PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
ESCOLA DE CIÊNCIAS DA SAÚDE E DA VIDA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

PEDRO LUIS DA SILVA

CARACTERIZAÇÃO DA IMUNOSSENESCÊNCIA NA ESCLEROSE MÚLTIPLA REMITENTE-RECORRENTE

Porto Alegre
2023

PEDRO LUIS DA SILVA

CARACTERIZAÇÃO DA IMUNOSSENESCÊNCIA NA ESCLEROSE MÚLTIPLA REMITENTE-RECORRENTE

Dissertação apresentada como requisito para obtenção do grau de Mestre pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Escola de Ciências da Saúde e da Vida da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientador

Prof. Dr. Moises Evandro Bauer

Porto Alegre

2023

Ficha Catalográfica

S586c Silva, Pedro Luis da

Caracterização da imunossenescência na esclerose múltipla remitente-recorrente / Pedro Luis da Silva. – 2023.
55f.
Dissertação (Mestrado) – Programa de Pós-Graduação em Biologia Celular e Molecular, PUCRS.

Orientador: Prof. Dr. Moises Evandro Bauer.

1. Esclerose Múltipla. 2. Iinflamação. 3. Imunosenescência. 4. Células T. I. Bauer, Moises Evandro. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da PUCRS
com os dados fornecidos pelo(a) autor(a).
Bibliotecária responsável: Clarissa Jesinska Selbach CRB-10/2051

PEDRO LUIS DA SILVA

CARACTERIZAÇÃO DA IMUNOSSENESCÊNCIA NA ESCLEROSE MÚLTIPLA REMITENTE-RECORRENTE

Dissertação apresentada como requisito para obtenção do grau de Mestre pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Escola de Ciências da Saúde e da Vida da Pontifícia Universidade Católica do Rio Grande do Sul.

Aprovada em: _____de_____de_____.

BANCA EXAMINADORA:

_____

_____

_____

Porto Alegre

2023

DEDICATÓRIA

A meus pais pelo constante apoio e amor.

Agradecimentos

Agradeço a minha mãe Maria Geneci da Silva por toda a ajuda.

Agradeço ao Professor Dr. Moises Evandro Bauer por ter me aceito com toda dedicação no seu grupo de orientandos. Além disso, agradeço as conversas e orientação de qual caminho seguir dentro da carreira profissional.

E agradeço ao meu colega Técnico Rodrigo Dorneles que sem dúvidas contribuíram para a finalização deste trabalho.

Agradeço a todos os demais colegas do laboratório, pessoas incríveis que tornaram essa jornada de dois anos mais feliz.

Resumo

A esclerose múltipla (EM) é uma doença inflamatória crônica e desmielinizante do sistema nervoso central. A fisiopatologia da EM resulta de interações complexas entre células T autorreativas e células B que levam a inflamação e desmielinização no sistema nervoso central (SNC). As taxas de envelhecimento (ou trajetórias) podem influenciar o adoecimento e mortalidade dos indivíduos. Indivíduos que envelhecem mais rapidamente, como visto na EM, apresentam um envelhecimento do sistema imune (imunossenescência) que pode comprometer a progressão clínica da doença. Um estado de inflamação crônica de baixo grau, que tem sido referido como inflammaging, também pode influenciar quadros clínicos na EM. Nesse trabalho, nós caracterizamos a imunossenescência na EM remitente-recorrente (EMRR) e investigamos sua associação com a progressão clínica. Vinte e dois pacientes com EMRR foram recrutados para avaliação no Serviço de Neurologia do Hospital São Lucas da PUCRS. Foi realizada uma análise abrangente de imunofenotipagem por citometria de fluxo para investigar subgrupos de linfócitos e monócitos, incluindo seu estado de ativação, fase de diferenciação, perfis de regulação, senescência e exaustão. As citocinas envolvidas com inflammaging (IL-1β, IL-8, IL-6 e TNF-α) ou regulação (IL-10) foram quantificadas no soro. O comprimento dos telômeros foi medido por PCR em tempo real, como marcador do envelhecimento biológico. Um total de 21 controles saudáveis e 25 pacientes foram recrutados e emparelhados por idade e sexo. Os pacientes com EMRR tinham linfócitos telômeros menores que os controles. Na avaliação de citocinas, não houve diferença entre os grupos. Os pacientes apresentaram proporções mais baixas de monócitos não clássicos (CD16brightCD14+) e clássicos (CD16-CD14bright), embora proporções aumentadas de monócitos intermediários (CD16+CD14bright) em comparação aos controles (todos $p<0,05$). Os subconjuntos NK (CD16+CD56dim e CD16lowCD56bright) não diferiram entre os indivíduos. Menores proporções de células B transicionais (fenótipo) e células B reguladoras (fenótipo) foram observadas em RMSS (todos $p<0,05$). Os pacientes apresentaram proporções menores de células T diferenciadas precocemente (CD27+CD28+), mas nenhuma alteração nas células intermediárias (CD27-CD28+) ou diferenciadas tardiamente ou senescentes (CD27-CD28-). Embora os pacientes apresentassem proporções significativamente menores de células T ativadas (CD25+) tanto nas células CD4+ quanto nas células CD8+ (todas $p<0,0001$),

não foram observadas alterações nas células T reguladoras (CD4+FoxP3+). Em relação aos checkpoints, os pacientes apresentaram proporções aumentadas de células CD4+PD-1+ em comparação aos controles ($p<0,01$), mas menores percentuais de células CD8+CTLA-4+ ($p<0,01$). Além disso, foram observadas proporções significativamente mais baixas de células T CD4+ e CD8+ expressando NKG2A ou NKG2D em pacientes (todos $p<0,05$). Embora a senescência precoce em RMSS tenha sido confirmada por telômeros encurtados e aumento de células T CD4+PD-1+, nenhuma alteração foi relatada em células T mais diferenciadas.

Palavras-chave: Esclerose Múltipla, inflamação, Imunosenescência e células T.

Abstract

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system. The pathophysiology of MS results from complex interactions between autoreactive T cells and B cells that lead to inflammation and demyelination in the central nervous system (CNS). Aging rates (or trajectories) can influence individuals' morbidity and mortality rates. Individuals aging more quickly, as seen in MS, have an aging immune system (immunosenescence) that may compromise the clinical progression of the disease. A state of chronic low-grade inflammation, which has been referred to as inflammaging, may also influence clinical pictures in MS. In this work, we further characterized immunosenescence in relapsing-remitting MS (RRMS) and investigated its association with clinical progression. Twenty-two patients with RRMS were recruited for evaluation at the Neurology Service of Hospital São Lucas at PUCRS. A comprehensive flow cytometric multicolor immunophenotyping analysis was performed to investigate subsets of lymphocytes and monocytes, including their activation state, differentiation states, regulatory, senescence, and exhaustion profiles. Cytokines involved in inflammation (IL-1beta, IL-8, IL-6 and TNF-alpha) or regulation (IL-10) were quantified in serum. Telomere length was measured by real-time PCR as a marker of biological aging. A total of 21 healthy controls and 25 patients were recruited and matched for age and sex. Patients with RRMS had shortened telomeres in mononuclear cells than controls. Concerning the cytokines, there were no differences between groups. Patients had lower proportions of non-classical (CD16brightCD14+) and classical (CD16-CD14bright) monocytes, although increased proportions of intermediate monocytes (CD16+CD14bright) compared to controls (all $p<0.05$). The NK subsets (CD16+CD56dim and CD16lowCD56bright) did not differ between individuals. Lower proportions of transitional B cells (CD19+ CD38HIGH) and regulatory B cells (CD19+CD27+CD38+) were observed in RMSS (all $p<0.05$). Patients had lower proportions of early-differentiated (CD27+CD28+) T cells, but no change in intermediate (CD27-CD28+) or late-differentiated or senescent (CD27-CD28-) cells. Although patients had significantly lower proportions of activated T cells (CD25+) in both CD4+ and CD8+ cells (all $p<0.0001$), no changes were observed in regulatory T cells (CD4+FoxP3+). Regarding checkpoint molecules, patients had increased proportions of CD4+PD-1+ cells compared to controls ($p<0.01$), but lower percentages of CD8+CTLA-4+ cells ($p<0.01$). In addition, significantly lower

proportions of CD4+ and CD8+ T cells expressing NKG2A or NKG2D were observed in patients (all $p<0.05$). Although early senescence in RMSS was confirmed by shortened telomeres and increased CD4+PD-1+ T cells, no changes were reported in more differentiated T cells.

Keywords: Multiple sclerosis, inflammation, immunosenescence and T cells.

Lista de abreviaturas

CBAs - Cytometric bead arrays

CIS - Síndrome clínica isolada

DMSO- Dimethyl sulfoxide

EDTA - ácido etilenodiamino

EM - Esclerose Múltipla

EMPP - Esclerose múltipla primariamente progressiva

EMRR - Esclerose múltipla remitente-recorrente

EMSP - Esclerose múltipla secundariamente progressiva

FBS - Soro fetal bovino

IFN – interferon

ILs – interleucinas

LCR- Líquido cefalorraquidiano

MS - Multiple Sclerosis

NK - Assassinas naturais

PBMCs - Células mononucleares do sangue periférico

PCR – Proteína C reativa

qPCR- Reação em cadeia da polimerase

SNC - Sistema nervoso central

TL - tamanho telomérico

TNF - Fator de necrose tumoral

TRECs - ciclos de excisão de células T

Tregs - Linfócitos T reguladores

SUMÁRIO

1. INTRODUÇÃO

1.1. Imunopatologia da Esclerose Multipla

A esclerose múltipla (EM) é uma doença crônica inflamatória e desmielinizante do sistema nervoso central (SNC). Estima-se que 2,5 milhões de pessoas em todo o mundo sofram dessa doença. No Brasil, a prevalência nacional não é conhecida. No entanto, alguns estudos regionais indicam que essa prevalência pode variar bastante entre as regiões equatoriais (1,36/100.000) e a região sudeste (18/100.000) (FILIPPI et al., 2018). A doença é mais comum entre as mulheres. Seus primeiros sintomas costumam ocorrer geralmente entre os 20 e 40 anos (COMPSTON; COLES, 2008). EM é uma causa potencial de deficiência física e cognitiva de longo prazo. Após 25 anos de diagnóstico inicial, aproximadamente 50% dos pacientes precisam de uso permanente de cadeiras de rodas (DENDROU; FUGGER; FRIESE, 2015)

A EM pode ser classificada em: CIS (síndrome clínica isolada), EM remitente-recorrente (EMRR), EM secundariamente progressiva (EMSP) e EM primariamente progressiva (EMPP)(LUBLIN et al., 2014). De acordo com estudos neuropatológicos, ocorrem lesões nas substâncias branca e cinzenta em todo o SNC, incluindo o cérebro, nervo ótico e medula espinhal. Do ponto de vista clínico, a EM pode apresentar-se de forma bastante heterogênea. Os sintomas neurológicos podem ser sensitivos (dormência, anestesia, dor), sensoriais (baixa acuidade visual), motores (fraqueza), cerebelares (ataxia), esfincterianos (retenção urinária e fecal), cognitivos (bradipsiquismo, depressão), refletindo principalmente a topografia do processo desmielinizante (LUBLIN et al., 2014)

