Journal of Neuroimmunology 338 (2020) 577107

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Increased plasma levels of mitochondrial DNA and pro-inflammatory cytokines in patients with progressive multiple sclerosis

Milena Nasi[a,*,1], Elena Bianchini[a,1], Sara De Biasi[b], Lara Gibellini[b], Anita Neroni[b], Marco Mattioli[b], Marcello Pinti[c], Anna Iannone[a], Anna Vittoria Mattioli[a,d], Anna Maria Simone[e], Diana Ferraro[f,g], Francesca Vitetta[f], Patrizia Sola[f], Andrea Cossarizza[b,d]

[a] Department of Surgery, Medicine, Dentistry and Morphological Sciences, University of Modena and Reggio Emilia, via Campi, 287, 41125 Modena, Italy
[b] Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, via Campi, 287, 41125 Modena, Italy
[c] Department of Life Sciences, University of Modena and Reggio Emilia, via Campi, 287, 41125 Modena, Italy
[d] National Institute of Cardiovascular Research, via Irnerio, 48, 40126 Bologna, Italy
[e] Neurology Unit, Carpi Hospital, AUSL Modena, via Molinari, 2, 41012, Carpi (MO), Italy
[f] Department of Neurosciences, Azienda Ospedaliero-Universitaria di Modena Ospedale Civile di Baggiovara, via Giardini, 1355, 41126 Baggiovara (MO), Italy
[g] Department of Biomedical, Metabolic and Neurosciences, University of Modena and Reggio Emilia, via Campi, 287, 41125 Modena, Italy

ARTICLE INFO

Keywords:
Progressive multiple sclerosis
Damage-associated molecular patterns
Mitochondrial DNA
High mobility group box protein 1
Pro-inflammatory cytokines

ABSTRACT

The role of damage-associated molecular patterns in multiple sclerosis (MS) is under investigation. Here, we studied the contribution of circulating high mobility group box protein 1 (HMGB1) and mitochondrial DNA (mtDNA) to neuroinflammation in progressive MS. We measured plasmatic mtDNA, HMGB1 and pro-inflammatory cytokines in 38 secondary progressive (SP) patients, 35 primary progressive (PP) patients and 42 controls. Free mtDNA was higher in SP than PP. Pro-inflammatory cytokines were increased in progressive patients. In PP, tumor necrosis factor-α correlated with MS Severity Score. Thus, in progressive patients, plasmatic mtDNA and pro-inflammatory cytokines likely contribute to the systemic inflammatory status.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease affecting the central nervous system (CNS), featured by a heterogeneous clinical course (Lublin et al., 2014). In MS, inflammation is an important component of neurodegeneration, and the mechanisms driving disease development deeply involve both innate and adaptive immune system (Dendrou et al., 2015; Mayo et al., 2012), as well as innate-like T lymphocytes (Bianchini et al., 2017; De Biasi et al., 2016). However, the stimulus (or stimuli) and mechanisms that trigger and maintain inflammatory processes are still a matter of debate. Increasing evidence exists for a role of molecules containing the so-called damage-associated molecular patterns (DAMPs). These molecules are normally present inside all cells and, when released into the extracellular milieu by damaged or dead/dying cells, they are able to elicit a sterile pro-inflammatory response by activating the innate immune system (Krysko et al., 2013).

Pioneering observations reported that high mobility group box protein 1 (HMGB1, a non-histone DNA-binding protein acting as transcriptional regulator and as nucleosome stabilizer) plays a role either in MS or in experimental autoimmune encephalomyelitis (Andersson et al., 2008). Recent studies have also suggested putative associations between DAMPs arising from mitochondrial (mt) components, particularly circulating cell-free mtDNA, and MS progression (Leurs et al., 2018; Lowes et al., 2019). Such DAMPs could induce the production of cytokines and other pro-inflammatory mediators that trigger and maintain the inflammatory environment, leading to a dysregulated and imbalanced cytokine network, well described in MS patients (Kallaur et al., 2017; Khaibullin et al., 2017).

