

SHORT REPORT

Peripheral levels of caspase-1 mRNA correlate with disease activity in patients with multiple sclerosis; a preliminary study

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Abstract

The cysteine protease caspase-1 plays a crucial part in the inflammatory process due to its ability to proteolytically activate proinflammatory cytokine precursors, such as interleukin (IL)-1 β and IL-18. Multiple sclerosis is a chronic inflammatory demyelinating disease of the CNS in which the pathogenic process is mainly orchestrated by proinflammatory cytokines.

The role of caspase-1 in multiple sclerosis was evaluated by measuring its mRNA levels in peripheral blood mononuclear cells (PBMCs) from seven patients with relapsing-remitting multiple sclerosis every 15 days over a 1 year period. The recorded levels were compared with clinical and MRI evidence of disease activity. Brain MRI was performed monthly in each patient.

Caspase-1 mRNA levels were significantly increased in PBMCs from patients with multiple sclerosis compared with healthy controls ($p < 0.001$). In patients with multiple sclerosis, a twofold to threefold increase of caspase-1 mRNA mean level was found in the week preceding an acute attack ($p < 0.05$). The magnitude of caspase-1 mRNA increase correlated with the number of new ($p = 0.01$) but not persisting gadolinium enhancing brain MRI lesions.

In conclusion, caspase-1 might be involved in the immune mediated process underlying CNS inflammation and might represent a suitable peripheral immunological marker of disease activity in multiple sclerosis.

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Keywords: multiple sclerosis; caspase-1; proinflammatory cytokines

The pathological hallmark of multiple sclerosis is the presence in the CNS of inflammatory infiltrates and patchy demyelinating lesions.¹ Several in vitro and in vivo studies indicate that proinflammatory cytokines play a major part in the pathogenic process of lesion formation.²

Due to their peculiarities (autocrine/paracrine activity, short half life, redundancy) these molecules, however, do not represent so far suitable surrogate multiple sclerosis markers of CNS inflammation.

The caspase family comprises 13 different cysteine protease members which are mainly involved in the apoptotic pathway.³ Among them caspase-1, which is activated by caspase-11 mediated proteolytic cleavage,⁴ is less involved in the apoptotic cascade but mainly in the inflammatory processes due to its role in regulating the cellular export of interleukin (IL)-1 β .⁵ The part played by caspase-1 in inflammation is also supported by several in vivo experimental studies. Caspase-1^{-/-} mice display an alteration in the export of several proinflammatory cytokines—namely IL-1 β , IL-1 α , IL-6, IL-18, and tumour necrosis factor (TNF) α ⁶—and are resistant to lipopolysaccharide induced endotoxic shock⁷ and to the induction of experimental pancreatitis.⁸ In vivo, pharmacological inhibition of caspase-1 protects mice from TNF α induced liver failure⁹ and collagen induced arthritis.¹⁰

Thus we evaluated the role of caspase-1 as a potential surrogate marker of CNS inflammation in multiple sclerosis by measuring its mRNA levels in peripheral blood mononuclear cells (PBMCs) from seven patients with relapsing-remitting multiple sclerosis followed up every 15 days for 1 year. Disease activity was evaluated by clinical examination and monthly enhanced brain MRI.

Methods

Peripheral blood mononuclear cells were obtained every 15 days for 1 year from seven patients (five women, two men; mean age 28.7, range 20-37 years) affected by clinically definite multiple sclerosis (mean disease duration 5.1, range 2-10 years; mean expanded disability status scale score at entry 2.3, range 1.0-3.5) according to the criteria of Poser *et al.*¹¹ The clinical course of the disease was relapsing-remitting in all patients, who were always treated with a 2 to 3 week course of steroids when experiencing a new attack of the disease. None of the patients reported infections during the follow up period. As a control

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group, PBMCs were obtained every 15 days for 2 months from five age matched healthy donors (three women, two men; mean age 31.8, range 27–34 years).

Brain MRI was performed monthly in all patients, with a 1.5 Tesla machine (Siemens SP63, Erlangen, Germany). A total of 5 mm contiguous axial T1 weighted (SE 600/17; field of view=230 mm, matrix size=198×256; two excitations) slices throughout the whole brain were obtained 5 to 7 minutes after the injection of gadolinium-DTPA (0.1 mmol/kg). New or persisting enhancing lesions were counted in each scan by one of us (MF), unaware of the patient's clinical status.

Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed as previously described.¹² Analysis of the results was performed with a phosphorimager (Molecular Dynamics; Image Quant Software, version 3.3). Values, expressed as arbitrary units (AU), were normalised against the housekeeping gene G6PDH. For intersubject comparisons, the normalised AU were further corrected using the normalised AU of the spots resulting from RT-PCR amplification of the monocytic/macrophage THP-1 cell line derived positive cDNA. To minimise experimental errors, all cDNA samples from a given patient were amplified simultaneously and a positive control, THP-1 cDNA, was included in all PCR experiments. The following primers and probes were used: caspase-1 (product: 771 bp),

antisense 5'- GAA ACA AAA GTC GGC AGA GA -3', sense 5'- TGG GAA GAG GTA GAA ACA TC -3', probe 5' TGG TAT TCG GGA AGG CAT TTG TGG 3'; G6PDH (product: 528 bp), antisense 5'- ACC ACC ATG GAG AAG GCT GG -3'; sense 5'- CTC AGT GTA GCC CAG GAT GC -3'; probe 5' GTG GAA GGA CTC ATG ACC ACA GTC CAT GCC 3'.

Results

Caspase-1 mRNA levels were measurable in all the 20 and 158 PBMC samples obtained from healthy donors and patients with multiple sclerosis respectively. The levels of caspase-1 mRNA ranged between 0.007 and 3.54 AU (mean (SD) 0.38 (0.92); median 0.027) in healthy donors and between 0.17 and 88.03 AU in patients with multiple sclerosis (mean (SD) 11.98 (16.83); median 6.30) ($p < 0.001$ *v* controls; Mann-Whitney). In multiple sclerosis, mean caspase-1 PBMC mRNA levels varied among patients and within the same patient: patient 1=5.55 (SD 4.03) (median 4.07; range 0.85–13.62); patient 2=9.61 (SD 12.16) (median 5.19; range 0.24–49.81); patient 3=8.06 (SD 4.69) (median 7.69; range 1.27–21.46); patient 4=7.49 (SD 8.17) (median 5.58; range 1.01–40.65); patient 5=2.97 (SD 5.81) (median 0.83; range 0.16–26.67); patient 6=13.80 (SD 16.45) (median 6.18; range 0.41–72.13); patient 7=32.05 (SD 26.79) (median 22.20; range 0.83–88.03).

Patients experienced 17 clinical attacks during the study (patients 1 and 7, three attacks; patient 2, one attack; patient 3, four attacks; patients 4, 5, and 6, two attacks) (mean annual exacerbation rate 2.4). To assess the relation between multiple sclerosis attacks and caspase-1 mRNA levels we analysed only samples obtained during the 4 weeks (-4w, -3w, -2w, -1w) preceding and the 2 weeks (+1w, +2w) after the attacks. During this time interval we obtained 42 PBMC samples at different time points (fig 1, A). The mean (SE) AU of caspase-1 mRNA registered during the -1w (10 samples) was 22.34 (7.44); this level was almost threefold higher than those registered during the -2w (four samples; mean 6.58 (1.44)), -3w (seven samples; mean 6.58 (2.21)), and -4w (six samples; mean 7.57 (3.04)) preceding the attacks ($p < 0.05$, Mann-Whitney). During the +1w (10 samples; mean 4.91 (1.43)) and +2w (five samples; mean 4.23 (0.61)) caspase-1 mRNA level progressively declined. To avoid biased results due to interpatient and inpatient variations of caspase-1 mRNA level, we further analysed the results obtained at weekly intervals by measuring increase of caspase-1 mRNA level over basal values (fig 1B). The basal value for each patient was the mean caspase-1 mRNA value registered in each patient during the whole follow up period. The induction over basal (mean (SE)) registered during -1w (1.41 (0.9)) was significantly higher than that recorded during -4w (0.49 (0.21); $p = 0.049$, Mann-Whitney), -3w (0.69 (0.18)), -2w (0.58 (0.38)). During +1w caspase-1 mRNA induction progressively