A fisiopatologia da EM resulta de interações complexas entre células T autorreativas e células B. Essas células são ativadas na periferia e cruzam a barreira hematoencefálica para produzir inflamação e desmielinização no SNC. Os clones autorreativos de linfócitos T CD8+ e CD4+ são reativos a proteínas expressas na bainha de mielina (JACOBSEN et al., 2002; SIEWERT et al., 2012). Do ponto de vista histopatológico, as lesões EM são caracterizadas por danos na bainha de mielina, acompanhada de infiltração de fagócitos e linfócitos T e B, principalmente na substância branca do cérebro, mas também na substância cinzenta. Além disso, o processo de neurodegeneração e atrofia cerebral ocorre concomitantemente à

desmielinização focal, e sua relação com o processo inflamatório ainda não foi totalmente elucidada (DENDROU; FUGGER; FRIESE, 2015)

As células Th1 e Th17 são os principais subtipos de células T CD4+ envolvidas na fisiopatologia da EM. A primeira linha de intervenções, incluindo IFN-gama e acetato de glatirâmer, modifica o perfil inflamatório Th1/Th17 para um perfil mais Th2 (Dendrou et al., 2015). À medida que o compartimento celular T se torna cada vez mais clonal durante o envelhecimento, alguns estudos sugerem uma propensão para a autorreatividade (GREBENCIUCOVA; BERGER, 2017). As células T CD8+, as células B autorreativas e as células T reguladoras (Treg) disfuncionais também podem contribuir para EM, assim como algumas células da imunidade inata. Esse conjunto de células autorreativas, mediadores pró-inflamatórios (como as citocinas e quimiocinas) e falha de mecanismos imunorreguladores fornece um ambiente favorável para desmielinização, com danos em oligodendrócitos e neuroaxonais (DENDROU; FUGGER; FRIESE, 2015). Na EM, independentemente da fase e atividade da doença, as células T CD8+ são observadas em números elevados, muito superiores às células T CD4 (CARBAJAL et al., 2015). As células T CD8+ são encontradas em grande abundância nos tecidos do SNC e no líquido cefalorraquidiano na EM. As células T CD8+ presentes tanto em lesões agudas como crônicas e secretam níveis elevados de IL-17 (KWONG et al., 2017). Estas células são encontradas em grande abundância nos tecidos do SNC e LCR (KWONG et al., 2017)

Dentre os mecanismos estudados de tolerância periférica, encontramos as células T reguladoras (Tregs): um subtipo de células T CD4+ que mantêm a tolerância aos autoantígenos, regulam a modulação do sistema imune e impedem a autoimunidade (KOHM et al., 2002). As Tregs são caracterizadas principalmente como CD4+CD25+Foxp3+ e são anti-inflamatórias. Na EM a frequência das células Treg não difere das dos indivíduos saudáveis, embora aspectos funcionais estejam prejudicados (ex., maturação e migração) na EM (LUDWIN et al., 2016). A falha de mecanismos imunorregulatórios (ex., Treg) fornece um ambiente favorável para a desmielinização, dano de oligodendrócitos e dano neuroaxonal (NAGPAL et al., 2018).

As células B também contribuem para a patogênese da EM, secretando autoanticorpos e citocinas pró-inflamatórias resultando na presença de bandas oligoclonais no líquido cefalorraquidiano e no parênquima cerebral. Essas bandas

oligoclonais de IgG são observadas no líquor e constituem a alteração imunológica de maior utilidade para o diagnóstico da EM. A prevalência desta alteração em doentes afetados por EM varia em diferentes populações (DISANTO et al., 2012). Nas placas desmielinizantes da EM, os plasmócitos são notados em grande número (WEKERLE, 2017). Os autoanticorpos anti-MOG são uma marca distintiva da EM infantil, bem como em alguns doentes com desordem do espectro óptico da neuromielite. É evidente que a ativação anormal de células B no CNS de doentes com EM sugere que as células B desempenham um papel na fisiopatologia da doença (WINGER; ZAMVIL, 2016)

2. Imunossenescência e a Esclerose Multipla

O envelhecimento do sistema imune, ou imunossenescência, está associado com remodelamento de aspectos enumerativos e funcionais que contribuem para aumento da morbilidade e mortalidade. O processo de imunossenescência é evidenciado pelo aumento de ocorrência de infecções, falhas nas respostas vacinais e reativações virais crônicas (OSTOLAZA IBÁÑEZ; CORROZA LAVIÑETA; AYUSO BLANCO, 2022). A imunossenescência leva a um aumento na morbidade e mortalidade em adultos, pois o envelhecimento celular está atrelado a uma diminuição no número de Células T naive e B, células NK e ruptura do equilíbrio pró e anti-inflamatório por alterações na produção de citocinas (ZANGHÌ et al., 2022). A imunossenescência engloba alterações relacionadas com a idade na composição e na função das vias adaptativas e inatas do sistema imune, paralelamente com o aumento níveis circulatórios de mediadores pró-inflamatórios, tais como a IL-6, o TNF-α e a PCR. Isso engloba um estado de inflamação crônica de baixo grau, que tem sido referido como *inflammaging*, podendo exacerbar quadros clínicos na EM (ZUROFF et al., 2022). No tema que nos interessa, os pacientes com EM apresentam características de um sistema imunológico envelhecido precocemente, que não condiz com a idade cronológica, e possivelmente associado com a fisiopatologia e progressão clínica da doença.

O processo de envelhecimento também afeta as células T. A timopoiese é a principal força de geração de novas células T naive. No entanto, o envelhecimento reduz a produção e maturação de células T no timo. A involução tímica inicia-se na adolescência e, por volta dos 45 anos, a timopoiese é severamente limitada (PALMER et al., 2017). A função tímica e a EM também apresentam uma relação inversa: com o avanço da idade a capacidade tímica decai enquanto a probabilidade de desenvolver EM aumenta. Em indivíduos saudáveis, a quantidade de ciclos de excisão de células T (TRECs) decai exponencialmente com o avanço da idade; em contraste, pacientes com EMRR ou EMPP já apresentam uma redução precocemente das TRECs (DUSZCZYSZYN et al., 2010; HAEGERT, 2011), indicando involução tímica precoce.

A falha na produção de novas células T favorece um mecanismo compensatório de auto proliferação na circulação e órgãos linfoides secundários. À medida que o

compartimento celular T se torna cada vez mais clonal durante o envelhecimento, alguns estudos sugerem uma propensão para a autorreatividade (GREBENCIUCOVA; BERGER, 2017). O estresse replicativo, promovido pela ativação crônica do sistema imune, pode contribuir para a senescência celular na EM. A senescência celular, tanto do envelhecimento natural quanto na EM, é fundamentada em três características: (1) trocas funcionais e fenotípicas, (2) encurtamento dos telômeros e (3) produção de grandes quantidades de citocinas inflamatórias (GREBENCIUCOVA; BERGER, 2017)

As células T reativas aos auto-peptídeos são geralmente eliminadas no timo para evitar a autorreatividade. Para evitar a autoimunidade, numerosas vias do ponto de controle (*checkpoints*) regulam a ativação das células T, atenuando as respostas autorreativas. No foco deste processo estão as vias do ponto de controle imunológico do antígeno 4 associado aos linfócitos T citotóxicos (CTLA-4) e da morte programada 1 (PD-1) (BUCHBINDER; DESAI, 2016). Pensa-se que as vias dos receptores CTLA-4 e PD-1 funcionam em diferentes fases de uma resposta imune. O CTLA-4 inibe as células T potencialmente autorreativas na fase inicial da ativação das células T naive. A via PD-1 regula as células T previamente ativadas, com ênfase nos tecidos periféricos, e está associada com exaustão celular e senescência (BRUNNER-WEINZIERL; RUDD, 2018).

Células T senescentes secretam citocinas pró-inflamatórias, como TNF-α e IL-6, contribuindo para a inflamação crônica (inflammaging) (GORONZY; WEYAND, 2019). A migração das células imunes entre o sangue, liquor e tecidos linfoides (tráfego celular) é um processo fundamental para as respostas imunes efetoras. Com o envelhecimento, observa-se mudanças nos principais subtipos linfocitários, que inclui:

- Diminuição das células T naive (CD45RA+);
- Aumento nas células T de memória (CD45RO+) e células NK;
- Redução na expressão de moléculas co-estimulatórias nas células T (GLOBERSON; EFFROS, 2000), expressando mais receptores de inibição e exaustão (ALONSO ARIAS et al., 2013).

De acordo com a expressão de moléculas co-estimulatórias na superfície das células T, podemos identificar vários estágios de maturação/desenvolvimento destas células (STRIOGA; PASUKONIENE; CHARACIEJUS, 2011):

- Recém-diferenciadas: CD27+CD28+CD57-CD45RA-
- Naïve: CD27+CD28+CD57-CD45RA+
- Estágio intermediário: CD27-CD28+CD57+CD45RA-
- Estágio senescente ou diferenciado tardio: CD27-CD28-CD57+ CD45RA+

Uma das características marcantes das trocas fenotípicas linfocitárias no envelhecimento é a perda da expressão da molécula co-estimulatória CD28. O CD28 é expresso na superfície das células T, constituindo um receptor essencial para a ativação inicial do linfócito T. Semelhantemente ao envelhecimento, existe uma expansão de células T CD4+CD28- no sangue periférico de pacientes com EM, quando comparados a controles saudáveis de mesma idade (BROUX et al., 2012; DEMA et al., 2021). Na deficiência da principal molécula co-estimulatória, as células T ganham capacidades pró-inflamatórias.

Outra característica da imunossenescência é a expansão dos linfócitos em estádio mais tardio de diferenciação, como os marcadores co-inibitórios KLRG1, PD-1 e LAG3 (ESCHBORN et al., 2021). Durante a senescência celular, as células T periféricas podem expressar receptores NK, como NKG2D e NKG2A, conferindo-lhes a capacidade de matar células alteradas ou estressadas de maneira independente do antígeno (SOTO-HEREDERO et al., 2023). Os receptores NKG2A nas células T desempenham um papel importante na regulação da resposta imune (KAULFUSS et al., 2023), e expansão de células T NKG2A+ foram vistas na COVID-19 e associadas com exaustão celular e agravamento da doença (ZHENG et al., 2020). Estudos mostraram que o receptor NKG2D está envolvido na ativação e função das células T citotóxicas (GROH et al., 2003; SOTO-HEREDERO et al., 2023). Isso poderia levar a uma resposta imune exacerbada e inflamação no sistema nervoso central, resultando em danos aos neurônios e à mielina (DAVID et al., 2023).

Atualmente há evidências do aumento de células B e células NK na EM, implicadas na sua patogênese (CENCIONI et al., 2021; GROSS et al., 2016; LARONI et al., 2016). A importância das células NK na EM está no início e progressão, pois elas auxiliam na destruição da bainha de mielina, bem como resolução do quadro clínico. A citotoxicidade das células NK, bem como as citocinas derivadas, participam na regulação das respostas imunes, bem como contribuindo para a patogênese da EM (HARVEY et al., 2009). As células NK são divididas em subconjuntos

funcionalmente distintos, baseados nos níveis de CD56 e de muitos outros receptores cruciais para realizar suas funções efetoras. As células NK CD56dim são efetoras altamente citotóxicas enquanto as células NK $CD56^{BRIGHT}$ constituem o subconjunto regulador que pode modular outras células imunes através da secreção de citocinas (GROSS et al., 2016; RAMMOHAN et al., 2020). A maioria das células NK circulantes é $CD56^{DIM}$CD16+, KIR+ e/ou NKG2A+ (GROSS et al., 2016; LARONI et al., 2016). As células NK $CD56^{BRIGHT}$ diminuem a resposta autoimunes no SNC. Observa-se números elevados de células NK $CD56^{BRIGHT}$ em indivíduos com EM tratados com IFN-β.