In recent years, the identification of molecules and mechanisms

Abbreviations: MS, multiple sclerosis; CNS, central nervous system; DAMPs, damage-associated molecular patterns; HMGB1, high mobility group box protein 1; mt, mitochondrial; TNF-α, tumor necrosis factor-α; IL, interleukin; IFN-γ, interferon-γ; SP, secondary progressive; PP, primary progressive; MSSS, Multiple Sclerosis Severity Score; EDSS, Kurztke's Expanded Disability Status Scale; CTR, controls; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; CSF, cerebrospinal fluid; RR, relapsing-remitting

* Corresponding author.
E-mail address: milena.nasi@unimore.it (M. Nasi).
[1] Milena Nasi and Elena Bianchini contributed equally to the study.

https://doi.org/10.1016/j.jneuroim.2019.577107
Received 3 October 2019; Received in revised form 24 October 2019; Accepted 4 November 2019
0165-5728/ © 2019 Published by Elsevier B.V.

contributing to the establishment and maintenance of inflammatory processes in MS has advanced, but studies concerning and correlating DAMPs like HMGB1 or free mtDNA and cytokine levels (as a marker of pro-inflammatory status) in MS patients are limited, and have often yielded discrepant results. Thus, we investigated the contribution of circulating HMGB1 and mtDNA, along with main pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8 and interferon (IFN)-γ to inflammation and neurodegeneration in the progressive forms of MS. To address this issue, we evaluated HMGB1, free mtDNA and pro-inflammatory cytokines in plasma samples from patients affected by primary or secondary progressive forms of MS, and assessed their correlation with disease progression.

2. Subjects and methods

2.1. Study subjects

Patients were recruited at the MS Center of the Neurology Clinic of the University Hospital-NOCSAE in Modena during their planned routine visits, in the framework of a recently published clinical study (De Biasi et al., 2016; De Biasi et al., 2019). All subjects gave written informed consent in agreement with the Declaration of Helsinki. The protocol of this study was approved by the Ethical Committee of Modena (Prot. 2483/CE).

We included all patients aged between 18 and 75 years old, and diagnosed with MS according to the 2010 revised McDonald criteria (Polman et al., 2011). Patients with concomitant infections (viral or bacterial) and with non-steroidal anti-inflammatory drug intake within the preceding 48 h were excluded. Patients treated with pulsed steroids within the last month, those on chronic corticosteroid or immunosuppressant therapy, and patients treated with immunomodulatory or immunosuppressant therapy in the previous 6 months were excluded as well.

We enrolled a total of 73 untreated, progressive MS patients: 38 with secondary progressive (SP) form and 35 with primary progressive (PP) form, all without clinical activity/superimposed relapses in the preceding 2 years. For each patient, the following parameters were recorded: demographic (sex, age) and clinical [disease duration, MS Severity Score (MSSS) and Kurztke's Expanded Disability Status Scale (EDSS)]. Forty-two healthy subjects, without a history of autoimmune diseases and immunosuppressant or corticosteroid therapy, were chosen as controls (CTR).

Table 1 summarizes the characteristics of MS patients and CTR enrolled in the study. SP patients had higher EDSS ($p = .016$; Mann-Whitney test) and disease duration ($p = .003$; Mann-Whitney test).

Venous blood was collected from each subject into EDTA tubes. Then, plasma was separated from blood cells according to standard procedures and stored at −80 °C until use.

Table 1
Demographical and clinical characteristics of progressive MS patients and CTR.

	CTR	SP	PP
Number	42	38	35
Age (years)*	53.00 ± 1.56	58.55 ± 1.11	58.70 ± 1.10
Gender (M/F)	18/24	13/25	12/23
Disease duration (months)*		291.30 ± 17.81	205.20 ± 19.10
MSSS*		6.33 ± 0.33	6.00 ± 0.39
EDSS*		6.47 ± 0.17	5.43 ± 0.31

Data are given as absolute numbers or as mean ± standard error of the mean (*).
CTR: healthy controls; SP: secondary progressive patients; PP: primary progressive patients; M/F: Male/Female; MSSS: Multiple Sclerosis Severity Score; EDSS: Expanded Disability Status Scale.

