2019IL-33 and its decoy sST2 in patients with Alzheimer’s Disease and Mild Cognitive Impairment

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Abstract

Background: Interleukin-33 is a cytokine endowed with pro- and anti-inflammatory properties that plays a still poorly defined role in the pathogenesis of a number of central nervous system (CNS) conditions including Alzheimer’s disease (AD). We analyzed this cytokine and its decoy receptor sST2 in Alzheimer’s disease (AD) and mild cognitive impairment (MCI).

Method: IL-33 and sST2 were analyzed in serum and CSF of AD and MCI patients, comparing the results to those obtained in age-matched healthy controls (HC).

Because of the ambiguous role of IL-33 in inflammation, the concentration of both inflammatory (IL-1β and IL-6) and anti-inflammatory (IL-10) cytokines was analyzed as well in serum and cerebrospinal fluid (CSF) of the same individuals. Finally, the effect of IL-33 in an in vitro system of Aβ42-stimulated monocytes was examined.

Results: As compared to HC: 1) IL-33 was significantly decreased in serum and CSF of AD and MCI; 2) sST2 was increased in serum of AD and MCI but was undetectable in CSF; 3) serum and CSF IL-1β concentration was significantly increased and that of IL-10 was reduced in AD and MCI whereas no differences were observed in IL-6. In vitro addition of IL-33 to LPS+Aβ 42-stimulated monocytes down regulated IL-1b generation in AD, MCI and HC and stimulated IL-10 production in HC alone. IL-33 addition also resulted in a significant reduction of NF-kB nuclear translocation in LPS+Aβ42-stimulated monocytes of HC alone.

Conclusions: These data support the hypothesis that IL-33 plays a complex anti-inflammatory role that is lost in AD- and MCI-associated neuroinflammation; results herein also suggest a possible use of IL-33 as a novel therapeutic approach in AD and MCI.
Interleukin-33 (IL-33) is a dual function cytokine produced by endothelial and epithelial cells as well as fibroblast, macrophages, adipocytes, smooth muscle and brain cells [1]. This cytokine is released as a full-length active protein that can be inactivated by caspase-1, -3 and -7-mediated cleavage [2-3] or can processed by different proteases into shorter forms characterized by diverse biological activities [4-6]. The regulation of IL-33 biological activity is complex as it is the result of the interplay between the extra- and intra-cellular forms of this cytokine and its ability to bind two different isoforms of ST2, its cognate receptor. Binding of extracellular IL-33 to ST2 on leukocytes, astrocytes and oligodendrocytes[7] mediates the biological effects of this cytokine [8]. On the other hand, when expressed intracellularly, IL-33 binds the p65 subunit of NF-kB [9]; the resulting IL-33/NF-kB p65 complex interferes with NF-kB-dependent transcription by impeding p65-mediated transactivation. This causes a negative modulation of NF-kB activity, with a dampening effect on inflammation. An additional actor that modulates the biological activity of IL-33 is the soluble form of ST2 (sST2), a decoy receptor. As is usually the case with decoy receptors, IL-33 binding to sST2 limits its the biological activity[10].

Th2 helper cells and mast cells express ST2 and respond to IL-33, and type 2 innate lymphoid cells (ILC2) are considered to be the signature IL-33-responsive cells [11]. Thus, IL-33 targets ILC2 to produce IL-5 and IL-13, resulting in the recruitment of eosinophils, the activation of DC, and Th2 differentiation [12-16]. A growing body of evidence suggests that IL-33 also controls the accumulation and effector function of regulatory T cell (Treg), either directly or indirectly via ILC2 activation and macrophage polarization [17-19]. Thus, IL-33 causes microglial and macrophage
polarization to an anti-inflammatory type-2 (M2) phenotype through the IL-33/ST2 signaling pathway; this results in IL-10 generation and reduces IL-1b and IL-6 production [20-22].