Os principais componentes da resposta inflamatória que acompanham as perturbações neurológicas são a micróglia e os macrófagos, células imunes inatas importantes para a regeneração do SNC. Estas células sofrem senescência de formas distintas, de forma negativa com impacto na resposta degenerativa e de reparação no envelhecimento do SNC (SHAW; GOLDSTEIN; MONTGOMERY, 2013). A compreensão de como o processo de envelhecimento afeta estes diferentes tipos de células deve revelar conhecimentos importantes sobre potenciais alvos mecanicistas que podem ser aproveitados para atenuação terapêutica dos processos neurodegenerativos como bem como a melhoria das atividades reparadoras no envelhecimento SNC (RAWJI et al., 2016).Tanto os macrófagos como as micróglias do SNC apresentam uma diminuição da fagocitose e da quimiotaxia com o envelhecimento, os efeitos da idade manifestam-se de forma diferente no que diz respeito ao seu perfil pró-inflamatório. Com o envelhecimento, os macrófagos são menos capazes de produzir uma resposta pró-inflamatória funcional. Contudo, a micróglia apresenta uma resposta pró-inflamatória exagerada, um fenômeno referido como "*microglia priming*" e associado com neuroinflamação (RAWJI et al., 2016).

2.1. Senescência replicativa

Alguns biomarcadores são relógios biológicos que se relacionam melhor com o processo de envelhecimento. O encurtamento telomérico é o marcador mais consistente do envelhecimento celular (BLACKBURN; EPEL; LIN, 2015). Telômeros são sequências repetidas de nucleotídeos da terminação linear dos cromossomos que são críticos para a manutenção e integridade do genoma. A extensão dos telômeros

é vista como um “relógio mitótico” porque, em cada ciclo celular, eles erodem progressivamente, até que a célula entre em estado de senescência e sofre apoptose. Porém vários fatores extrínsecos (ex., dieta, estresse oxidativo e inflamação crônica) promovem a erosão telomérica e aceleraram, portanto, o relógio biológico. Nos últimos anos, demonstrou-se que telômeros encurtados se associam com a progressão clínica de várias doenças e constituem importantes relógios biológicos. Os telômeros são estruturas núcleo-proteicas encontradas no final de cada cromossomo. As principais funções são a manutenção e proteção da informação genética, bem como a regulação da capacidade de replicação celular (SARETZKI, 2018). O comprimento dos telômeros diminui com a idade como um processo fisiológico e sujeito a fatores genéticos e de estilo de vida. Isto refere-se à questão de que durante cada ciclo de replicação de DNA, as sequências finais do DNA não são totalmente replicadas. Como resultado, há uma perda da sequência genômica com cada divisão celular. Consequentemente, os telômeros humanos diminuem (MARIONI et al., 2016). O encurtamento telomérico acelerado pode promover a senescência e apoptose celular. Os telômeros são vulneráveis ao estresse oxidativo (com maior produção de espécies reativas do oxigênio, ROS), que está aumentado no tabagismo, estresse psicossocial e associado com sobrepeso e hábitos alimentares (MARIONI et al., 2016; SARETZKI, 2018; VON ZGLINICKI, 2002). Além disso, a inflamação crônica e as infecções virais persistentes são bem conhecidas por estarem associadas ao aumento do atrito telomérico (BÜHRING et al., 2021)

Em comparação com os controles saudáveis, o telômero mais curto em leucócitos tem sido associado a várias doenças, incluindo doenças cardiovasculares, transtorno bipolar, diabetes tipo 2, doença de Alzheimer e doenças autoimunes, tais como artrite reumatoide (ANITHA et al., 2019). Pacientes com EM apresentam telômeros mais encurtados, sendo a erosão telomérica associada com a progressão clínica da doença (BÜHRING et al., 2021). O encurtamento dos telômeros pode ser causado por um defeito na expressão e função da telomerase. A telomerase é uma enzima que atenua o atrito e a erosão dos telômeros e, portanto, na sua ausência, as sequências teloméricas são incompletamente duplicadas (BLACKBURN; EPEL; LIN, 2015).

A ativação imunológica crônica pode também interferir com os mecanismos de manutenção dos telômeros. Por outro lado, ficou demonstrado que as infecções virais persistentes afetam a dinâmica da TL e provocam a senescência nas células imunes (BELLON; NICOT, 2017). O acúmulo de células senescentes anérgicas (sem capacidade proliferativa) tem consequências adversas, uma vez que estas células ocupam nichos celulares e secretam citocinas pró-inflamatórias. Além disso, é de notar que a organização da cromatina e a regulação genética são também moduladas por encurtamento telômero, mesmo muito antes do início dos sinais de danos no DNA. Isto sugere que o envelhecimento biológico está relacionado com a inflamação e a neurodegeneração na EM e que a avaliação da TL como um biomarcador da imunossenescência pode fornecer informações úteis sobre o curso individual da doença. Contudo, mais estudos são necessários para compreender melhor a complexa relação entre o envelhecimento e a patogenia da EM (BÜHRING et al., 2021).

3. JUSTIFICATIVA

A esclerose múltipla (EM) é uma doença autoimune associada com o envelhecimento, apresenta um perfil inflamatório crônico que provoca danos físicos, cognitivos e progressivamente incapacitantes. Devido ao seu curso crônico, leva a prejuízos econômicos significativos para o SUS. O fenótipo EMRR, que se caracteriza por recorrência: início subagudo de sintomas ou sinais neurológicos focais, associado ao aparecimento de novas lesões desmielinizantes.

A EM vem sendo associada com um envelhecimento imune precoce, contribuindo negativamente para a evolução clínica da doença. Logo, precisamos entender mais profundamente como a imunossenescência interage com a EM. Visto as constatações acima, a evolução clínica da doença pode estar comprometida devido à presença constante de inflamação e envelhecimento acelerado. Este projeto pretende demonstrar as relações complexas entre a fisiopatologia e biomarcadores periféricos conhecidos do envelhecimento acelerado.

4. HIPÓTESES

4.1. HIPÓTESE 0 (H0):

Não há associação entre a esclerose múltipla e a imunossenescência.

4.2. HIPÓTESE 1 (H1, Alternativa):

Há associação entre a esclerose múltipla com biomarcadores de imunossenescência.

5. OBJETIVOS

5.1. Objetivo geral

Caracterizar a imunossenescência na esclerose múltipla.

5.2. Objetivos específicos

1. Recrutar pacientes com EMRR e controles saudáveis.

2. Avaliar o tamanho telomérico das células mononucleares do sangue periférico.

3. Identificar subtipos dos monócitos e linfócitos (T, B e NK).

4. Identificar fenótipos linfocitários associados com a senescência, exaustão e regulação celular.

5. Avaliar as citocinas inflamatórias e regulatória no soro.

6. Correlacionar os biomarcadores do envelhecimento precoce e inflamatórios com gravidade clínica.

6. ARTIGO CIENTÍFICO

INTERNATIONAL IMMUNOPHARMACOLOGY, a ser submetido

COMPREHENSIVE IMMUNE PROFILING CONFIRMS PREMATURE IMMUNOSENESCENCE IN MULTIPLE SCLEROSIS

Pedro Luis da Silva[a,b], Juliete Nathali Scholl[a,b], Carolina Dias Cezar[a,b], Lucas Kich Grun [c], Mailton P. Madruga[a,b], Gisele Hansel[d], Giordani Rodrigues Dos Passos [d], Douglas K. Sato[d] and Moisés Evandro Bauer[a,b]

[a] Laboratory of Immunobiology, School of Health and Life Sciences, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brasil.

[b] Graduate program in Cellular and Molecular Biology, School of Health and Life Sciences, PUCRS, Porto Alegre, Brasil.

[c] Programa de Pós-Graduação em Saúde da Criança e do Adolescente e Laboratório de Fisiologia Respiratória, Escola de Medicina, PUCRS, Porto Alegre, Brasil.

[d] The Brain Institute of Rio Grande do Sul, Porto Alegre, PUCRS, Porto Alegre, Brazil,

Corresponding author: Professor M.E. Bauer, Pontifical Catholic University of Rio Grande do Sul. Av. Ipiranga, 6681, building 12 (4th floor). Porto Alegre, RS 90619-900, Brazil. E-mail: mebauer@pucrs.br .

ABSTRACT

INTRODUCTION: Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system. Premature aging of the immune system (immunosenescence) leads to increased morbidity and mortality in adults, and it has been associated with MS. Here, we have further characterized differentiation stages of T cells, activated and regulatory lymphocytes and monocytes, as well as checkpoints involved with T-cell exhaustion and senescence in relapsing-remitting MS (RRMS) and age-matched controls. **METHODS:** serum and peripheral blood mononuclear cells (PBMCs) were isolated and comprehensively immunophenotyped by multicolor flow cytometry. Serum cytokines (TNF-α, IL-1β, IL-8, IL-6 and IL-10) were determined by cytometric bead assays (CBAs). Telomere length of PBMCs was measured by real-time PCR. **RESULTS:** Eighty-nine leukocyte subsets and phenotypes from lymphocytes and monocytes were identified. PBMCs of RRMS patients had shortened telomeres than controls ($p<0.05$). Cytokines did not differ between patients and controls. Patients had lower proportions of non-classical ($CD16^{bright}CD14^{+}$) and classical monocytes ($CD16\text{-}CD14^{bright}$) albeit increased proportions of intermediate monocytes ($CD16+CD14^{bright}$) as compared to controls (all $p<0.05$). NK subsets ($CD16^{+}CD56^{dim}$ and $CD16^{low}CD56^{bright}$) did not differ between subjects. Lower proportions of transitional B cells ($CD19+\ CD38^{bright}$) and regulatory B cells (CD19+CD27+CD38+) were observed in RMSS (all $p<0.05$). Patients had lower proportions of early-differentiated T cells ($CD27^{+}CD28^{+}$) but no changes in intermediate ($CD27^{-}CD28^{+}$) or late-differentiated cells ($CD27^{-}CD28^{-}$). Although patients had significantly lower proportions of activated T ($CD25^{+}$) in both $CD4^{+}$ or $CD8^{+}$ cells (all $p<0.0001$), no changes were observed in regulatory T cells ($CD4^{+}FoxP3^{+}$). Regarding the checkpoints, patients had increased proportions of $CD4^{+}PD\text{-}1^{+}$ cells as compared to controls ($p<0.01$), but lower percentages of $CD8^{+}CTLA\text{-}4^{+}$ cells ($p<0.01$). Also, significantly lower proportions of $CD4^{+}$ and $CD8^{+}$ T cells expressing NK receptors (NKG2A or NKG2D) were observed in patients (all $p<0.05$). **CONCLUSIONS:** Although early senescence in RMSS was confirmed by shortened TL and expansion of aged-exhausted T cells, no changes were reported in more differentiated T cells.