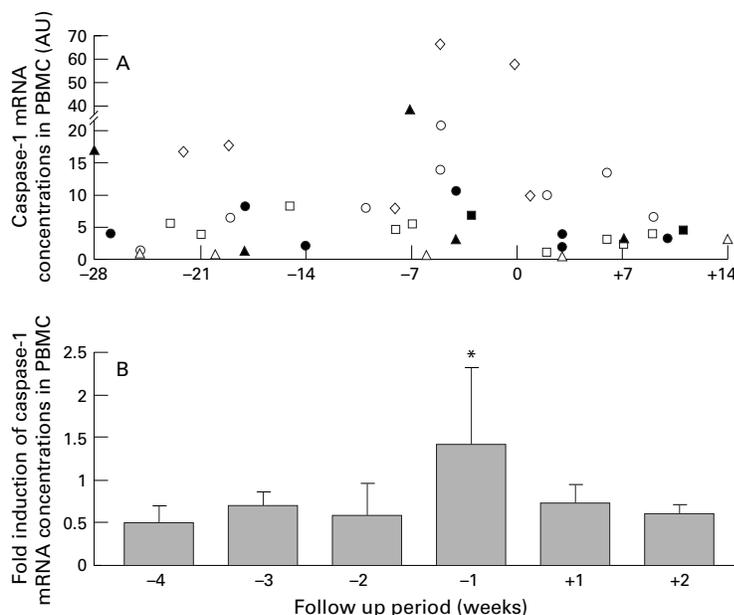


Figure 1 Relation between caspase-1 mRNA levels measured in PBMCs from patients with relapsing-remitting multiple sclerosis and clinically documented acute attacks. In panel A each dot represents a single measurement of PBMC caspase-1 mRNA levels (expressed as AU) and each symbol represents a patient with multiple sclerosis. In patient 1 (open squares) symbols are from three different attacks, in patient 2 (closed squares) from one attack, in patient 3 (open circles) from four attacks, in patient 4 (closed circles) from two attacks, in patient 5 (open triangles) from two attacks, in patient 6 (closed triangles) from two attacks, and in patient 7 (open diamonds) from three attacks (see results section for patients' numbering). Days of blood sampling preceding (-) or following (+) the acute attacks are on the x axis. The same symbol present twice at a given follow up day always represents blood sampling performed during two distinct acute attacks in the same patient (open and closed circles at days -5 and +3). Panel B shows the mean (SE) fold increase over basal of caspase-1 mRNA level measured at weekly intervals during each of the 4 weeks (-4w, -3w, -2w, -1w) preceding and the 2 weeks (+1w, +2w) after the 17 multiple sclerosis attacks. Increase of caspase-1 mRNA during the -1w was significantly higher ($p = 0.05$) compared with values registered during -4w.

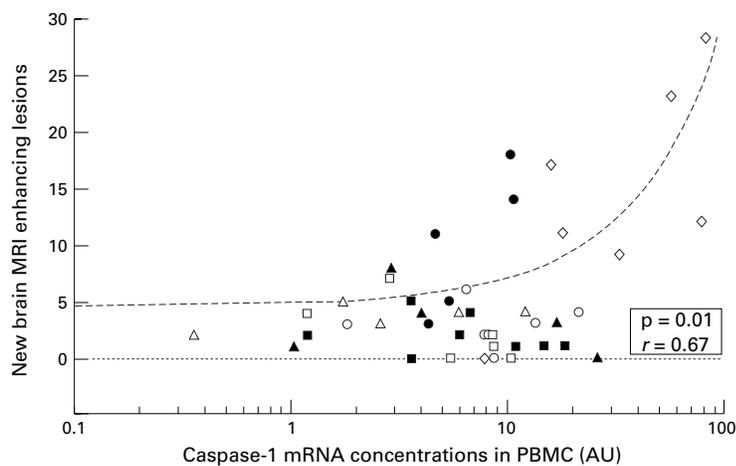


Figure 2 Relation between brain MRI enhancing lesions and caspase-1 mRNA levels measured in PBMCs from all patients with multiple sclerosis during the follow up period. The panel shows the significant correlation between new enhancing lesions and caspase-1 mRNA levels ($p=0.01$). Each dot represents a single scan and each symbol represents a single patient (the patients' symbols are the same as in fig 1). Scans performed during periods of steroid treatment have been excluded from the correlation analyses.

declined (0.73 (0.21)) and reached the levels registered at the beginning of the month preceding the attack in the +2w (0.60 (0.11), fig 1B).