IL-33 plays a yet poorly understood role in the pathogenesis of Alzheimer's disease (AD), a condition where deposition of extracellular amyloid beta (Ab) plaques in the brain and neuronal cell death accompany neuroinflammation. In AD patients the concentration of proinflammatory cytokines, including interleukin IL-1b is increased, possibly as a result of the activation of the NLRP3 inflammasome, and immune regulatory mechanisms, including those mediated by Tregs and PDL-1, are impaired [23-27]. A more subtle form of neuroinflammation is also present in mild cognitive impairment (MCI), a subjective and objective decline in cognitive performance that is greater than expected for an individual's age and education level, but does not meet criteria for the diagnosis of AD [28]. Elderly MCI patients are at high-risk for developing AD, this situation thus represents a borderline condition between normal aging and AD [29].

Correlates of MCI conversion to AD are still poorly defined, even if neuroinflammation is strongly suspected to play a role in this process [29-33]. Recent observations suggest a beneficial role of IL-33 in AD-associated neuroinflammation. To summarize: 1) ex-vivo results in cellular models of AD indicated that over expression of IL-33 decreases Aβ secretion; and 2) autoptic data indicate that IL-33 is significantly reduced in brains of AD patients. Notably, in the APP/PS1 animal model of AD, IL-33 administration was shown to restore the phagolysosomal activity of the microglia, thus enhancing amyloid β (Aβ) clearance, and to polarize monocytes to an anti-inflammatory phenotype. This resulted in a significant amelioration of AD symptoms [34], suggesting the possibility that IL-33
is a neuroprotective cytokine in AD [35]. This hypothesis nevertheless is not supported by other results suggesting that IL-33 is present in high concentrations in the neuropathological lesions of AD brain and can exacerbate AD-associated neuroinflammation [36]. In the attempt to clarify the role of the ST2/IL-33 axis in AD we analyzed these proteins in AD, MCI and HC individuals and explored the effect of IL-33 supplementation in an in vitro system of Aβ-stimulated monocytes.

materials and methods

Patients and controls

Ninety elderly Italian individuals were enrolled in the study by the Neurology Department of the IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Italy. Thiry patients had a diagnosis of AD, 30 patients had a diagnosis of mild cognitive impairment (MCI) and 30 individuals were age and sex matched healthy controls (HC). The clinical diagnosis of AD was performed according to NINCDS-ADRDA work group criteria [28] and further revisions [37], the clinical diagnosis of MCI fulfilled Petersen’s operational criteria [29]. Neuropsychological evaluation was performed with a Mini-Mental State Examination (MMSE) [38] and Clinical Dementia Rating Scale (CDR) [39].

All patients underwent a clinical interview, neurological and neuropsychological examination, routine blood tests, brain MRI, and lumbar puncture (LP). The mean age of AD patients (13 males and 17 females) was 74.3 years (age range 54-88 years) and that of MCI patients (8 males and 22 females) was 75.3 years (age range 63-84 years). All the AD patients were enrolled in a Multidimensional Stimulation Rehabilitation program designed for this pathology. Finally, the thirty age- and sex-
matched elderly subjects (HC) who were included in the study were selected according to the SENIEUR protocol for immuno-gerontological studies of European Community’s Control Action Programme on Aging [40]; their MMSE score was \( \geq 28 \).

The study was approved by the Ethics Committee of Don Gnocchi Foundation and informed written consent was obtained from all the included subjects before study initiation.

**Serum and CSF**

Serum was collected in vacutainer tubes containing serum separator (Becton Dickinson and Co., Rutherford, NJ, USA) and were centrifuged at 3,000 rpm for 10 min to separate sera; CSF samples were collected by lumbar puncture. Serum and CSF were used immediately or stored at -80°C.

**Blood sample collection and cell separation**

Whole blood of a subset of individuals (5 AD, 5 MCI and 5 HC) was collected in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) (Becton Dickinson). Peripheral blood mononuclear cells (PBMC) were separated on lympholyte separation medium (Cedarlane, Hornby, Ontario, CA) and washed twice in PBS at 1500 rpm for 10 min; viable leukocytes were determined using a TC20 Automated Cell Counter (Biorad, Hercules, CA, USA).