Keywords: Multiple sclerosis, cytokines, immunosenescence and T cells.

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS). The pathophysiology of MS involves complex interactions between autoreactive B and T cells (DENDROU; FUGGER; FRIESE, 2015), leading to the destruction of myelin sheaths crucially involved with neurological disability in patients. In MS, regardless of disease stage and activity, CD8 T cells are seen in much higher numbers than CD4 T cells (CARBAJAL et al., 2015). As the T cell compartment becomes increasingly clonal during aging, some studies suggest a propensity for self-reactivity (GREBENCIUCOVA; BERGER, 2017). Peripheral effector T cells (including Th17 cells), myeloid cells and dysfunctional regulatory T cells may also contribute to MS onset and progression. Failure of immunoregulatory mechanisms provides a favorable environment for demyelination, oligodendrocyte damage and neuroaxonal damage (DENDROU; FUGGER; FRIESE, 2015).

MS has been associated with accelerated or premature aging of the immune system (immunosenescence) (BOLTON; SMITH, 2018). In adults with MS, this premature immunosenescence resembles that seen in the elderly and can be characterized by: (1) thymic involution, (2) telomere shortening, (3) production of large amounts of inflammatory cytokines and (4) expansion of more differentiated T and B cells (BÜHRING et al., 2021; GREBENCIUCOVA; BERGER, 2017). Of note, age-related dysfunctional T cells (ex., CD28-) may arise from repeated antigenic stimulation, acquire cytotoxic functions, and secrete increased levels of pro-inflammatory mediators (such as cytokines and chemokines). In fact, expansions of CD8+CD28- T cells have been repeatedly observed in healthy older adults (BAUER; FUENTE, 2016) as well as in adults with autoimmune disorders. In rheumatoid arthritis, CD28- T cells have been found in the synovial tissue and associated with disease progression (BAUER, 2020). To what extent these age-related changes are implicated with disease progression in MS remains to be established.

Changes in natural killer (NK) and myeloid cells may also be involved with disease onset and progression in MS. Human NK cells may be subdivided into different populations based on the relative expression of the surface markers CD56 and CD16: the $CD56^{bright}$ $CD16^{dim/-}$ (regulatory) and $CD56^{dim}$ $CD16^{+}$ (cytotoxic) (GROSS et al., 2016). The $CD56^{bright}$ NK cells could be involved with reduced autoimmune responses in the CNS, as increasing numbers of $CD56^{bright}$ NK cells were associated with reducing the number of active

lesions on MRI (MARTIN et al., 2010). To what extent these changes are involved with early immunosenescence in MS remain to be established.

Here, we have further characterized the immunosenescence in RRMS patients, by a comprehensive immune profiling of peripheral lymphocytes and monocytes, as well as by investigating telomere attrition, and circulating inflammatory cytokines. In addition, we associated these changes with clinical features.

MATERIALS & METHODS

Subjects and clinical assessment

From March 2021 until March 2022, we recruited 25 patients with an Expanded Disability Status Scale (EDSS) score ≤ 5 and diagnosed with RRMS (highly active) from the Neurology Service of São Lucas Hospital (PUCRS). Twenty-one age- and sex-matched healthy controls were also recruited from local community. The exclusion criteria included other chronic autoimmune diseases, infections, neoplasia, metabolic diseases. Clinical assessment was performed by a highly trained neurologists (GRDP and DKS). In this study, highly active relapsing MS was defined as one relapse in the previous year and ≥1 T1 gadolinium enhancing (Gd+) lesion or ≥ 9 T2 lesions while on treatment with another DMT or ≥ 2 relapses in the previous year whether on DMT or not. All participants signed a written consent form before inclusion. The study was approved by the Institutional Review Board (CAAE: 36048820.0.0000.5336).

Isolation of peripheral blood mononuclear cells

Peripheral blood (16 mL) was into EDTA tubes through venous puncture (always between 10-12 a.m.). The samples were centrifuged for 15 minutes at 400g. Blood plasma was separated and stored at -80°C for cytokine quantification. Peripheral blood mononuclear cells (PBMCs) were isolated through density gradient using Ficoll-Paque™ PLUS (GE Healthcare, Milwaukee, USA) by centrifugation for 30 minutes at 400g. PBMCs were counted via microscopy (100x). Viability exceeded 95%, judged from their ability to exclude Trypan Blue (Sigma, St. Louis, USA). PBMCs were suspended in fetal bovine serum (FBS) containing 10% of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA) and stored at -80°C.

Immunophenotyping

Ten patients and 11 controls were included in these analyses. Cells were washed with cytometry buffer (PBS containing 1% bovine serum and 0.01% sodium azide) and stained for 30 min with the following of monoclonal antibodies (all from BD Biosciences, San Jose, USA):

anti-CD3, anti-CD4, anti-CD8, anti-CD28, anti-CD27, anti-CD57, anti-NKG2D, anti-NKG2A, anti-CD19, anti-PD-1, anti-LAG-3 anti-FOXP3, anti-CD56, anti-CD5, anti-CTLA-4, anti-CD38, anti-CD14, and anti-CD16. The following fluorochromes were used: FITC, PE, APC, APC-H7, PerCP-Cy5.5, BV421, and BV510. After staining, cells were washed, resuspended, and analyzed in a FACS Canto II equipped with three lasers. A Minimum of 30,000 events were gated by size and granularity. Data were analyzed using FlowJo v10 software (Tree Star Inc., Ashland, USA).

Cytokines

Serum cytokine levels (TNF-α, IL-10, IL-6, IL-1β, IL-12p70 and IL-8) were measured by cytometric bead arrays (CBAs) using the Human Inflammatory Cytokine Kit, accordingly to the manufacturer's guidelines (BD Biosciences, Brazil). Data were adquired in FACSCanto II (Becton Dickinson, USA). Quantitative plasma and supernatant levels were estimated by the FCAP Array v3.0 software (Soft Flow Inc., Pecs, Hungary). Minimum detection limits in plasma for these assays were TNF-α = 1.03 pg/ml, IL-10 = 1.49 pg/ml, IL-6 = 1.68 pg/ml, IL-1β = 1.44 pg/ml, IL-8 = 3.63 pg/ml, and IL-12p70 = 1.21 pg/ml. Minimum detection limits in stimulated supernatant for these assays were TNF-α = 109.46 pg/ml, IL-10 = 49.89 pg/ml, IL-6 = 480.39 pg/ml, IL-1β = 340.97 pg/ml, IL-8 = 6088.3 pg/ml, and IL-12p70 = 1.21 pg/ml.

DNA extraction

The genomic DNA (gDNA) was isolated from 2×10^6 PBMCs via a kit (PureLink® Genomic DNA Mini Kit, Invitrogen, USA), according to the manufacturer's instructions. The purified gDNA was eluted and purity/concentration was assessed by NanoDrop Lite (Thermo Fisher, USA), with an absorbance ratio set at 260/280 nm.

Relative telomere length

The relative telomere length was determined here as previously described (CAWTHON, 2002) (GRUN et al., 2018). In short, two qPCR reactions were performed for each sample: amplifying a telomere sequence (T) and a single-copy autosomal gene, encoding the ribosomal acid phosphoprotein P0 36B4 (S). All reactions were determined in triplicates through the Platinum® Taq DNA polymerase enzyme (Invitrogen, USA) in a 96-well 7500 Fast Real-Time PCR System instrument (Applied Biosystems). Data was assessed by the comparative cT (cycle threshold) method (ΔΔcT) (LIVAK; SCHMITTGEN, 2001) and expressed as relative T/S Ratio.

Statistical analysis

All variables were tested for normality of distribution using Shapiro–Wilk test. For categorical variables, differences between groups were compared using chi-square (X^2) or Mann-Whitney tests. For continuous variables, differences between groups were performed by unpaired t test or Mann-Whitney, when indicated. Correlation analysis was performed using Spearman tests. Analyses were performed using GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA). The significance level was set at p-value < 0.05 (two-tailed).

RESULTS

Demographic and Clinical Characteristics

The demographic and clinical characteristics are summarized in Table 1. They had a median of two relapses (range, 1-3) in the previous year and 40% had been treated with a disease-modifying drug (none with immune reconstitution therapy). At baseline, brain lesion count was 11-20 in 30%, 21-40 in 40% and uncountable in 30%, with Gd-enhancing lesions in 50%.

Table 1. Demographic and clinical characteristics of subjects.

	Controls (n=21)	**MS (n=25)**	**p-value**
Age (years)	32.14 ± 4.93	30.16 ± 5.80	0.22
Sex (n)			0.50
Female	14.00	14.00	NS
Disease duration (months)		14.98 ± 8.393	--
EDSS		2.00 ± 1.13	
Drugs (%)			
Nothing		28.00	
Fumarate		12.00	
Teriflunomide		12.00	
Natalizumab		40.00	
Glatiramer acetate		8.00	

Telomere attrition

Here, we have investigated the TL of peripherical mononuclear cells as objective marker of biological aging. TL was found significantly reduced in PBMCs of MS patients compared with age-matched healthy controls (Figure 1).

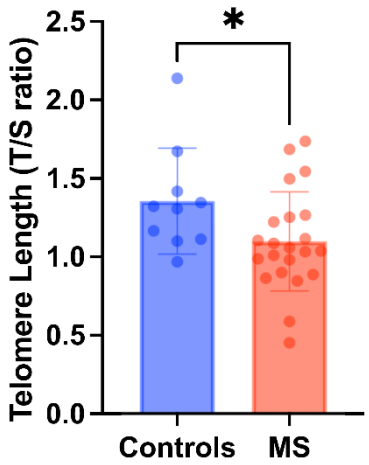

Figure 1. Telomere length in peripheral blood mononuclear cells (PBMCs). Data were analyzed by unpaired T test. Statistically significant difference is indicated: * $p < 0.05$.

Serum cytokines

We have analyzed serum pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-8 and IL-10), as they have previously been associated with *inflammaging* (ALMANAN et al., 2020) and disease progression (BUGBEE; WANG; GOMMERMAN, 2023). However, patients had similar cytokine levels than controls (Table 2).

Table 2. Serum cytokines. Data is shown as mean ± SD.

	Controls (n=11)	**MS (n=12)**	**p-value**
TNF-α	1.55 ± 0.33	1.50 ± 0.26	0.95
IL-6	2.02 ± 0.41	2.22 ± 0.66	0.51
IL-10	1.96 ± 0.23	1.87 ± 0.42	0.25
IL-1β	1.99 ± 0.24	1.85 ± 0.25	0.21
IL-8	4.61 ± 1.13	5.45 ± 1.71	0.24

Monocytes and NK cells

The percentages of classical ($CD14^{bright}CD16^{-}$) and non-classical ($CD14^{+}CD16^{bright}$) monocytes were reduced in patients compared with controls (Figure 2). In contrast, RRMS patients had increased proportions of intermediate monocytes ($CD14^{bright}CD16^{dim}$) than controls. There were no differences regarding NK cells and their subsets (Figure 3).

