, and the venus tag did not substantially alter this process (Fig. 2*B* and Fig. S1). To investigate whether tau-venus foci recapitulated the pathological characteristics of tauopathies, we used immunostaining for phosphorylated and fibril-specific epitopes. A pan-tau antibody, HT7, colocalized with tau-venus in both untreated cells and seeded cells (Fig. S2). In contrast, tau-venus foci that occurred after seeding, but not soluble tau-venus, were stained by antibodies that depend on phosphorylation of their antigens, anti-pS422 (Fig. S2) (16) and AT100 (pT212, pS214, pT217), which is specific for pathological tau structures (Fig. 2*C*) (17). To confirm that intracellular expressed tau-venus, rather than seed, was the target of hyperphosphorylation, we challenged cells with tau assemblies bearing point mutation S422A. Phosphorylation at S422 was detected within 12 h of challenge (Fig. 2*D*), confirming aggregation and hyperphosphorylation of intracellular expressed tau. These observations demonstrate a seeding assay that recapitulates both the aggregation and hyperphosphorylation observed in diseased brains in a widely used human cell line. Furthermore, the results support the close temporal association of intracellular aggregation and hyperphosphorylation.

Cytoplasmic Delivery of Tau Is Rate-Limiting to Seeding. Previous observations in cell-based models of tau seeding support the hypothesis that entry to the cell, rather than aggregation itself, is rate-limiting (9). We used cationic lipids (Lipofectamine 2000; LF) to promote uptake and disrupt endosomal membranes. LF substantially increased both the rate and overall level of aggregation in our assay (Fig. 2*E*), using automated image analysis (Fig. S2). The data suggest that seeded aggregation is a rapid process that occurs largely within 12 h, whereas uptake and endosomal rupture are inefficient and, in the absence of LF, rate-limiting. LF increased the percentage of seeded cells in a manner proportional to its ability to deliver a luciferase-encoding plasmid (Fig. 2*F* and Fig. S3), consistent with intracellular delivery of tau assemblies being the cause of increased seeding by LF. In the presence of LF, seeding increased in a dose-dependent manner with tau concentration over more than three orders of magnitude (Fig. 2*G*). Statistically significant seeding, relative to a