**Cell cultures**

PBMC (1x10^6/ml) were cultured in RPMI 1640 supplemented with 10% human serum, 2mM L-glutamine, and 1% penicillin (Invitrogen Ltd, Paisley, UK) and incubated at 37°C in a humidified 5% CO₂ atmosphere for 2 hours in a 12 wells plate for monocyte adhesion. After 2 hours, non- adhering PBMC were harvested and discarded and monocytes grown on plate were either culture in medium alone
(unstimulated) or were or primed with 2mg/ml Lipopolysaccharide (LPS) for 2 hours \textbf{(Sigma-Aldrich, St. Louis, MO, USA)} before stimulation with 10mg/ml of 1-42 amyloid-beta peptide (\textit{Ab}_{42}) \textbf{(Sigma-Aldrich)} in the absence/presence of 10ng/ml of Human Recombinant IL-33 (Biolegend, San Diego, CA, USA) for 24h at 37°C in a humidified 5\% CO\textsubscript{2} atmosphere. After 24 hour, supernatants were collected and stored at -20°C; adhering cells (monocytes) were collected and prepared for FlowSight analysis.

\textbf{Enzyme-linked immunosorbent assay (ELISA)}

IL-33 (catalog number D3300B, sensitivity: 1.51 pg/mL, assay range: 3.1-200 pg/mL, minimum detectable dose (MDD): ranged from 0.069-1.51 pg/mL), sST2 (catalog number DST200, sensitivity 13.5 pg/mL, assay range: 31.3-2000 pg/mL, MDD: ranged from 2.45-13.5 pg/mL), IL-1b (catalog number DLB50, sensitivity: 1 pg/mL, assay range: 3.9-250 pg/ml MDD: less than 1 pg/mL), IL-6 (catalog number D6050, sensitivity:0.70 pg/mL, assay range: 3.1-300 pg/ml MDD: less than 0.70 pg/mL) and IL-10 (catalog number D1000B, sensitivity: 3.90 pg/mL, assay range: 7.8-500 pg/mL, MDD: less than 3.90 pg/mL) concentration was analyzed in serum and CSF by commercially-available ELISA according to the manufacturer’s recommendations (Quantikine Immunoassay; R&D Systems, Minneapolis, MN, USA or Thermo Fisher Scientific, Waltham, MA, USA). A plate reader (Sunrise, Tecan, Mannedorf, Switzerland) was used and optical densities (OD) were determined at 450/620 nm. All samples were performed in duplicates. The same methods were used to measure IL-1b, IL-6 and IL-10 in unstimulated and in LPS-primed and \textit{Ab}_{42}^- stimulated-PBMC supernatants in the presence/absence of recombinant IL-33 (see above).

\textbf{Western blot}
A Qubit Protein Assay Kit was used for protein extraction; total protein amount was measured using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). Equal amounts of protein (15 µg) from each sample, or 2 ng of artificial truncated IL-33 (amino acids 112-270) (20 KD) (Recombinant Human IL33 carrier-free, Biolegend, San Diego, CA, USA) used as positive control to confirm that the bands correspond to IL-33 full length (amino acids 1-270) (34-30 KD), to the cleaved inactive forms (amino acids 1-178) (22-20 KD) and (amino acids 179-270) (13-12 KD) [2-3], or, finally, to the cleaved active forms (amino acids 95-270) (amino acids 99-270) and (amino acids 109-270) (19-15 KD) [4], were separated by 10% SDS-PAGE and transferred to PVDF membranes (Genscript). A pre-stained marker, broad range 11-190 KDa (Cell signaling, Danvers, MA, USA), was also used, PVDF membrane were treated by ONE-HOUR western detection kit (Genscript) and proteins were visualized using a Chromosensor TMB substrate (Genscript). After protein transfer, PVDF membranes were incubated with Pretreatment Solution for 5 min RT and then with the WB solution and an a-human IL-33 Ab (Nessy-1)(Abcam, Cambridge, UK) for 40 min. After three washes membranes were developed with a TMB substrate.