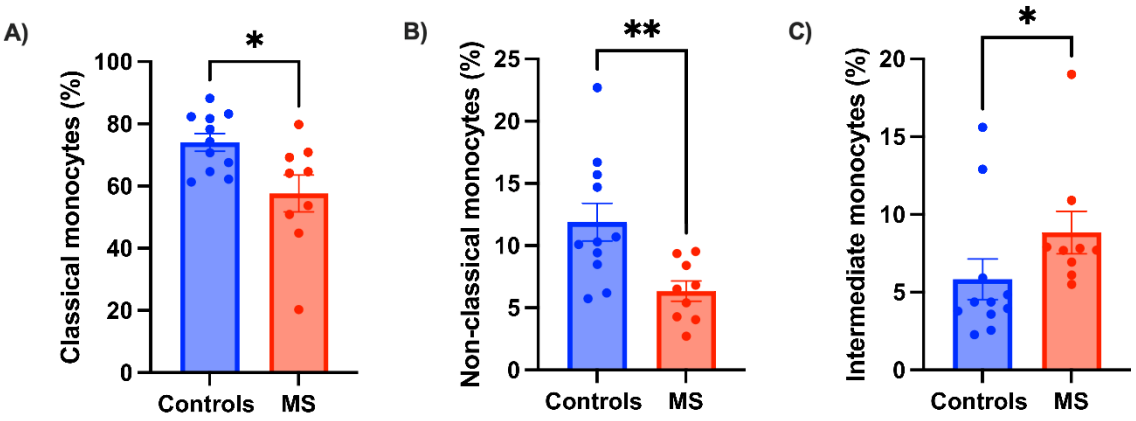

Figure 2. Peripheral monocyte subsets. Subpopulations were defined as classical ($CD14^{bright}CD16^{-}$), non-classical ($CD14^{+}CD16^{bright}$) and intermediate ($CD14^{bright}CD16^{dim}$). Data is shown as mean ± SD. Data were analyzed by unpaired T-tests and Mann-Whitney (intermediate). Statistically significant differences are indicated: * $p < 0.05$, ** $p < 0.01$.

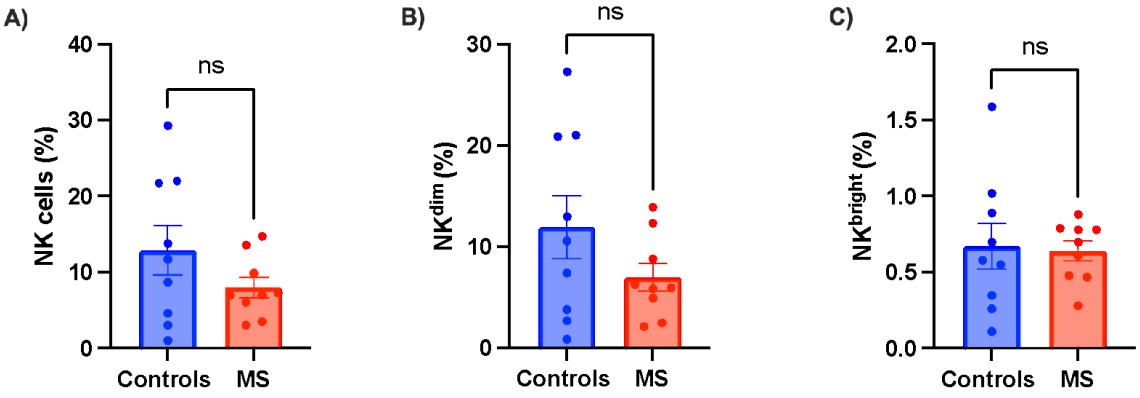

Figure 3. Peripheral NK cells and their subpopulations. Subpopulations were defined as the $CD56^{bright}$ $CD16^{dim/-}$ (regulatory) and $CD56^{dim}$ $CD16^{+}$ (cytotoxic). Data were analyzed by unpaired T tests and are shown as mean ± SD.

B-cell subsets

When analyzing B lymphocytes, MS patients and controls showed similar proportions of total B cells (CD19+), naïve B cells (CD19+CD27-), memory B cells (CD19+CD27+CD38+) and CD5+ B cells (Figure 4). However, there was a decreased proportion of transitional (CD27- $CD38^{BRIGHT}$CD19+) and regulatory B cells ($CD38^{BRIGHT}$CD19+) in MS patients than controls.

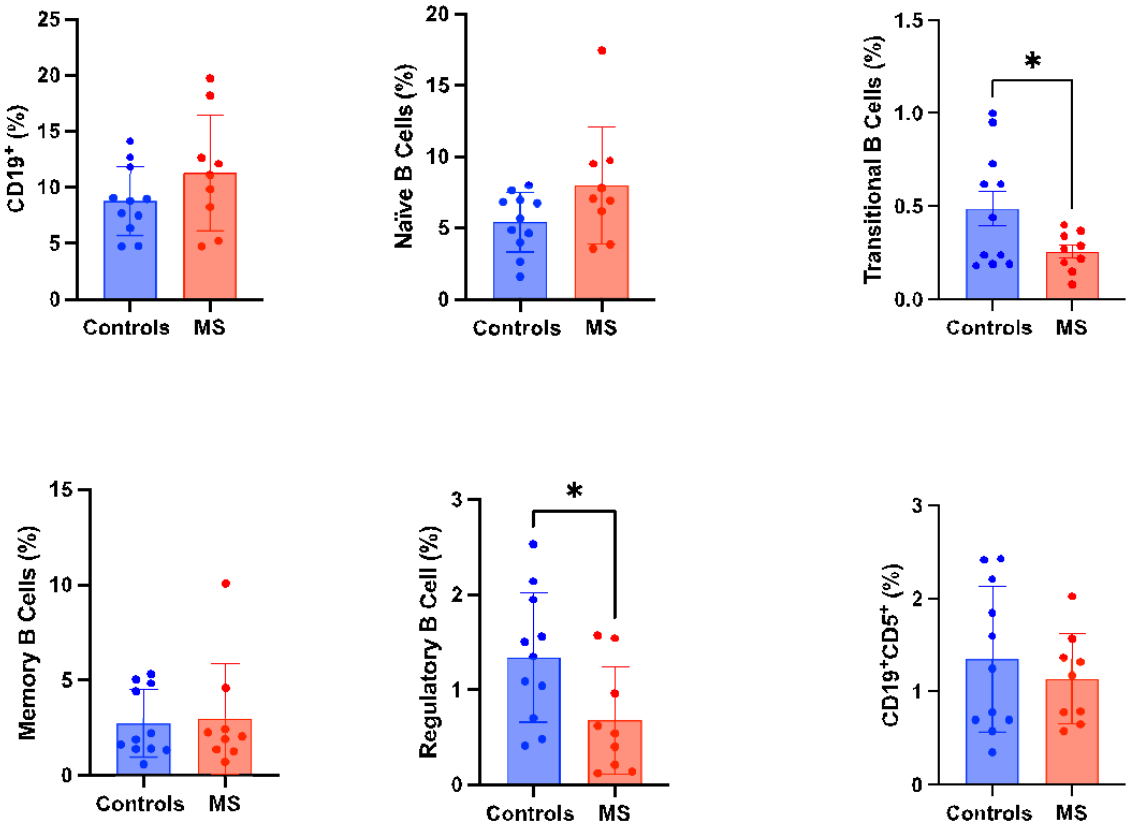

Figure 4. Peripheral total B cells (CD19+) and their subpopulations. Data were analyzed by unpaired T tests and are shown as mean ± SD. Statistical significant difference is indicated: * $p < 0.05$.

Activated and regulatory profiles (including checkpoints)

We investigated different peripheral lymphocyte subsets associated with activation and regulatory profiles. Both groups had similar proportions of CD4+ and Treg T cells (CD4+ and CD8+), Figure 5. However, there was a decrease in the proportions of CD3+CD8+, CD3+CD8+CD25+ (recent activation) and CD3+CD4+CD25+ (recent activation) T cells in patients compared to controls.

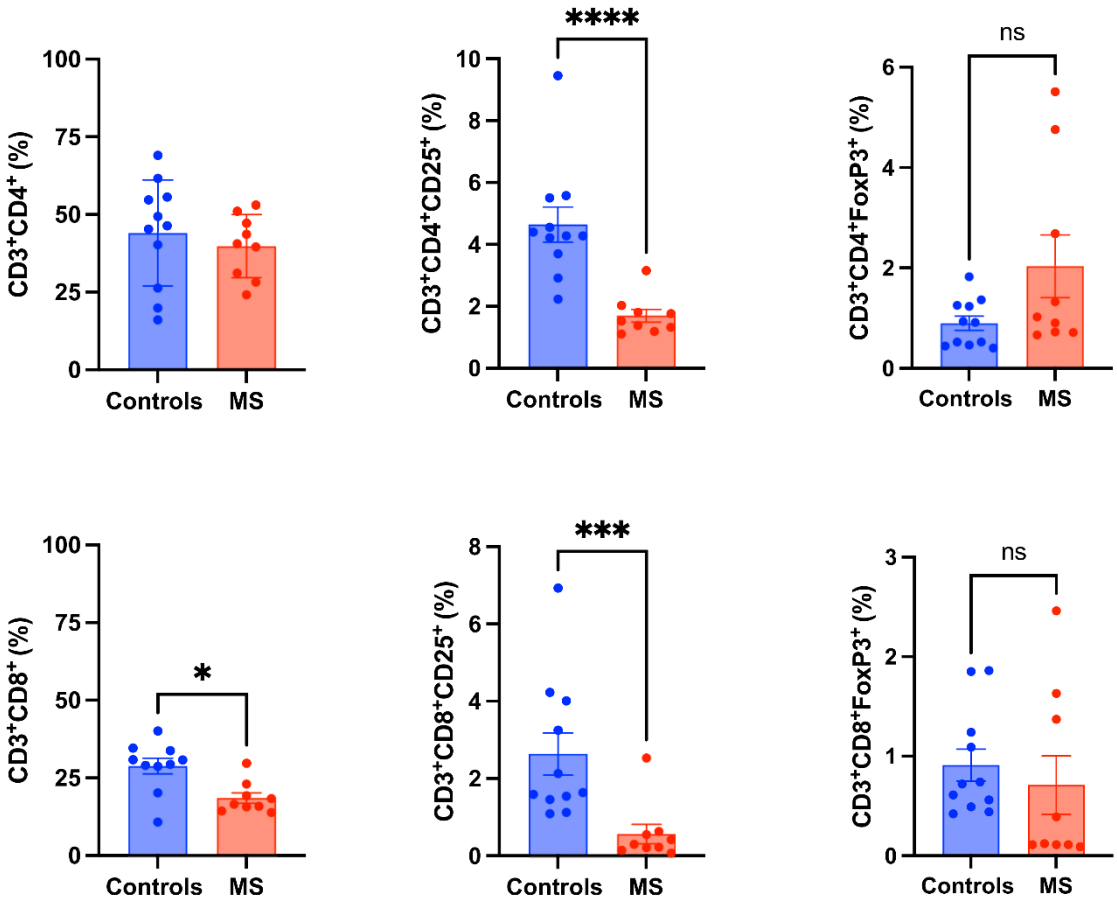

Figure 4. Peripheral activated and regulatory T cells. Data were analyzed by Mann-Whitney tests and are shown as mean ± SD. Statistical significant differences are indicated: * $p < 0.05$, *** $p < 0.001$ and **** $p < 0.0001$.