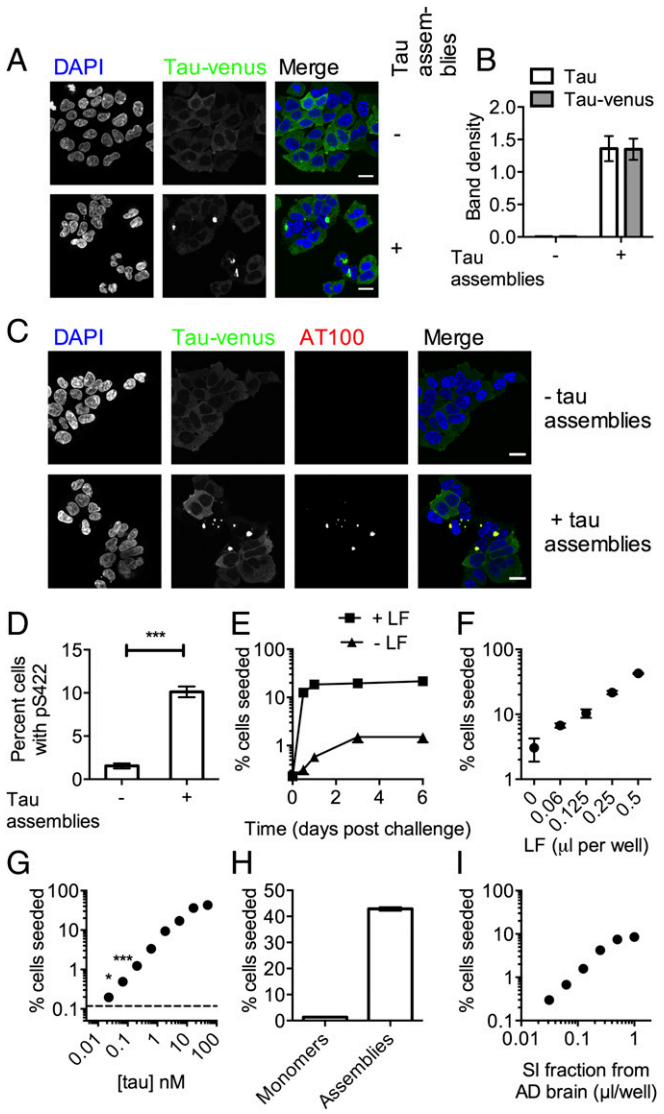

Fig. 2. Exogenously added tau assemblies induce aggregation and phosphorylation of intracellular tau. (*A*) P301S tau-venus expressed in HEK293 cells with or without addition of heparin-assembled recombinant P301S tau to culture supernatant. (*B*) Sarkosyl insoluble tau 3 d after challenge with P301S tau assemblies or mock treatment. Levels determined by Western blot and normalized to total tau. (*C*) Cells treated as in *A* stained with phosphorylated tau fibril-specific antibody AT100. (*D*) Levels of staining with anti-pS422 12 h postchallenge with P301S/S422A tau assemblies. (*E*) Time course of seeding by 100 nM P301S tau assemblies in the presence or absence of 0.5 µL LF. (*F*) Percentage HEK293 cells that exhibit aggregated tau-venus 3 d postchallenge with 200 nM P301S tau assemblies with indicated dose of LF. (*G–I*) Percentage of cells seeded after challenge in the presence of LF with (*G*) a titration of P301S tau assemblies, (*H*) 50 nM monomeric or aggregated P301S tau, or (*I*) AD brain sarkosyl insoluble (SI) fraction. *Unpaired *t* test $P < 0.05$; ***$P < 0.0001$. Error denotes SEM. In *B*, *D*, and *F*, $n = 3$ replicate experiments; in *E* and *G–I*, $n = 9–24$ fields per condition. (Scale bars, 20 µm.)

LF-treated control, was detected using 23-pM tau assemblies. In contrast, the addition of monomeric tau did not induce seeding (Fig. 2*H*). The sarkosyl-insoluble fraction of Alzheimer's diseased brain tissue was also competent at inducing seeding (Fig. 2*I*). These data demonstrate a sensitive and quantitative assay using full-length tau that responds to the presence of tau assemblies, both recombinant and clinically derived, by propagating intracellular, phosphorylated tau aggregates. Moreover, they demonstrate that cytosolic entry is rate-limiting and that once inside the cell, trace amounts of seed, left unrestricted, are capable of rapidly initiating tau aggregation.

Antibodies Prevent Seeding but Poorly Block Entry. Next, we used our assay to identify antibodies that reduce seeding. A panel of phosphorylation-independent anti-tau and control antibodies (Table S1) was screened, of which mouse monoclonal IgG1, 5A6 (18), and rabbit polyclonal antibody, BR134 (19), were found to prevent seeding by recombinant P301S tau assemblies in the absence of LF (Fig. 3 *A* and *B*). The ability of 5A6 and BR134 to prevent binding and uptake of AlexaFluor488-labeled tau assemblies to cells was tested. Binding (at 4 °C) and uptake (at 37 °C) were marginally reduced (~20%) by 5A6, but not by BR134 (Fig. 3*C*). Consistent with previous observations (20), these results suggest that entry-blocking contributes to neutralization. However, for both 5A6 and BR134, the majority of neutralization was not accounted for by this mechanism. Moreover, neutralization was observed when assembled P301S tau-antibody complexes were introduced by LF (Fig. 3*D*). Together, these results suggest that neutralization by 5A6 and BR134 occurs largely postentry.