**Cell culture and NFkB /7AAD intracellular staining**

Monocytes that were either unstimulated or LPS-primed and Ab42- stimulated in the presence/absence of 10ng/ml of recombinant IL-33 were analyzed to evaluate nuclear translocation of NF-kB; the Amnis® NF-kB Translocation Kit was used according to the manufacturer’s recommendations (Merck KGaA,
Darmstadt, Germany). Briefly, cells were fixed, permeabilized and stained with Anti-Hu NF-
κB(p50) Alexa Fluor® 488 for 30 min RT. After incubation, monocyte were washed, fixed and
10ml of 7AAD were added.

FlowSight Analysis

The FlowSight (Amnis Corporation, Seattle, WA, USA) is equipped with two lasers
operating at 488 and 642 nm, two camera and twelve standard detection channels.
It simultaneously produce side scatter (darkfield) images, one or two transmitted
light (brightfield) images, and up to ten channels of fluorescence imagery of every
cell. FlowSight acquires 2000 cells/second and operates with a 1mm pixel size
(∼20X magnification) allowing visualization of fluorescence from the membrane,
cytoplasm, or nucleus. The IDEAS image analysis software allows quantification of
cellular morphology and fluorescence at different cellular localizations by defining
specific cellular regions (masks) and mathematical expressions that uses image
pixel data or masks (feature) by different wizards. Analysis of NF-kB translocation
was performed by Nuclear Localization Wizard using Similarity Feature. Briefly,
nuclear translocation has occurred if the NF-κB and nuclear fluorescence signals
overlap with similar shapes. The Bright Detail Similarity is designed to specifically
to compare the small bright image detail of two images and can be used to quantify
the co-localization of two probes (NF-kB and 7AAD) in a defined region. The
Similarity score is the log transformed Pearson’s Correlation Coefficient and it is a
measure of the degree to which two images are linearly correlated within a masked
region and is calculated on double positive region (NF-kB+7AAD+).

Statistical analysis
Quantitative data were not normally distributed (Shapiro-Wilk test) and were summarized as median and Interquartile Range (IQR) (25° and 75° percentile). Comparisons between groups were performed used a Kruskal-Wallis ANOVA for each variable. Comparisons among the different groups were made using a 2-tailed Mann-Whitney U test performed for independent samples. Data analysis was performed using the MedCalc statistical package (MedCalc Software bvba, Mariakerke, Belgium).

results

**IL-33 isoforms**

Analysis of the different isoforms of IL-33 (full-length; cleaved inactive; cleaved active) was performed by Western blotting in serum and CSF of AD, MCI and HC individuals. Results showed that the full-length IL-33 protein (30kDa band) was present in all the serum and CSF samples analyzed with the exception of two AD sera. In these two particular samples, a 20 KD band, corresponding to the cleaved (c), inactive form of IL-33 [3] was observed. These results are shown in Figure 1A e 1B.

**IL-33 and sST2 serum and CSF concentration**

IL-33 concentration was decreased in serum and CSF of both AD and MCI individuals compared to HC (p value vs. HC: serum, AD p= 0.02; MCI p=0.04; CSF, AD p=0.01; MCI p=0.009). In contrast with these results, serum concentration of the IL-33 decoy receptor sST2 was significantly increased in AD and MCI compared to HC (p value vs. HC: p=0.02 and p= 0.01 respectively) (Figure 2). CSF sST2 concentration was below the limit of detection of the assay (33pg/ml) in all the analyzed samples (data
IL-1b, IL-6 and IL-10 serum and CSF concentration

IL-1b serum concentration was significantly increased in AD and MCI sera compared to HC (p=0.01 in both cases) whereas that of IL-10 was significantly reduced in both groups of patients compared to HC (p=0.04 in both cases). No significant differences were detected when IL-6 serum concentration was compared between the three groups. These results are shown in Figure 3.