2.2. Quantification of circulating mtDNA

Plasma content of cell-free mtDNA was measured by quantitative real-time PCR as previously described (Nasi et al., 2016; Pinti et al., 2014). PCR was performed using CFX96 Touch (BioRad, Hercules, CA, USA); all analyses were performed in triplicate.

2.3. Analysis of HMGB1 and cytokine plasma levels

We quantified plasma levels of TNF-α, IL-1β, IL-6, IL-8 and IFN-γ by Human HS Cytokine A Custom Premix 5 Plex Magnetic Luminex Performance Assay (R&D, Minneapolis, MN, CAN); HMGB1 was measured by Human HMGB1 ELISA Kit (Fine Test, Zone, Wuhan, China).

2.4. Statistical analysis

Quantitative variables between groups were compared by Mann-Whitney test. An explorative Spearman correlation test and a subsequent linear regression analysis were performed to investigate possible correlations between demographic/clinical parameters and experimental data. A p value $< .05$ was considered statistically significant. Data shown in graphs are represented as the mean ± SEM. Statistical analyses were performed using Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA).

2.5. Protein interaction network analysis

Interactions between cytokines selected among those differently expressed between MS patients and CTR were analyzed by the bioinformatics tool Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, version 11.0), using high confidence (score 0.7) (Franceschini et al., 2013).

3. Results

3.1. Plasmatic mtDNA is higher in SP patients compared to PP patients

We could measure circulating mtDNA in plasma samples from 26 PP patients, 34 SP patients and 30 CTR, comparable for age ($p > .05$). As shown in Fig. 1A, free mtDNA was significantly higher in SP patients than PP patients ($p = .0040$), and also higher than CTR.

3.2. Pro-inflammatory cytokine levels are increased in plasma from progressive MS patients

The quantification of HMGB1 and pro-inflammatory cytokines was performed on 25 PP patients, 25 SP patients and 30 CTR. No significant differences were found in plasmatic HMGB1 between CTR and MS patients or between SP and PP patients, which displayed similar levels (CTR = 1175.45 ± 84.55 pg/mL, SP = 1177.78 ± 108.54 pg/mL, PP = 1077.06 ± 81.34 pg/mL; data not shown). In PP, plasma concentrations of TNF-α and IL-8 were higher compared to CTR or compared to SP and CTR, respectively ($p = .0229$, $p = .0073$ and $p = .0195$; Fig. 1B and F), while all progressive MS patients had higher levels of IL-6 and IL-1β than CTR (SP: $p = .0038$ and $p = .0002$, PP: $p = .0471$ and $p = .0240$; Fig. 1C and D). Despite not reaching statistically significant differences, IFN-γ levels showed trends towards increased expression in PP patients than in SP patients and CTR, as well as in SP patients than CTR (Fig. 1E).

STRING analysis revealed that the cytokines up-regulated in MS plasma samples were strongly connected, and highlighted the relevance of TNF-α, positioned in the center of the cluster (Fig. 2A).