Concerning the analysis of checkpoints, MS patients had increased proportions of exhausted CD4+PD-1+ T cells than controls (Figure 5). However, patients had lower percentages of CD8+CTLA-4+ T cells compared with controls.

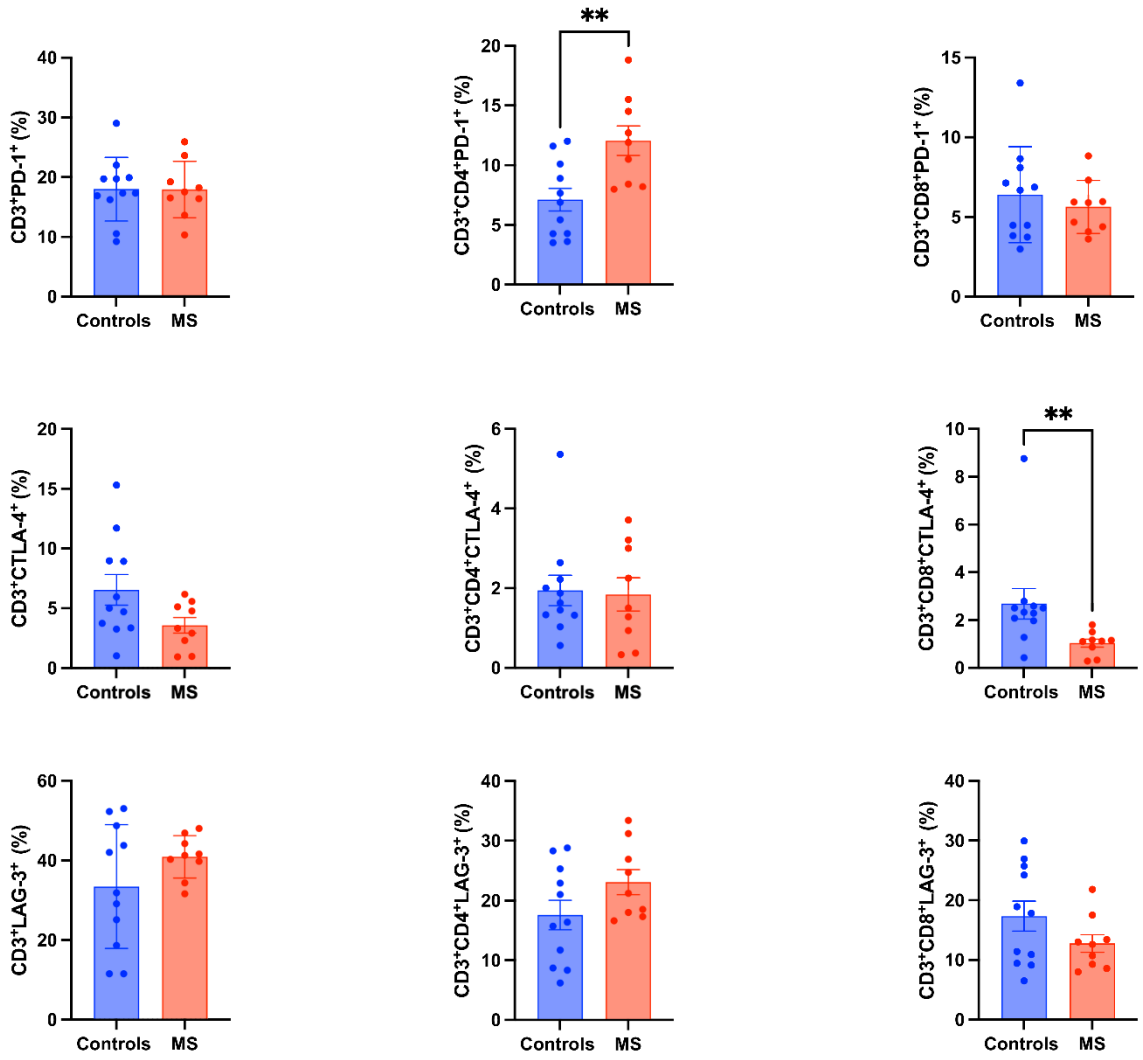

Figure 5. Checkpoints in peripheral T cells. Data were analyzed by Mann-Whitney tests and are shown as mean ± SD. Statistical significant differences are indicated: ** $p < 0.01$.

Stages of T-cell differentiation

Different stages of T-cell differentiation can be described based on the expression of cell surface co-stimulatory molecules: early differentiated (CD28+CD27+CD57-), intermediate

(CD28+CD27-CD57+), and late differentiated/senescent cells (CD28-CD27-CD57+) (STRIOGA; PASUKONIENE; CHARACIEJUS, 2011). Patients had reduced proportions of CD4+CD27+CD28+ naïve T cells than controls (Figure 6). Patients and controls had similar proportions of intermediate or late differentiated T cells.

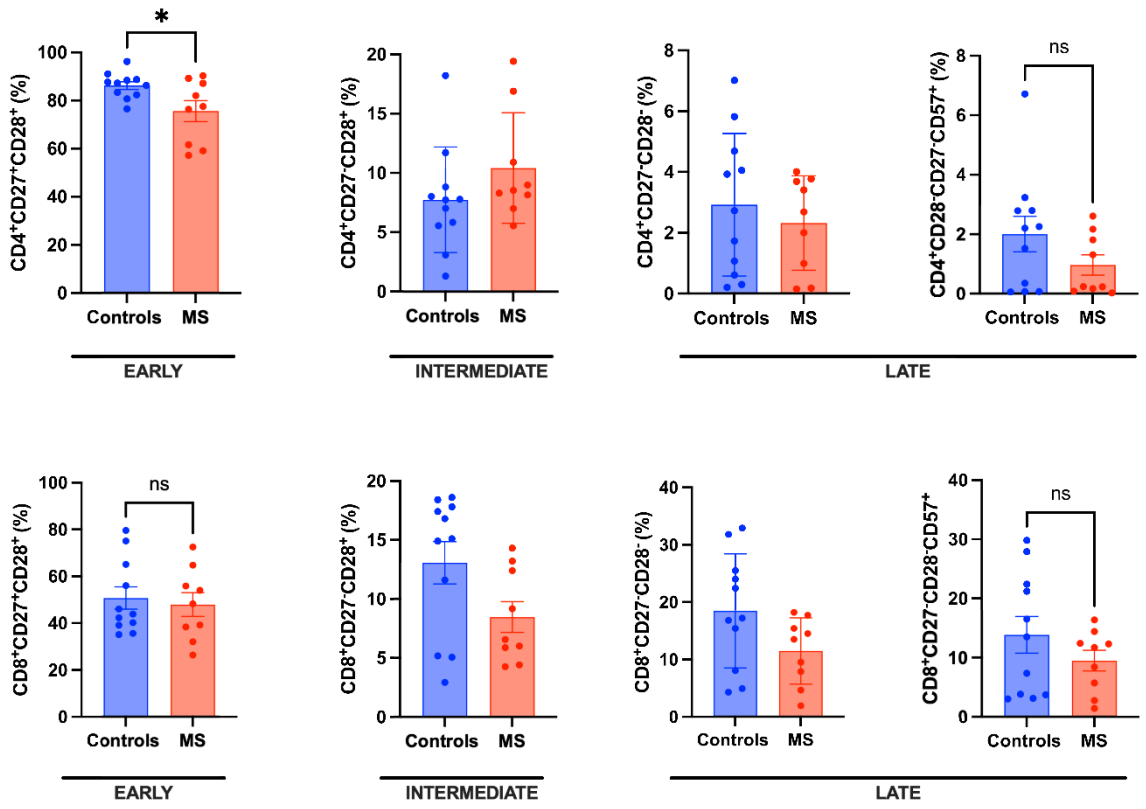

Figure 6. Different stages of T-cell differentiation. Data were analyzed by unpaired t tests and are shown as mean ± SD. Statistical significant differences is indicated: * $p < 0.05$.

Senescence-associated receptors

During cellular senescence, peripheral T cells may express NK receptors, such as NKG2D and NKG2A, conferring them the ability to kill altered or stressed cells in an antigen-independent manner (SOTO-HEREDERO et al., 2023). Patients had reduced proportions of most T and NK cells expressing NKG2A and NKG2D receptors than controls (Figure 7). Only NKG2A expression in CD4+ T cells did not differ between groups.

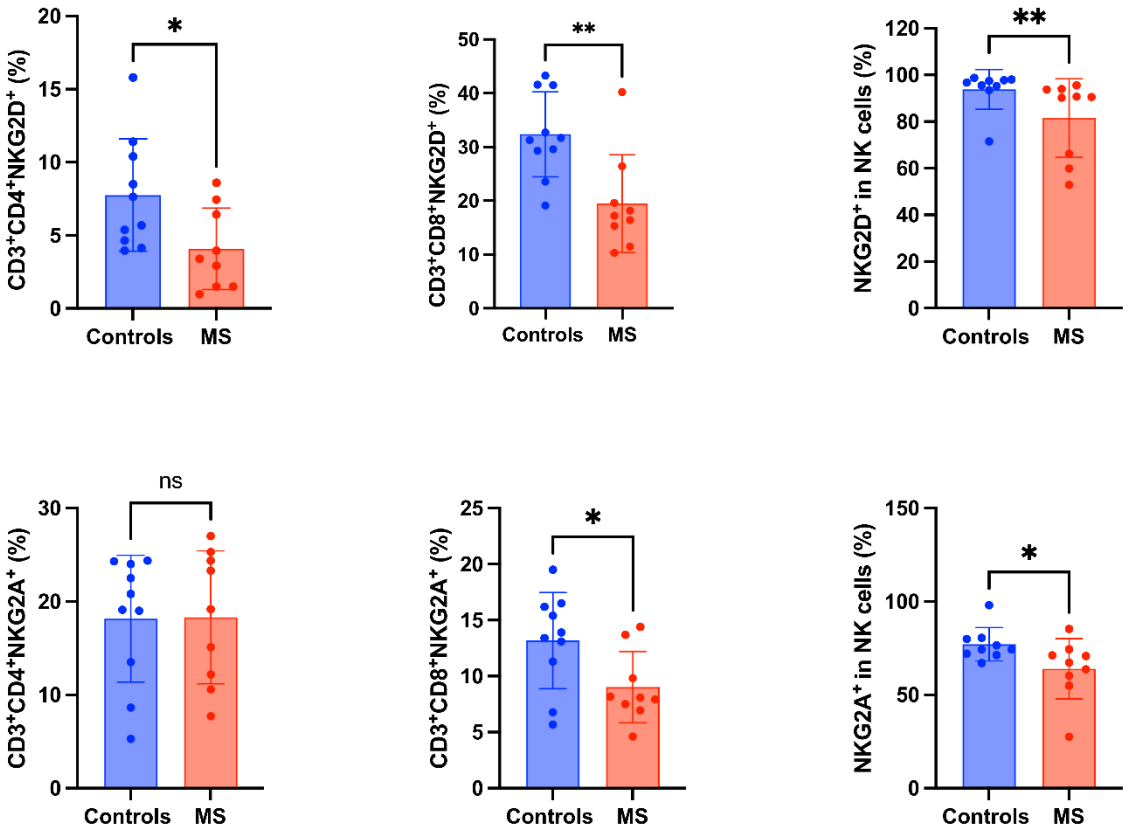

Figure 6. Expression of NK receptors in peripheral T and NK cells. Data were analyzed by unpaired t tests and are shown as mean ± SD. Statistical significant differences are indicated: * $p < 0.05$, and ** $p < 0.01$.