TRIM21 Neutralizes Tau Seeding. Cytosolic Fc receptor TRIM21 mediates the highly efficient postentry neutralization of antibody-bound nonenveloped viruses and soluble toxic tau precursors (1, 2, 13, 21). To investigate whether tau seeding can be neutralized by TRIM21, we used CRISPR/Cas9 to disrupt *TRIM21* in HEK293 cells expressing P301S tau-venus and confirmed protein knockout by immunoblot (Fig. 4*A*). HEK293 cells take up tau aggregates by macropinocytosis (22), but lack expression of FcγRs, permitting analysis of neutralization in the absence of cell surface antibody receptors. Disruption of *TRIM21* by CRISPR/Cas9 reduced the ability of mouse monoclonal antibody 9C12 to neutralize adenovirus infection (Fig. 1*E*), as has previously been demonstrated by small hairpin RNA and genetic ablation (2). Neutralization of tau seeding

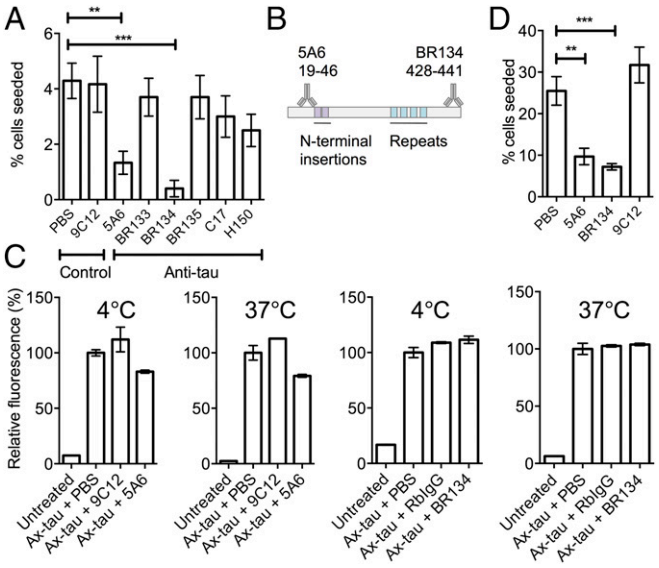

Fig. 3. Neutralization of tau seeding can occur independent of entry blocking. (*A*) Percentage HEK293 tau-venus cells seeded 5 d after challenge with P301S tau assemblies that were mixed with indicated antibodies for 1 h before addition to culture supernatant. (*B*) Tau epitopes identified by 5A6 and BR134. (*C*) Relative fluorescence of HEK293 cells treated with Alexa488-labeled tau assemblies at indicated temperature. (*D*) Percentage HEK293 tau-venus cells seeded after challenge with 100 nM P301S tau assemblies incubated with indicated antibody in the presence of LF. **Unpaired *t* test $P < 0.01$; ***$P < 0.0001$. Error denotes SEM. In *A* and *G*, $n = 10–20$ fields per condition; *C–F*, duplicate experiments. (Scale bars, 20 µm.)