During the study we obtained 75 enhanced brain MR scans. Four patients had 12 monthly scans, two patients had 11 monthly scans, and one patient had five monthly scans. A total of 459 enhancing lesions were found in 64 scans (326 lesions were new and 97 were persisting from at least the previous scan); no enhancing lesions were detected in the remaining scans. A total of 235 new enhancing and 66 persisting lesions registered in 43 scans from the patients with multiple sclerosis (ranges: 5–8 scans per patient, 0–28 new enhancing lesions per patient, 0–17 persisting lesions per patient) were eligible to be correlated with caspase-1 mRNA PBMC levels being done in follow up periods in which the patients were not under steroid treatment from at least 1 month. We found a significant relation between the number of new enhancing lesions per scan and caspase-1 PBMC mRNA levels ($r=0.67$; $p=0.01$, regression analysis, fig 2). However, when we correlated caspase-1 mRNA levels with the number of persisting lesions from the previous scans we found no correlation ($r=0.10$; $p=0.49$, regression analysis) (data not shown).

Discussion and conclusions

Caspase-1 cleaves IL-1 β and IL-18 precursor proteins into their secretory forms and it is therefore essential in the early steps of the inflammatory process.³ Due to the central part played by inflammation in multiple sclerosis and due to the presence of IL-1 β in blood derived macrophages surrounding the demyelinating plaques, it is conceivable to suggest that caspase-1 might also be involved in the blood derived CNS confined inflammatory process underlying lesion formation in multiple sclerosis.

We first serially measured caspase-1 transcription rate in PBMCs obtained from healthy

donors and patients with multiple sclerosis. Transcription rate of caspase-1 was strongly and significantly increased in PBMCs from patients with multiple sclerosis compared with healthy controls ($p<0.001$) despite the limitations due to the semiquantitative nature of the RT-PCR technique. We then analysed the fluctuations of the transcription rate of caspase-1 over time in patients with relapsing-remitting multiple sclerosis and correlated the values obtained with clinical and MRI evidence of disease activity. We purposely excluded data, obtained during the period of steroid treatment as caspase-1 transcription rate as well as the detection of gadolinium enhancing lesions are severely impaired by steroids.^{13 14} We found that caspase-1 mRNA levels (a) peak significantly ($p<0.05$) only in the week preceding an acute attack, (b) subside immediately after the beginning of an acute attack although steroid mediated down regulation of caspase-1 transcription rate cannot be excluded, and (c) correlate with the number of new but not persisting enhancing lesions ($p=0.01$). Although we cannot exclude the possibility that our results have been partially biased by a subset of the patients with multiple sclerosis analysed, there is enough overlap between clinical and MRI evidence to suggest that caspase-1 transcription rate is influenced by the cell mediated peripheral inflammatory events underlying the formation of CNS confined inflammatory lesions in multiple sclerosis. Because caspase-1 activity is mainly confined to monocyte/macrophage cells it is, however, tempting to speculate that the association we found might be due to the early peripheral inflammatory events activating demyelinating lesion forming blood derived macrophages. The involvement of caspase-1 in the early phase of inflammation but not in the perpetuation of the process is a well known phenomenon. The treatment of chemically induced peritonitis with caspase-1 pharmacological inhibitors fails when the treatment is started after the beginning of the inflammatory process.¹⁵ Moreover, we recently confirmed this finding in myelin-oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis.¹⁶

Studies performed in knock-out mice showed that caspase-1 regulates not only the cellular export of IL-1 β and IL-18 but also of IL-1 α , IL-6, and TNF α , although these three cytokines are not substrates for caspase-1.⁶ The measurement of one of these cytokines should therefore reflect caspase-1 measurement. We did not find any correlation between TNF α and caspase-1 mRNA levels in the weeks preceding and after an acute multiple sclerosis attack ($r=0.09$; $p=0.56$, regression analysis; data not shown). This apparent paradox is possibly explained by the fact that cytokines are usually “consumed” around the site of production/action (CNS, lymph nodes) and accumulate within the circulation only when overproduced or underconsumed. Thus cytokine detection in biological fluids in organ specific immune mediated diseases (for example,

multiple sclerosis) is “unpredictable” and “unuseful” as regards its use as a surrogate marker of disease activity.¹²

In conclusion, our data support a role for caspase-1 in the systemic inflammatory processes leading to CNS confined inflammatory lesions. Its role as a putative marker of disease activity can be therefore envisaged. Data on a larger series of patients with multiple sclerosis is, however, mandatory to confirm our findings as the great variability shown by caspase-1 mRNA levels could limit the clinical applicability of such measurement. Quantitative assays, such as those measuring caspase-1 at the protein level (ELISA), rather than the semiquantitative RT-PCR, could further help to better understand the role of this cysteine protease as a putative marker of acute multiple sclerosis.

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