In CSF, IL-1b concentration was significantly augmented in AD and MCI compared to HC (p=0.02 and p= 0.01 respectively). IL-6 concentration was slightly augmented and IL-10 concentration was marginally reduced in AD and MCI compared to what was seen in HC but these differences did not reach statistical significance (Figure 3).

Recombinant IL-33 differentially modulates IL-1b, IL-6 and IL-10 production by LPS primed and Ab42-stimulated PBMC

Monocytes of AD, MCI and HC individuals (n=8 in all cases) were LPS primed and Ab42-stimulated and IL1b, IL-6 and IL-10 production was measured by ELISA.

Whereas the production of these cytokines was minimal (<20 pg/ml in all cases) and comparable between the three groups of individuals analyzed when monocytes were unstimulated (medium alone) or when IL-33 was added to the culture medium, LPS-primed and Ab42-stimulated-monocytes of AD and MCI patients produce significantly augmented quantities of IL-1b (median: AD= 230 ng/ml; MCI= 140ng/ml ) compared to HC (median: 62 ng/ml; AD vs. HC p=0.006; MCI vs. HC p=0.03). Notably, IL-10

not shown).
production by cells stimulated in the same conditions was significantly reduced in AD (median: 129 ng/ml) compared to HC individuals (median: 245 ng/ml) (p=0.01). Addition of IL-33 to cell cultures resulted in a significant reduction of the production of both IL-1β (median: 36 pg/ml; p=0.04) and IL-6 (median: 616 pg/ml; p=0.01) in HC, and of IL-1β alone in MCI (median: 23 pg/ml; p=0.02) and, although without reaching statistical significance. Finally, LPS+Ab42-stimulated IL-10 production was not modified by IL-33 in AD and MCI, but was significantly increased in HC (median: 585 pg/ml; p=0.02) (Figure 4).

These results support the idea that IL-33 reduces the generation of pro-inflammatory cytokines and stimulates that of anti-inflammatory cytokines, favoring the establishment of an anti-inflammatory milieu, and suggest that IL-10-mediated anti-inflammatory mechanisms are impaired/exhausted in AD and MCI.

**Recombinant IL-33 reduces NF-kB nuclear translocation**

Unstimulated as well as LPS primed and Ab42-stimulated-monocyte of AD (n=5), MCI (n=5) and HC individuals (n=5) were analyzed to verify the effect of IL-33 on NF-kB nuclear translocation using the FlowSight technology. No differences in NF-kB nuclear translocation were detected in unstimulated cells (data not shown); upon antigenic stimulation, the IL-33 was observed to significantly reduce NF-kB nuclear translocation (p=0.01) in HC alone (Figure 5). These results suggest that this is a pivotal IL-33 anti-inflammatory mechanism that is possibly lost in MCI and AD.

**Discussion**

Alzheimer’s disease is a highly prevalent form of dementia characterized by the
accumulation of extracellular amyloid beta plaques in the brain, neuronal cell death and neuroinflammation. The pathogenesis of AD-associated neuroinflammation includes an increased production of proinflammatory cytokines, possibly driven by the excessive activation of the inflammasome, a reduced activity of Treg lymphocytes, and the dysregulation of immune-mediated mechanisms of tolerance. Recent results suggest that IL-33 plays an important role as well in the pathogenesis of AD. IL-33 is released by damaged cell and can be produced by mast cells, macrophages and dendritic cells either as an active, full length or as an inactive differently cleaved form. Notably, although only present in a small minority of samples, the cleaved, biologically inert form of IL-33 was present in serum of a subset of AD patients alone.