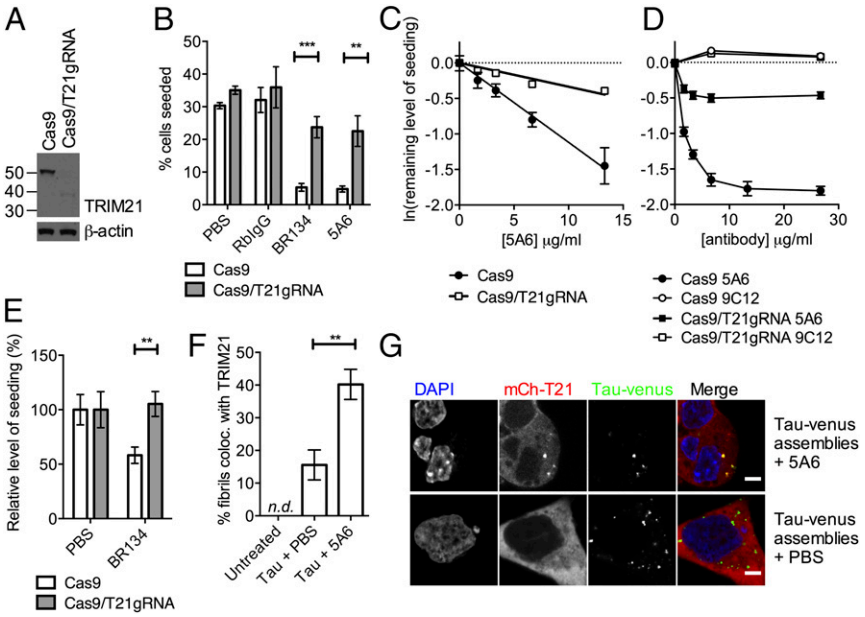

Fig. 4. Tau seeding is neutralized via TRIM21. (*A*) Immunoblot for TRIM21 in HEK293 tau-venus cells treated with control vector expressing Cas9 only or with additional TRIM21-directed guide RNA. (*B*) Relative levels of seeding by recombinant P301S tau assemblies after treatment with indicated antibody in the presence of LF. (*C*) Titration of 5A6 against tau seeding in HEK293. (*D*) Titration of 5A6 or isotype control antibody 9C12 against tau seeding. (*E*) Relative level of seeding by AD brain sarkosyl insoluble fraction alone or in complex with BR134 in HEK293 cells in the presence of LF. (*F*) Percentage of tau aggregates within cell boundaries that colocalize with myc-TRIM21. (*G*) Confocal microscope images of cells expressing mCherry-TRIM21 fixed 1 h after application of tau-venus assemblies purified from human cells in the presence of LF. (Scale bar, 5 μm.) **Unpaired *t* test $P < 0.01$; ***$P < 0.0001$. Error denotes SEM. In *B–E*, $n = 8$–12 fields per condition; in *G*, $n = 13$ fields per condition. n.d., none detected.

by 5A6 and BR134 was significantly reduced in cells that lacked TRIM21 (Fig. 4*B*). TRIM21-mediated neutralization of adenovirus proceeds without a lag phase and is dose-dependent until reaching a plateau termed the "persistent fraction." This represents a proportion of infection that cannot be prevented, but whose level is dependent on TRIM21 expression levels (2, 23). TRIM21-mediated neutralization of tau seeding occurred with log-linear dynamics and without a detectable lag-phase (Fig. 4*C*). This suggests that, as in the case of adenovirus neutralization, a threshold antibody stoichiometry is not required, but that neutralization increases incrementally with antibody occupancy. A persistent fraction was observed for tau neutralization, whose level was increased in cells lacking TRIM21 (Fig. 4*D*). Thus, neutralization of tau seeding occurs with similar dynamics as neutralization of adenovirus infection. To determine whether the nature of the tau seed affects neutralization, we tested whether TRIM21 can neutralize the sarkosyl-insoluble fraction from AD brain, which contains tau fibrils. We found that BR134 reduced seeding in unmodified HEK293 cells, but provided no protection when *TRIM21* was deleted (Fig. 4*E*). Thus, tau assemblies from recombinant and clinical samples can be neutralized in the intracellular domain in a similar manner to viral infection.

TRIM21 Is Rapidly Recruited to Antibody-Bound Tau Assemblies. The fast kinetics of seeded tau aggregation imply that for TRIM21 to be effective, it must be rapidly recruited to incoming antibody-coated seeds. To test this, we challenged HEK293 cells expressing myc-tagged TRIM21 with tau assemblies and examined the localization of TRIM21 after 4 h (Fig. S4 and Fig. 4*F*). Alternatively, we challenged HEK293 cells expressing mCherry-TRIM21 with the sarkosyl-insoluble fraction prepared from seeded tau-venus-expressing cells and fixed cells at 1 h (Fig. 4*G*). In both cases, TRIM21 adopted a disperse cytoplasmic distribution in cells that were treated with unlabeled tau assemblies. However, TRIM21 reorganized to bright foci surrounding antibody-coated tau assemblies during challenge. As has been observed for antibody-bound viruses and bacteria (1, 3), a subset of antibody-bound P301S tau assemblies did not colocalize with TRIM21 and is likely endosomal. We conclude that TRIM21 is recruited to tau assemblies shortly after their entry to the cytoplasm.