3.3. Correlations among plasma and clinical parameters

When correlating experimental data with demographic/clinical

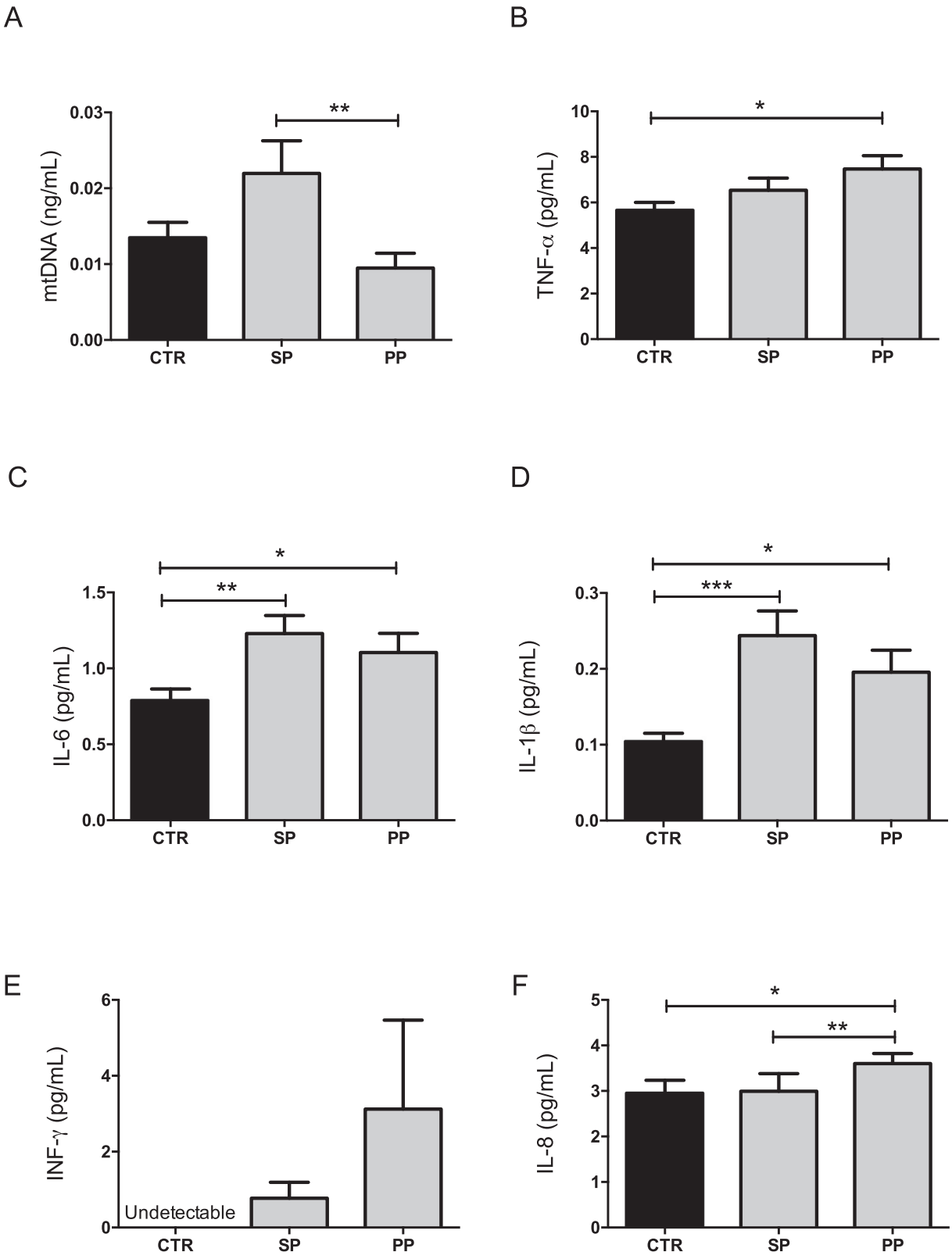

Fig. 1. Plasma levels of free mtDNA and pro-inflammatory cytokines in progressive MS patients and CTR.
(A) Quantification of cell-free mtDNA in plasma from SP MS ($N = 34$), PP MS ($N = 26$) and CTR ($N = 30$). (B) TNF-α, (C) IL-6, (D) IL-1β, (E) IFN-γ and (F) IL-8 concentrations in plasma from SP MS ($N = 25$), PP MS (N = 25) and CTR (N = 30).
CTR: healthy controls; SP: secondary progressive patients; PP: primary progressive patients. *$p < .05$; **$p < .01$; ***$p < .001$.