Discussion

Here, we provided an in-depth analysis of immunosenescence in MS, assessed by comprehensive multicolor flow cytometry (89 phenotypes identified), TL, and cytokines involved with *inflammaging*. Telomere shortening is an objective marker of cellular senescence. In the present study, in agreement with the current state of the art, PBMCs from patients had shortened telomeres than cells from controls. In addition, telomere shortening has been associated with greater disability and brain atrophy (KRYSKO et al., 2019), supporting the hypothesis that biological aging contributes to the progression of MS. A shorter TL was previously associated with higher EDSS scores in MS (BÜHRING et al., 2021; MA et al., 2022; SHU; LI; ZHU, 2022). Increased telomeric erosion in MS can be explained by the chronic inflammatory and oxidative process of this pathology, as well as by defects in telomerase activity ((BÜHRING et al., 2021).

Aging has been associated with chronic low-grade systemic inflammation (termed *inflammaging*), demonstrated by increased levels of pro-inflammatory mediators (cytokines, chemokines, and acute phase proteins) that increase the risk for age-related morbidity and mortality (DE MARTINIS et al., 2005; FRANCESCHI et al., 2006). Here, we have analyzed levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β and IL-8) and IL-10, as they have previously been associated with *inflammaging* (ALMANAN et al., 2020) and disease progression in MS (BUGBEE; WANG; GOMMERMAN, 2023). However, no changes were reported for the studied serum cytokines. Similarly, a previous study found similar serum IL-6 levels between MS patients and controls (VANDEBERGH; DUBOIS; GORIS, 2022), indicating little relevance in the context of the pathology. Contrary to these findings, previous studies found increased serum levels of TNF-α (GILIO et al., 2022; STEPHAN et al., 2022),IL-6 (SY et al., 2023), IL-8 (BURMAN et al., 2022; MELAMUD et al., 2022), and IL-10 (GILIO et al., 2022; TALBOT et al., 2022) in RRMS than healthy controls. In addition, serum levels of IL-1β were also found to be lower in unmedicated MS patients compared to controls (MELAMUD et al., 2022). The current treatment may have normalized the cytokines in our study, as both natalizumab and glatinavir have immunomodulatory functions (OREJA-GUEVARA et al., 2012). It is necessary to highlight that natalizumab can reduce IL-6 levels in the CSF of MS patients (MELLERGÅRD et al., 2010), therefore being an intervening variable for the analyses.

In our study, we reported reduced proportions of classical ($CD14^{BRIGHT}CD16$-) and non-classical ($CD14+CD16^{BRIGHT}$) monocytes, as well as an increase in intermediate cells

($CD14^{BRIGHT}CD16^{DIM}$) in MS patients compared to controls. Previous studies have reported these cells to be increased in MS compared to healthy controls and associated with pro-inflammatory parameters (COULOUME et al., 2021). Differences between studies can be explained by the clinical characteristics of the cohorts, as high disease activity was associated with the expansion of these cells (COULOUME et al., 2021). Peripheral blood monocytes secrete prostaglandins before MS lesions. During clinical activity, the expression of activation markers was associated with increased levels of IL-1 and TNF-α (REDER et al., 1998). Furthermore, differential expression of chemokine receptors may redeploy these monocytes to the CNS – in MS, it is recognized that CCR1, CCR2 and CCR5 are among the most important receptors involved in such migration. These receptors are primarily expressed in classical monocytes, but intermediate and non-classical monocytes can also migrate to the CNS and promote the infiltration of other cells as well as tissue destruction (CARSTENSEN et al., 2020). Recently, there has been shown an increase in three subtypes of monocytes in the blood of MS patients, of note in non-classical monocytes (GJELSTRUP et al., 2018; HASCHKA et al., 2020).

B cells are thought to contribute to MS pathophysiology because of their roles as antigen-presenting cells and via producing autoantibodies. Peripheral B cells in MS can activate autoreactive T cells, leading to an imbalance in the production of pro- and anti-inflammatory cytokines as well as increasing levels of co-stimulatory molecules (HECKER et al., 2023)). Here, we observed reduced proportions of transitional B cells ($CD27\text{-}CD38^{BRIGHT}CD19+$) and regulatory B cells ($CD38^{BRIGHT}CD19+$) in RRMS. Transitional B cells may act as a link between immature B cells and peripheral mature B cells (CHUNG; SILVERMAN; MONROE, 2003). This could contribute to exacerbations of the disease, as these cells can exert inhibitory control functions overactivated T cells (MIMPEN et al., 2021).

In this study, we investigated subpopulations of activated and regulatory T cells (including checkpoint expression). In this study, the CD3+CD8+, CD3+CD8+CD25+ and CD3+CD4+CD25+ populations were decreased in MS, despite similar percentages of Treg cells between patients and controls. Furthermore, we found an expansion of CD4+PD-1+ T cells in MS. These cells participate in peripheral tolerance in MS - reductions in these subpopulations have been associated with disease exacerbations (Liu et al., 2023). Therefore, we speculate that low proportions of activated T cells and increased PD-1+ cells are associated with effective control of peripheral immune activation promoted by patient treatments

(CARMO, 2019; FOLEY, 2010). Furthermore, PD-1+ T cells are more differentiated (aged) and have been seen expanded in healthy aging (SOTO-HEREDERO et al., 2023).. However, MS patients had a reduction in CD8+CTLA-4+ T cells compared to controls. CTLA-4 is an inhibitory receptor expressed primarily by T cells and is therefore a critical regulator of T cell homeostasis and self-tolerance (VAN COILLIE; WIERNICKI; XU, 2020). The final balance of several checkpoints may ensure better control of the disease.

Regarding the differentiation stages of T cells, we found a decrease in the percentage of newly differentiated cells (CD3+CD4+CD28+CD27+) in MS. However, this data may have been biased since some patients were using natalizumab, which can decrease this cell type (IANNETTA et al., 2016). In relation to the more senescent T subtypes, which express NK cell markers, we observed reductions in the proportions of T and NK cells expressing NKG2A+ and NKG2D+ in MS. These data contrast with previous studies that identified expansions of senescent T cells in MS (TOMAS-OJER et al., 2022). CD4+NKG2D+ cells have already been associated with a pro-inflammatory profile and neuroinflammation (Ruck et al., 2013). NKG2D+ was found in a lower proportion in patients with RRMS, but with greater interaction with astrocytes (CARMENA MORATALLA et al., 2023). Although we did not investigate these cell subsets in the CNS, a decreased expression of NKG2 receptors could be associated with less neuroinflammation or better disease control (RANGANATH et al., 2020; SCHWICHTENBERG et al., 2021; WISGALLA et al., 2022).

Our data should be interpreted considering some limitations. The sample sizes are relatively small, and this should be considered a pilot study. The cross-sectional design may preclude causal relationships. Future prospective studies are necessary to explore whether the senescence-related immune changes are found previous or consequent of the disease onset. In conclusion, although early senescence in RMSS was confirmed by shortened TL and increased aged/exhausted $CD4^{+}PD\text{-}1^{+}$ T cells, no changes were reported in more differentiated T cells. More studies are necessary to replicate these findings.

Conflict of interests

The authors declare that they have no competing financial interests or personal relationships that could appear to influence the work reported in this article.

7. References

ALMANAN, M. et al. IL-10–producing Tfh cells accumulate with age and link inflammation with age-related immune suppression. **Science Advances**, v. 6, n. 31, 31 jul. 2020.

BAUER, M. E. Accelerated immunosenescence in rheumatoid arthritis: impact on clinical progression. **Immunity & Ageing**, v. 17, n. 1, p. 6, 9 dez. 2020.

BAUER, M. E.; FUENTE, M. D. LA. The role of oxidative and inflammatory stress and persistent viral infections in immunosenescence. **Mechanisms of Ageing and Development**, v. 158, p. 27–37, set. 2016.

BOLTON, C.; SMITH, P. A. The influence and impact of ageing and immunosenescence (ISC) on adaptive immunity during multiple sclerosis (MS) and the animal counterpart experimental autoimmune encephalomyelitis (EAE). **Ageing Research Reviews**, v. 41, p. 64–81, jan. 2018.

BUGBEE, E.; WANG, A. A.; GOMMERMAN, J. L. Under the influence: environmental factors as modulators of neuroinflammation through the IL-10/IL-10R axis. **Frontiers in Immunology**, v. 14, 3 ago. 2023.

BÜHRING, J. et al. Systematic Review of Studies on Telomere Length in Patients with Multiple Sclerosis. **Aging and disease**, v. 12, n. 5, p. 1272, 2021.

BURMAN, J. et al. Cerebrospinal fluid cytokines after autologous haematopoietic stem cell transplantation and intrathecal rituximab treatment for multiple sclerosis. **Brain Communications**, v. 5, n. 1, 29 dez. 2022.

CARBAJAL, K. S. et al. Th Cell Diversity in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. **The Journal of Immunology**, v. 195, n. 6, p. 2552–2559, 15 set. 2015.

CARMENA MORATALLA, A. et al. Specific alterations in NKG2D+ T lymphocytes in relapsing-remitting and progressive multiple sclerosis patients. **Multiple Sclerosis and Related Disorders**, v. 71, p. 104542, mar. 2023.

CARMO, L. DA SILVA. **Esclerose múltipla: tratamentos farmacológicos e perspectivas futuras**. sao paulo: USP, 2019.

CARSTENSEN, M. et al. Activated monocytes and markers of inflammation in newly diagnosed multiple sclerosis. **Immunology & Cell Biology**, v. 98, n. 7, p. 549–562, 5 ago. 2020.

CAWTHON, R. M. Telomere measurement by quantitative PCR. **Nucleic Acids Research**, v. 30, n. 10, p. 47e–447, 15 maio 2002.

CHUNG, J. B.; SILVERMAN, M.; MONROE, J. G. Transitional B cells: step by step towards immune competence. **Trends in Immunology**, v. 24, n. 6, p. 342–348, jun. 2003.

COULOUME, L. et al. Mass Cytometry Identifies Expansion of T-bet+ B Cells and CD206+ Monocytes in Early Multiple Sclerosis. **Frontiers in Immunology**, v. 12, 4 maio 2021.

DE MARTINIS, M. et al. Inflamm-ageing and lifelong antigenic load as major determinants of ageing rate and longevity. **FEBS letters**, v. 579, n. 10, p. 2035–2039, 11 abr. 2005.

DENDROU, C. A.; FUGGER, L.; FRIESE, M. A. Immunopathology of multiple sclerosis. **Nature Reviews Immunology**, v. 15, n. 9, p. 545–558, 7 set. 2015.

FOLEY, J. Recommendations for the selection, treatment, and management of patients utilizing natalizumab therapy for multiple sclerosis. **The American journal of managed care**, v. 16, n. 6 Suppl, p. S178-83, jun. 2010.

FRANCESCHI, C. et al. Inflamm-aging: An Evolutionary Perspective on Immunosenescence. **Annals of the New York Academy of Sciences**, v. 908, n. 1, p. 244–254, 25 jan. 2006.