TRIM21 Is the Sole Protective Fc Receptor in a Human Neuronal Cell Line. It has previously been demonstrated that antibody receptors FcγRII/III expressed on the surface of neurons in brain slices mediates uptake of uncomplexed anti-tau antibodies (24, 25). The internalized antibodies are proposed to interact with cellular tau pools in a compartment that stains positive for markers of the endolysosome. Such a mechanism could potentially affect the seeded aggregation of cytosolic tau, either by increasing the uptake of tau–antibody immune complexes to neurons or by diverting immune complexes to endolysosomal degradation pathways. We therefore addressed whether cell surface Fc receptors enhance or interfere with seeding/neutralization in a human neuronal cell line. We established a seeding assay in the human neuroblastoma cell line SHSY-5Y by expressing P301S tau-venus in a similar manner as in HEK293s (Fig. S5). Seeding was observed in both the presence and absence of lipofectamine and cells bearing seeded tau aggregates stained positive with AT100. The presence of excess IgG or an antibody that blocks FcγRII/III did not alter observed levels of seeding by tau–antibody complexes (Fig. 5*A*). These data are suggestive of an absence of FcγR activity in these cells. Directly testing for the expression FcγRs in SHSY-5Y cells revealed minimal or no expression for both high-affinity (CD64, FcγRIB) and low-affinity (CD32 FcγRIIA; CD16a, FcγRIIIA; CD16b, FcγRIIIB) Fc receptors, similar to HEK293s (Fig. 5*B*). In contrast, we observed strong FcγRI and FcγRII expression in the monocyte-derived cell line THP-1, consistent with its role as an antigen-presenting cell. Conversely, *TRIM21* mRNA was detected in all cell lines at comparable levels. These findings are in agreement with data from cultured mouse neurons and transcriptomic databases, where cell surface FcγR expression in neurons is found to be very low or absent (14, 26). Tau neutralization in SHSY-5Y cells occurred similarly to HEK293s. In both the presence and absence of LF, seeding was neutralized by 5A6, but significantly reduced on TRIM21 depletion (Fig. 5 *C–E*). Thus, TRIM21 appears to be the only FcR expressed and functional in neurons, and therefore the sole receptor capable of effecting the neutralization of prion-like proteins that replicate in the cytoplasm of neurons.

Neutralization of Tau Seeding Is Proteasome- and VCP-Dependent. To investigate whether neutralization of tau seeding follows a similar cofactor dependency as virus neutralization, we treated cells with the proteasomal inhibitor MG132. Under these conditions, neutralization of tau seeding was significantly reduced, although not completely inhibited (Fig. 6*A*). To examine the contribution of

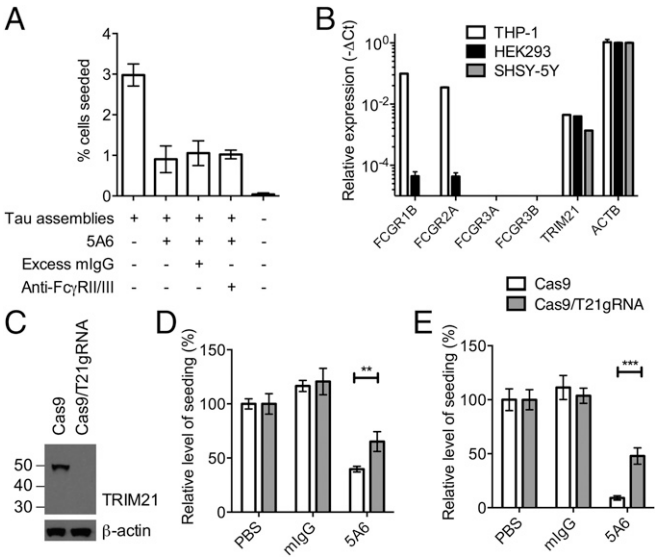

Fig. 5. TRIM21 is the only protective Fc receptor in SHSY-5Y neuroblastoma cells. (*A*) Levels of seeding in SHSY-5Y cells treated as indicated in the absence of LF. Excess mouse IgG (mIgG) or an antibody that blocks the low-affinity FcγRII and FcγRIII did not alter levels of seeding after challenge with tau-5A6 complexes. (*B*) Levels of indicated mRNA transcripts in HEK293, SHSY-5Y, and the monocyte cell line THP-1. Relative levels were determined by RT-qPCR, using the −ΔCt method relative to β-actin (ACTB). (*C*) Immunoblot for TRIM21 in SHSY-5Y cells that were treated with Cas9 only or with an additional TRIM21-directed guide RNA. (*D* and *E*) Relative levels of seeding in SHSY-5Y cells after challenge with tau assemblies that were pretreated with PBS, nonspecific mouse IgG, or tau-specific antibody 5A6 in the (*D*) absence and (*E*) presence of LF. **Unpaired *t* test $P < 0.01$; ***$P < 0.0001$. Error denotes SEM. In *A* and *E*, $n = 12$ fields per condition; in *B*, mean of triplicate cDNA preparations; in *D*, $n = 24$ fields per condition.