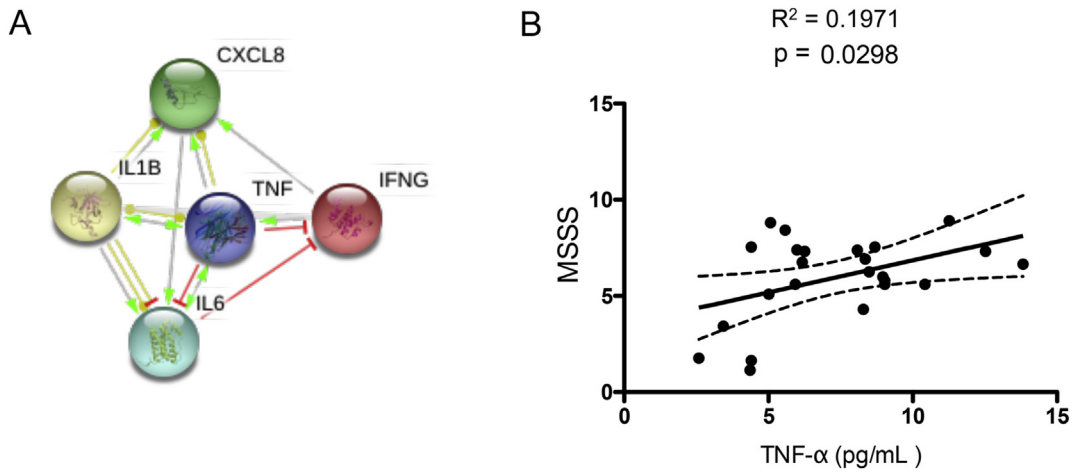

Fig. 2. STRING analysis and correlations between experimental and clinical parameters.
(A) Plasma STRING analysis for the interactions between TNF-α, IL-6, IL-1β, IFN-γ and IL-8: green = activation; red = inhibition; yellow = transcriptional regulation.
(B) Scatterplot and linear regression analysis between plasma levels of TNF-α and MSSS in PP MS (N = 25).
MSSS: Multiple Sclerosis Severity Score. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

parameters, we observed that in PP plasma levels of TNF-α directly correlated with MSSS (p = .0298, R^2 = 0.1971; Fig. 2B).

4. Discussion

Understanding the molecules and mechanisms triggering and maintaining inflammatory processes in MS is crucial for the identification of new, suitable therapeutic targets. DAMPs such as circulating HMGB1 and mtDNA are receiving increasing attention as molecules able to elicit sterile inflammation in several conditions characterized by a chronic inflammatory status (Cossarizza et al., 2011; Pinti et al., 2014; Pinti et al., 2012). Here, we observed that plasmatic mtDNA was higher in SP than PP, while HMGB1 levels did not vary between progressive MS patients and CTR or between SP and PP patients.

Recent studies have associated decreased levels of circulating cell-free mtDNA in the cerebrospinal fluid (CSF) from patients afflicted by Alzheimer's and Parkinson's disease with neurodegeneration (Podlesniy et al., 2013; Pyle et al., 2015). Similarly, reduced concentrations of circulating mtDNA were detected in the CSF of end-stage progressive MS with evidence of neurodegeneration (Lowes et al., 2019). Conversely, a significantly higher level of CSF cell-free mtDNA was found in relapsing-remitting (RR) MS patients, as well as an inverse correlation between circulating mtDNA concentration and time since disease onset, suggesting that free mtDNA level may reflect early, active inflammatory activity (Varhaug et al., 2017).

We are well aware that variations in plasmatic mtDNA can be due to the release of DAMPs from different organs and cell types. However, although CSF better mirrors the inflammatory processes occurring inside the CNS, plasma concentrations of cell-free mtDNA likely indicate the general degree of inflammatory status. Thus, our findings suggest that free mtDNA released in plasma could contribute to the systemic inflammatory status of patients, and that SP form is characterized by a higher inflammatory milieu. In particular, the observation that circulating mtDNA levels did not show statistically significant differences between PP patients and CTR, but were increased in SP patients compared to PP patients could indicate that the implication of free mtDNA in inflammation is secondary and associated to a chronic stage of the disease where cell shrinkage may play a role, possibly peculiar of SP form. In any case, this finding could suggest that a difference exists between the two progressive forms of MS, even though these two phenotypes encompass the same disease spectrum and are considered clinically overlapping (Ontaneda et al., 2017).