IL-33 is endowed with both pro- and anti-inflammatory properties, hence the presence of contrasting data indicating both an increase and a reduction of IL-33 in AD. We investigated the role of IL-33 in AD pathogenesis both by directly measuring the concentration of this cytokine and its decoy receptor in biological fluids of AD and MCI patients and by analyzing the effect of IL-33 addition in an in vitro model of Ab\textsubscript{42}-stimulated monocytes of patients and healthy controls.

Results herein show that the IL-33/ST2 axis is deeply impaired in MCI and AD. Thus, IL-33 concentration in serum and CSF was significantly reduced whereas the concentration of sST2, the IL-33-specific decoy receptor, was significantly increased in the same patients. In AD, thus, lower amounts of IL-33 are produced and they cannot optimally bind their cognate receptor on the surface of cells, as they are trapped by soluble decoys.

That reduced concentrations of IL-33 are associated with inflammation was indirectly confirmed by the observation that IL1b concentrations were significantly
increased whereas that of IL-10 was greatly reduced in the same AD and MCI patients. The anti-inflammatory role of IL-33 was further reinforced by the observation that IL-33 supplementation resulted in the reduction of IL-1b and IL-6 generation by Ab-stimulated cells of AD and MCI. Importantly, IL-33 increased IL-10 production by cells of HC alone, suggesting that the ability of monocytes of AD and MCI individuals to secrete IL-10 in response to IL-33 is deeply defective or exhausted.

IL-1b is augmented in AD. This cytokine promotes amyloid plaque deposition, reduces phagocytic activity by the microglia, stimulates the hyperphosphorylation of t protein, and affects synaptic plasticity. IL-10 production, on the other hand, has repeatedly been shown to be reduced in AD patients [24,41,42], in whom the IL-10 gene SNPs associated with higher production of this cytokine are also less frequently detected [33]. Results herein could thus at least partially justify the genesis of the proinflammatory milieu seen in AD and MCI as the consequence of the reduced amounts of IL-33 seen in these patients.

Notably, IL-33 significantly down-modulated NF-kB nuclear translocation in cells of HC alone, indicating this as a possible mechanisms by which IL-33 favors the maintenance of an anti-inflammatory milieu in individuals in whom dementia is not present. Thus, IL-33 was shown to have a regulatory effect on the NF-kB pathway, which is mediated by its ability to binding the NF-kB p65 subunit [9]. The IL-33/NF-kB p65 complex impedes p65-mediated transactivation; this down regulates NF-kB activity, with a dampening effect on inflammation. The observation that this effect was lost in cells of MCI and AD individuals suggests that the role of IL-33 in the pathogenesis of AD- and MCI-associated inflammation is multifaceted, as this cytokine would exert its effect both at the intracellular and at the extracellular
level. To summarize: 1) reduction of IL-33 production per se, 2) increased
concentrations of the sST2 decoy receptor, and 3) an impairment of the ability of IL-
33 to induce NF-kB nuclear translocation are different mechanism that can explain
the proinflammatory role played by IL-33 in AD and MCI.

IL-33 was also reported to induce the polarization of monocytes toward an M2 anti-
inflammatory phenotype; these monocytes produce IL-10, whose concentration, as
indicated above, is greatly reduced in AD and MCI individuals [43-44]. M2
polarization by IL-33 is possibly explained by the observation that the IL-33 binding
to ST2 negatively regulates TLR signaling by competing with MyD88 [45-46]. The IL-
33/ST2 complex thus suppresses IL-1b generation and the down-stream activation of
the TLR signaling pathway by sequestration of MyD88 with the consequence of
further inhibiting NF-kB and activating MAP kinases [46]. IL-33 indeed activates
ERK1\2 and STAT3, facilitating binding of STAT3 to the -1954 to – 1936 bp sequence
upstream of the IL-10 transcription start site, thereby promoting its transcription in
macrophages [47]. Although we did not analyze M1 and M2 monocytes in this study,
previously published data indicate that M2 cells are significantly reduced in AD. The
reduction of M2 monocytes seen in AD could explain why IL-33 supplementation did
not increase IL-10 production by monocytes of AD and MCI individuals in our in vitro
system and could be a third way to justify why the lower quantities of IL-33 seen in
AD results in inflammation in these patients.