the molecular unfoldase VCP, we used the inhibitor N^2,N^4-dibenzylquinazoline-2,4-diamine (DBeQ) (27) or its derivative ML240 (28). Treatment with these compounds almost completely reversed neutralization (Fig. 6 *B* and *C*). Depletion of VCP by siRNA also reduced levels of neutralization, confirming VCP dependence (Fig. 6 *D* and *E*). These results demonstrate that neutralization of tau seeding occurs with similar cofactor dependencies as viral neutralization. However, the results suggest a more prominent role for VCP compared with the proteasome, as inhibition of neutralization was greater with inhibitors of the former. VCP has a role in both proteasomal and macroautophagy degradation pathways. We therefore tested whether TRIM21 also exerts neutralization via autophagy. We used siRNA depletion of the ubiquitin-like protein ATG12, whose conjugation to ATG5 is an upstream requirement for the formation of autophagosomes, to test the effect of impaired autophagy on intracellular neutralization. However, despite preventing the formation of GFP-LC3 puncta after starvation (Fig. S6), ATG12 depletion did not impair the neutralization of tau seeding by 5A6 (Fig. 6*F*). The role of VCP in neutralization of tau seeding is therefore likely to be independent of autophagy. Further supporting this finding, chemical inhibition of autophagy or lysosomal acidification did not prevent neutralization of tau seeding (Fig. 6*G* and Fig. S6). Overall, these results demonstrate that, similar to viruses, antibody-labeled tau aggregates are neutralized in a proteasome- and VCP-dependent manner in the intracellular environment.

Discussion

We have developed high-content quantitative assays for measuring cytosolic tau aggregation in situ. Whereas the introduction of tau assemblies to the cytoplasm induced rapid aggregation, the presence of anti-tau antibodies diverted tau seeds to a neutralization pathway that substantially reduced their seeding capacity (diagram in Fig. S7). This inhibition relied on the detection of antibody-bound tau by the cytosolic Fc receptor TRIM21, which exerted neutralization activity in concert with known intracellular neutralization machinery, the molecular unfoldase VCP and the proteasome. Intracellular neutralization occurred rapidly, before tau hyperphosphorylation, and was observed for tau assemblies prepared from recombinant or AD brain-derived sources. These results demonstrate that, despite lacking PAMPs, pathogenic protein assemblies can be recognized and destroyed by the cell if presented in context with a danger signal, intracellular antibody. Thus, intracellular antiviral immunity can be repurposed to protect against self-propagating protein misfolding.

Large tau aggregates are refractory to proteasomal activity, and their turnover is thought to rely primarily on macroautophagy (29, 30). In contrast, seeds are reported to be short fibrils containing >10 copies of tau (31). Our results suggest that tau seeds are substrates for proteasomal degradation if targeted rapidly after cell entry. Thus, targeting seeds rather than established aggregates may be a more effective strategy for preventing spreading tau aggregation. Importantly, we observed that the inhibition of tau aggregation was more dependent on VCP than the proteasome. This is in contrast to adenovirus neutralization, in which proteasome and VCP activities are equally critical (1, 5). VCP has been linked to both proteasomal degradation and autophagy; however, we did not observe any reduction in the neutralization of aggregation under conditions where autophagy was prevented. Instead, it is possible that VCP exerts its effect by unfolding tau seeds to prevent them from catalyzing aggregation. This would be consistent with the enzyme's known unfoldase activity (32), and with altered tau conformations being the drivers of seeded aggregation.

The discovery that antibodies are capable of preventing cytoplasmic tau aggregation is significant to the development of immunotherapy for AD and other tau pathologies. In transgenic mouse models, passive transfer of tau-specific antibodies can reduce tau pathology (20, 33–35). Similar approaches in humans are being widely studied as potential treatments for neurodegeneration (36).