Hence, plasmatic mtDNA could be the secondary and unspecific result of cell shrinkage associated to disease. Another possibility is that free mtDNA could come from nervous tissue mt damage. Alternatively, taking into account the previously unrecognized observation that peripheral blood leukocytes are able to rapidly eject interferogenic mtDNA as web filament structures upon appropriate stimulation (Ingelsson et al., 2018), we hypothesize that mtDNA webs could be the source of the increased circulating mtDNA, but this speculation needs further deepening.

As for HMGB1, other investigators variably reported increased (Malhotra et al., 2015) or unchanged (Sternberg et al., 2016) serum levels in MS patients compared to healthy subjects. In particular, Malhotra et al. observed the highest HMGB1 serum levels in RR MS when stratifying patients for clinical forms, and reported an increased HMGB1 serum concentration in SP than CTR, with a similar trend in PP (Malhotra et al., 2015). However, the discrepancies with our findings could be attributed to the different and smaller cohort of patients enrolled in that study.

We also found that in PP plasma levels of TNF-α and IL-8 were higher compared to CTR or compared to both SP and CTR, respectively, while all progressive MS patients had increased concentrations of IL-6 and IL-1β. In addition, similarly to TNF-α and IL-8 patterns, IFN-γ levels showed trends towards increased expression in PP patients than in SP patients and CTR, as well as in SP patients than CTR, even if not reaching statistically significant differences. Several studies have investigated the levels of many cytokines in CSF, serum and plasma samples from MS patients with different forms and treatments of the disease (Kallaur et al., 2017; Khaibullin et al., 2017; Martins et al., 2011), often yielding conflicting results, but overall demonstrating a marked increase of pro-inflammatory molecules in MS patients. Of note, most of the previous reports investigated the RR form of MS or comprised in the progressive group both SP and PP patients. Our results further highlight the imbalanced cytokine network that exists in progressive MS patients and that likely modulates disease activity and progression, and suggest the possibility to develop agents targeting cytokines or their receptors as novel therapies for MS. As expected because of the common inflammatory nature shared by the mediators investigated and their well-known relationships, STRING analysis confirmed the existence of strong interactions among the cytokines differently expressed by SP and PP, accounting, at least in part, for the alterations we observed in their cytokine profile.

Statistically significant correlations between cytokine levels and

demographic/clinical parameters were found between plasma levels of TNF-α and MSSS in PP. An association between the CSF level of TNF-α and disease severity and progression in chronic progressive MS patients has been known since 1991, supporting the role for this cytokine in the progression of MS (Sharief and Hentges, 1991). Interestingly, the STRING analysis highlights the relevance of TNF-α, positioned in the center of the connections with the up-regulated cytokines, even if this analysis took into account a low number of molecules. This correlation was present only in PP patients, shedding light again on the differences concerning some inflammatory components in the SP and PP forms, probably due to the different disease course that leads to that endpoint.

Overall, our study has some limitations, including limited DAMP testing, poor cytokine profiling, scarce correlation with *in vivo* cell or humoral immunological response of patients, and poor testing of Toll-like receptor 9 activation. However, our data indicate that in progressive MS increased plasma levels of free mtDNA, in synergy with pro-inflammatory cytokines, likely contribute to the systemic inflammatory status of these patients, though the inflammatory component could be differently activated in the two progressive forms of the disease.

Acknowledgements

We gratefully thank all patients who donated their blood for this study. Elena Bianchini is a 2019 post-doctoral fellow of Fondazione Umberto Veronesi.

Funding

This work was supported by the Italian Multiple Sclerosis Foundation (FISM) [grant “Mitochondrial DAMPs in Multiple Sclerosis: a pilot study” to AC, code 2017/R/10]. Blood was collected during the FISM projects 2014/R/16 and 2016/R/20 to MP.

Declaration of Competing Interest

None.

References

Andersson, A., Covacu, R., Sunnemark, D., Danilov, A.I., Dal Bianco, A., Khademi, M., Wallström, E., Lobell, A., Brundin, L., Lassmann, H., Harris, R.A., 2008. Pivotal advance: HMGB1 expression in active lesions of human and experimental multiple sclerosis. J. Leukoc. Biol. 84, 1248–1255.