IL-33 has repeatedly been suspected to be involved in the pathogenesis of CNS
diseases. In particular the administration of recombinant IL-33 was shown to
promote recovery in a mouse model of autoimmune encephalomyelitis (EAE) [48-49]
and to provide neuroprotection in a mouse model of contusion spinal cord injury
(SCI) [43]. In the APP/PS1 animal model of AD, IL-33 attenuated AD pathology and
memory deficit and stimulated the polarization of microglia in an anti-inflammatory direction [50-51]. Finally, recent data obtained in a small groups of MCI who did or did not convert to AD over time [31] showed that higher amounts of IL-33-producing CD14+ monocytes were seen in AD-non converters, in whom percentages of CD14+/IL-33+ cells positively correlated with the volumes of both left and right hippocampus.

conclusions
IL-33 was shown to have a neuroprotective role in AD secondary to the reduction of Ab secretion and the activation of Ab phagocytosis by the microglia [35]. Findings herein offer an immune explanation as well to the protective role of IL-33 in AD; these results warrant the investigation of this cytokine in treatment and rehabilitation programs for AD.

abbreviations

AD: Alzheimer’s disease
MCI: Mild Cognitive Impairment
HC: Healthy Controls
IL: Interleukin
CSF: Cerebro Spinal Fluid
Aβ: amyloid beta
TLR: Toll like receptor
PBMC: peripheral blood mononuclear cell
sST2: soluble ST2
declarations

Ethics approval and consent to participate
All patients and controls gave informed consent according to a protocol approved by the local ethics committee of the Don Gnocchi Foundation

Consent for publication
Not Applicable

Availability of data and material
The authors confirm that the data supporting the findings of this study are available within the article. The raw data of this study are available from the corresponding author [M.S.] on request.

Competing interests
The authors declare that they have no conflict of interest

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Authors' contributions
MS, IM and MC conceived and designed the research; MS, IM, FR, and FP performed the experiments; CF, DG, and ES are responsible for the clinical cohorts of patients; MS, IM and MC analyzed the data and prepared the manuscript.
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Figures
Figure 1

IL-33 isoforms Interleukin-33 in serum (A) and CSF (B) of representative AD, MCI.
Figure 2

IL-33 and sST2 IL-33 and sST2 concentration in serum and CSF of AD, MCI and age-matched HC individuals (N=30 individuals in each group). The box plots show the interquartile range with the median indicated by a horizontal line within the box. The whiskers extend to the most extreme data points that are not considered outliers. Outliers are represented by individual points. The numbers above the box plots indicate the statistical significance of the differences between groups, with AD, MCI, and HC indicating Alzheimer's disease, mild cognitive impairment, and healthy controls, respectively.
IL-1β, IL-6 and IL-10 concentration in serum (u
Figure 4

IL-1β, IL-6 and IL-10 production by LPS-primed and Aβ-stimulated monocytes of AD, MCI and age- and sex-matched HC.
**Figure 5**

**NF-kB Nuclear translocation** Panel A: Representative images of NF-kB nuclear translocation. The images show the pattern of NF-kB nuclear translocation in unstimulated (MED) and LPS-primed and Aβstimulated conditions. The similarity scores indicate the degree of colocalization between NF-kB and the nuclear dye, with higher scores indicating better colocalization.

Panel B: MED and LPS+Aβ comparison. The histograms show the frequency distribution of NF-kB translocated cells. The MED group has a higher percentage of untranslocated cells (62%) and fewer translocated cells (38%) compared to the LPS+Aβ group (27% translocated).

Panel C: Nuclear translocation in AD sample, panel D: MCI sample, and panel E: HC sample. The box plots show the distribution of translocated monocyte percentage among different groups and conditions. The p-value (p=0.01) indicates a significant difference in translocation percentages between the conditions.