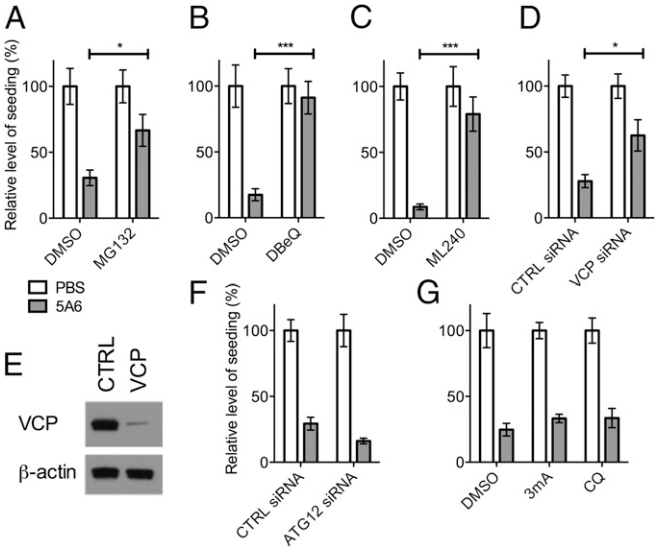

Fig. 6. Neutralization of tau seeding occurs via established TRIM21 degradative pathways. (*A*) Effect of proteasome inhibitor MG132 on neutralization by 5A6. (*B–D*) Effect of VCP inhibitors (*B*) DBeQ, (*C*) ML240, or (*D*) VCP knockdown on neutralization by 5A6. (*E*) Immunoblot for VCP and β-actin after VCP-directed or control (CTRL) siRNA treatment. (*F* and *G*) Effect of (*F*) ATG12 depletion by siRNA or (*G*) inhibitors 3-methyladenine (3mA) and chloroquine (CQ) on neutralization of tau seeding. *Unpaired *t* test $P < 0.02$; ***$P < 0.0001$. Error denotes SEM. In *B–E*, $n = 12$ fields per condition.

However, the mechanisms by which antibodies exert their activity is unclear. Mechanisms that have been described include the clearance by FcγRs expressed on microglia (37), the blockade of tau assemblies from entering neurons (20), the uptake of antibodies in an FcγRII/III-dependent manner and subsequent colocalization with tau in an endolysosomal compartment (24), and the uptake of uncomplexed antibodies by neurons via unknown mechanisms and degradation of soluble tau toxic precursors via TRIM21 (13). The relevance of mechanisms in which interaction with an FcγR is required has recently been questioned by the finding that an anti-tau antibody mutated to prevent FcγR binding remains protective in mice (26). This is consistent with our in vitro findings that FcγR interactions neither promote nor inhibit tau aggregation in neuronal cell lines. Use of mutated "'effectorless" antibodies may be favorable in therapeutic settings, as it would minimize the proinflammatory effects of extracellular tau immune complexes. Importantly, however, the mutations used to render antibody effectorless do not affect the TRIM21 binding site, and would therefore maintain full levels of TRIM21 binding. Observations of antibody protection in mouse models of tau pathology are therefore consistent with Trim21-mediated protection. In vivo experiments in a *Trim21* null setting are necessary to determine the contribution of cytoplasmic degradation and neutralization to immunotherapeutic protection against tau pathology.

Our results demonstrate that antibodies can direct a potent TRIM21-dependent neutralizing response immediately after cytosolic entry of tau seeds. The selective targeting of pathological proteins in the intracellular domain by antibodies may provide an effective therapeutic strategy.

Materials and Methods

Seeding Assays. A full description of experimental techniques is provided in *SI Materials and Method*. Recombinant, heparin-assembled tau preparations were added to HEK293 or SHSY-5Y cells expressing P301S tau-venus in 96-well plates. LF was added at 0.5 μL per well where indicated. Seeded cells were enumerated by high-content imaging.

Adenovirus Infection. Mouse primary neurons and other cells were challenged with E1E3-deleted human adenovirus type 5 vector in the presence or absence of anti-hexon antibody 9C12, as before.

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