Bianchini, E., De Biasi, S., Simone, A.M., Ferraro, D., Sola, P., Cossarizza, A., Pinti, M., 2017. Invariant natural killer T cells and mucosal-associated invariant T cells in multiple sclerosis. Immunol. Lett. 183, 1–7.

Cossarizza, A., Pinti, M., Nasi, M., Gibellini, L., Manzini, S., Roat, E., De Biasi, S., Bertoncelli, L., Montagna, J.P., Bisi, L., Manzini, L., Trenti, T., Borghi, V., Mussini, C., 2011. Increased plasma levels of extracellular mitochondrial DNA during HIV infection: a new role for mitochondrial damage-associated molecular patterns during inflammation. Mitochondrion 11, 750–755.

De Biasi, S., Simone, A.M., Nasi, M., Bianchini, E., Ferraro, D., Vitetta, F., Gibellini, L., Pinti, M., Del Giovane, C., Sola, P., Cossarizza, A., 2016. iNKT cells in secondary progressive multiple sclerosis patients display pro-inflammatory profiles. Front. Immunol. 7, 555.

De Biasi, S., Simone, A.M., Bianchini, E., Lo Tartaro, D., Pecorini, S., Nasi, M., Patergnani, S., Carnevale, G., Gibellini, L., Ferraro, D., Vitetta, F., Pinton, P., Sola, P., Cossarizza, A., Pinti, M., 2019. Mitochondrial functionality and metabolism in T cells from progressive multiple sclerosis patients. Eur. J. Immunol. https://doi.org/10.1002/eji.201948223.

Dendrou, C.A., Fugger, L., Friese, M.A., 2015. Immunopathology of multiple sclerosis. Nat. Rev. Immunol. 15, 545–558.

Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., Lin, J., Minguez, P., Bork, P., von Mering, C., Jensen, L.J., 2013. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 41, D808–D815.

Ingelsson, B., Soderberg, D., Strid, T., Soderberg, A., Bergh, A.C., Loitto, V., Lotfi, K., Segelmark, M., Spyrou, G., Rosén, A., 2018. Lymphocytes eject interferogenic mitochondrial DNA webs in response to CpG and non-CpG oligodeoxynucleotides of class C. Proc. Natl. Acad. Sci. U. S. A. 115, E478–E487.

Kallaur, A.P., Oliveira, S.R., Simao, A.N.C., Alfieri, D.F., Flauzino, T., Lopes, J., de Carvalho Jennings Pereira, W.L., de Meleck Proença, C., Borelli, S.D., Kalmen-Maciel, D.R., Maes, M., Reiche, E.M.V., 2017. Cytokine profile in patients with progressive multiple sclerosis and its association with disease progression and disability. Mol. Neurobiol. 54, 2950–2960.

Khaibullin, T., Ivanova, V., Martynova, E., Cherepnev, G., Khabirov, F., Granatov, E., Rizvanov, A., Khaiboullina, S., 2017. Elevated levels of proinflammatory cytokines in cerebrospinal fluid of multiple sclerosis patients. Front Immunol. 8, 531.

Krysko, O., Love Aaes, T., Bachert, C., Vandenabeele, P., Krysko, D.V., 2013. Many faces of DAMPs in cancer therapy. Cell Death Dis. 4, e631.

Leurs, C.E., Podlesniy, P., Trullas, R., Balk, L., Steenwijk, M.D., Malekzadeh, A., Piehl, F., Uitdehaag, B.M., Killestein, J., Van Horssen, J., Teunissen, C.E., 2018. Cerebrospinal fluid mtDNA concentration is elevated in multiple sclerosis disease and responds to treatment. Mult. Scler. 24, 472–480.

Lowes, H., Pyle, A., Duddy, M., Hudson, G., 2019. Cell-free mitochondrial DNA in progressive multiple sclerosis. Mitochondrion 46, 307–312.

Lublin, F.D., Reingold, S.C., Cohen, J.A., Cutter, G.R., Sorensen, P.S., Thompson, A.J., Wolinsky, J.S., Balcer, L.J., Banwell, B., Barkhof, F., Bebo, B., Calabresi, P.A., Clanet, M., Comi, G., Fox, R.J., Freedman, M.S., Goodman, A.D., Inglese, M., Kappos, L., Kieseier, B.C., Lincoln, J.A., Lubetzki, C., Miller, A.E., Montalban, X., O'Connor, P.W., Petkau, J., Pozzilli, C., Rudick, R.A., Sormani, M.P., Stüve, O., Waubant, E., Polman, C.H., 2014. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 83, 278–286.

Malhotra, S., Fissolo, N., Tintore, M., Wing, A.C., Castillo, J., Vidal-Jordana, A., Montalban, X., Comabella, M., 2015. Role of high mobility group box protein 1 (HMGB1) in peripheral blood from patients with multiple sclerosis. J. Neuroinflammation 12, 48.

Martins, T.B., Rose, J.W., Jaskowski, T.D., Wilson, A.R., Husebye, D., Seraj, H.S., Hill, H.R., 2011. Analysis of proinflammatory and anti-inflammatory cytokine serum concentrations in patients with multiple sclerosis by using a multiplexed immunoassay. Am. J. Clin. Pathol. 136, 696–704.

Mayo, L., Quintana, F.J., Weiner, H.L., 2012. The innate immune system in demyelinating disease. Immunol. Rev. 248, 170–187.

Nasi, M., Cristani, A., Pinti, M., Lamberti, I., Gibellini, L., De Biasi, S., Guazzaloca, A., Trenti, T., Cossarizza, A., 2016. Decreased circulating mtDNA levels in professional male volleyball players. Int. J. Sports Physiol. Perform. 11, 116–121.

Ontaneda, D., Thompson, A.J., Fox, R.J., Cohen, J.A., 2017. Progressive multiple sclerosis: prospects for disease therapy, repair, and restoration of function. Lancet 389, 1357–1366.

Pinti, M., Mussini, C., Cossarizza, A., 2012. Mitochondrial DNA: a proinflammatory 'enemy from within' during HIV infection? Cell Death Dis. 3, 307.

Pinti, M., Cevenini, E., Nasi, M., De Biasi, S., Salvioli, S., Monti, D., Benatti, S., Gibellini, L., Cotichini, R., Stazi, M.A., Trenti, T., Franceschi, C., Cossarizza, A., 2014. Circulating mitochondrial DNA increases with age and is a familiar trait: implications for “inflamm-aging”. Eur. J. Immunol. 44, 1552–1562.

Podlesniy, P., Figueiro-Silva, J., Llado, A., Antonell, A., Sanchez-Valle, R., Alcolea, D., Lleo, A., Molinuevo, J.L., Serra, N., Trullas, R., 2013. Low cerebrospinal fluid concentration of mitochondrial DNA in preclinical Alzheimer disease. Ann. Neurol. 74, 655–668.

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., Fujhara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F.D., Montalban, X., O'Connor, P., Sandberg-Wollheim, M., Thompson, A.J., Waubant, E., Weinshenker, B., Wolinsky, J.S., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann. Neurol. 69, 292–302.

Pyle, A., Brennan, R., Kurzawa-Akanbi, M., Yarnall, A., Thouin, A., Mollenhauer, B., Burn, D., Chinnery, P.F., Hudson, G., 2015. Reduced cerebrospinal fluid mitochondrial DNA is a biomarker for early-stage Parkinson’s disease. Ann. Neurol. 78, 1000–1004.

Sharief, M.K., Hentges, R., 1991. Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. N. Engl. J. Med. 325, 467–472.

Sternberg, Z., Sternberg, D., Chichelli, T., Drake, A., Patel, N., Kolb, C., Chadha, K., Yu, J., Hojnacki, D., 2016. High-mobility group box 1 in multiple sclerosis. Immunol. Res. 64, 385–391.

Varhaug, K.N., Vedeler, C.A., Myhr, K.M., Aarseth, J.H., Tzoulis, C., Bindoff, L.A., 2017. Increased levels of cell-free mitochondrial DNA in the cerebrospinal fluid of patients with multiple sclerosis. Mitochondrion 34, 32–35.