GANJI, A. et al. Effects of interferon and glatiramer acetate on cytokine patterns in multiple sclerosis patients. **Cytokine**, v. 126, p. 154911, fev. 2020.

GILIO, L. et al. Fatigue in Multiple Sclerosis Is Associated with Reduced Expression of Interleukin-10 and Worse Prospective Disease Activity. **Biomedicines**, v. 10, n. 9, p. 2058, 23 ago. 2022.

GJELSTRUP, M. C. et al. Subsets of activated monocytes and markers of inflammation in incipient and progressed multiple sclerosis. **Immunology and Cell Biology**, v. 96, n. 2, p. 160–174, fev. 2018

.

GREBENCIUCOVA, E.; BERGER, J. R. Immunosenescence: the Role of Aging in the Predisposition to Neuro-Infectious Complications Arising from the Treatment of Multiple Sclerosis. **Current Neurology and Neuroscience Reports**, v. 17, n. 8, p. 61, 1 ago. 2017.

GROSS, C. C. et al. Regulatory Functions of Natural Killer Cells in Multiple Sclerosis. **Frontiers in Immunology**, v. 7, 19 dez. 2016.

GRUN, L. K. et al. TRF1 as a major contributor for telomeres' shortening in the context of obesity. **Free Radical Biology and Medicine**, v. 129, p. 286–295, dez. 2018.

HASCHKA, D. et al. Expansion of Neutrophils and Classical and Nonclassical Monocytes as a Hallmark in Relapsing-Remitting Multiple Sclerosis. **Frontiers in Immunology**, v. 11, 29 abr. 2020.

HECKER, M. et al. Transcriptome alterations in peripheral blood B cells of patients with multiple sclerosis receiving immune reconstitution therapy. **Journal of Neuroinflammation**, v. 20, n. 1, p. 181, 2 ago. 2023.

IANNETTA, M. et al. Natalizumab Affects T-Cell Phenotype in Multiple Sclerosis: Implications for JCV Reactivation. **PLOS ONE**, v. 11, n. 8, p. e0160277, 3 ago. 2016.

KRYSKO, K. M. et al. Telomere Length Is Associated with Disability Progression in Multiple Sclerosis. **Annals of Neurology**, v. 86, n. 5, p. 671–682, 2 nov. 2019.

LIU, P.-J. et al. Characterization of antigen-specific CD8+ memory T cell subsets in peripheral blood of patients with multiple sclerosis. **Frontiers in Immunology**, v. 14, 4 maio 2023.

LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of Relative Gene Expression Data Using Real−Time Quantitative PCR and the 2−ΔΔCT Method. **Methods**, v. 25, n. 4, p. 402–408, dez. 2001.

MA, Y. et al. Telomere length and multiple sclerosis: a Mendelian randomization study. **International Journal of Neuroscience**, p. 1–5, 13 jul. 2022.

MARTIN, J. F. et al. An IL-2 Paradox: Blocking CD25 on T Cells Induces IL-2–Driven Activation of CD56bright NK Cells. **The Journal of Immunology**, v. 185, n. 2, p. 1311–1320, 15 jul. 2010.

MELAMUD, M. M. et al. Multiplex Analysis of Serum Cytokine Profiles in Systemic Lupus Erythematosus and Multiple Sclerosis. **International Journal of Molecular Sciences**, v. 23, n. 22, p. 13829, 10 nov. 2022.

MELLERGÅRD, J. et al. Natalizumab treatment in multiple sclerosis: marked decline of chemokines and cytokines in cerebrospinal fluid. **Multiple Sclerosis Journal**, v. 16, n. 2, p. 208–217, 9 fev. 2010.

MIMPEN, M. et al. Proportions of circulating transitional B cells associate with MRI activity in interferon beta-treated multiple sclerosis patients. **Journal of Neuroimmunology**, v. 358, p. 577664, set. 2021.

OREJA-GUEVARA, C. et al. TH1/TH2 Cytokine profile in relapsing-remitting multiple sclerosis patients treated with Glatiramer acetate or Natalizumab. **BMC Neurology**, v. 12, n. 1, p. 95, 18 dez. 2012.

RANGANATH, T. et al. Characterization of the Impact of Daclizumab Beta on Circulating Natural Killer Cells by Mass Cytometry. **Frontiers in Immunology**, v. 11, 24 abr. 2020.

REDER, A. T. et al. Monocyte activation in multiple sclerosis. **Multiple Sclerosis Journal**, v. 4, n. 3, p. 162–168, 2 jun. 1998.

RUCK, T. et al. CD4+NKG2D+ T Cells Exhibit Enhanced Migratory and Encephalitogenic Properties in Neuroinflammation. **PLoS ONE**, v. 8, n. 11, p. e81455, 25 nov. 2013.

SCHWICHTENBERG, S. C. et al. Fingolimod Therapy in Multiple Sclerosis Leads to the Enrichment of a Subpopulation of Aged NK Cells. **Neurotherapeutics**, v. 18, n. 3, p. 1783–1797, 9 jul. 2021.

SHU, M.-J.; LI, J.; ZHU, Y.-C. Genetically predicted telomere length and multiple sclerosis. **Multiple Sclerosis and Related Disorders**, v. 60, p. 103731, abr. 2022.

SOTO-HEREDERO, G. et al. Extremely Differentiated T Cell Subsets Contribute to Tissue Deterioration During Aging. **Annual Review of Immunology**, v. 41, n. 1, p. 181–205, 26 abr. 2023.

STEPHAN, D. et al. TWEAK and TNFα, Both TNF Ligand Family Members and Multiple Sclerosis-Related Cytokines, Induce Distinct Gene Response in Human Brain Microvascular Endothelial Cells. **Genes**, v. 13, n. 10, p. 1714, 24 set. 2022.

STRIOGA, M.; PASUKONIENE, V.; CHARACIEJUS, D. CD8+ CD28− and CD8+ CD57+ T cells and their role in health and disease. **Immunology**, v. 134, n. 1, p. 17–32, set. 2011.

SY, M. et al. N-acetylglucosamine inhibits inflammation and neurodegeneration markers in multiple sclerosis: a mechanistic trial. **Journal of Neuroinflammation**, v. 20, n. 1, p. 209, 13 set. 2023.

TALBOT, J. et al. Relationship between cerebrospinal fluid biomarkers of inflammation and tissue damage in primary progressive multiple sclerosis. **Multiple Sclerosis and Related Disorders**, v. 68, p. 104209, dez. 2022.

TOMAS-OJER, P. et al. Characterization of Antigen-Induced CD4+ T-Cell Senescence in Multiple Sclerosis. **Frontiers in Neurology**, v. 13, 3 fev. 2022.

VAN COILLIE, S.; WIERNICKI, B.; XU, J. Molecular and Cellular Functions of CTLA-4. Em: [s.l: s.n.]. p. 7–32.

VANDEBERGH, M.; DUBOIS, B.; GORIS, A. Effects of Vitamin D and Body Mass Index on Disease Risk and Relapse Hazard in Multiple Sclerosis. **Neurology - Neuroimmunology Neuroinflammation**, v. 9, n. 3, p. e1165, 7 maio 2022.

WISGALLA, A. et al. Alterations of NK Cell Phenotype During Pregnancy in Multiple Sclerosis. **Frontiers in Immunology**, v. 13, 4 jul. 2022.

8. CONSIDERAÇÕES FINAIS

Os resultados apresentados nessa dissertação apoiam parcialmente a hipótese da imunossenescência prematura na EM. Embora a senescência precoce em RMSS tenha sido confirmada por telômeros encurtados e aumento de células T CD4+PD-1+, nenhuma alteração foi relatada em células T mais diferenciadas ou nos níveis de citocinas periféricas associadas com *inflammaging* (TNF-α, IL-1β, IL-6 e IL-8).

O estudo teve um tamanho amostral reduzido, portanto deve ser considerado um piloto. O presente trabalho faz parte de um ensaio clínico aberto para investigar efeitos da cladribina oral (que depleta linfócitos) na reconstituição imune após 12 meses (estudo CLAREMI). No momento da participação dos pacientes neste estudo (ponto 1), nenhum sujeito havia sido medicado com cladribina. Por este motivo se faz necessário um estudo com maior tamanho amostral para melhor elucidar as questões sobre a EM e sua possível relação com a imunossenescência. Além de que vale ressaltar que os pacientes eram todos EMRR, sendo interessante avaliar em pacientes que tem outras apresentações clínicas e estadiamentos. Outro ponto interessante seria realizar um estudo multicêntrico pois, como já foi discutido, a imunossenescência é influenciada por fatores genéticos e ambientais. Ter um comparativo entre pacientes com estilos de vida e geologicamente distintos seria de grande valia para contextualizar a doença na dinâmica sociocultural do paciente.

Um dos principais vieses desse estudo foi o uso de medicações que poderiam influenciar as análises. Entre os medicamentos usados: o fumarato (imunomodulador) reduz a ativação de células imunes e a liberação de citocinas pró-inflamatórias(GOLD et al., 2012); teriflunomida (imunomodulador) bloqueia a proliferação dos linfócitos (DEMIREL OZBEK et al., 2023); o natalizumabe (anticorpo monoclonal) previne a transmigração dos leucócitos mononucleares através do endotélio para o tecido parenquimal inflamado(CIANO-PETERSEN et al., 2023) e o acetato de glatirâmer (imunomodulador), que modula para que as células apresentadoras de antígenos não exerçam sua função evitando a resposta inflamatória (KASINDI et al., 2022).

Outro viés a ser observado é que os pacientes não estavam em crise quando realizaram a coleta, sendo interessante essa coleta para se ter um comparativo entre a doença ativa e inativa. Em perspectivas futuras se faz necessário elucidações de

como a imunossenescência interage com a EM. Qual a dinâmica relacionada aos sintomas da EM, pois ela diverge entre os sintomas dos pacientes.

9. REFERÊNCIAS

ALMANAN, M. et al. IL-10–producing Tfh cells accumulate with age and link inflammation with age-related immune suppression. **Science Advances**, v. 6, n. 31, 31 jul. 2020.

ALONSO ARIAS, R. et al. Intensity of the Humoral Response to Cytomegalovirus Is Associated with the Phenotypic and Functional Status of the Immune System. **Journal of Virology**, v. 87, n. 8, p. 4486–4495, 15 abr. 2013
.
ANITHA, A. et al. Telomeres in neurological disorders. Em: [s.l: s.n.]. p. 81–132.

BAUER, M. E. Accelerated immunosenescence in rheumatoid arthritis: impact on clinical progression. **Immunity & Ageing**, v. 17, n. 1, p. 6, 9 dez. 2020.

BAUER, M. E.; FUENTE, M. D. LA. The role of oxidative and inflammatory stress and persistent viral infections in immunosenescence. **Mechanisms of Ageing and Development**, v. 158, p. 27–37, set. 2016.

BELLON, M.; NICOT, C. Telomere Dynamics in Immune Senescence and Exhaustion Triggered by Chronic Viral Infection. **Viruses**, v. 9, n. 10, p. 289, 5 out. 2017.

BLACKBURN, E. H.; EPEL, E. S.; LIN, J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. **Science**, v. 350, n. 6265, p. 1193–1198, 4 dez. 2015.

BOLTON, C.; SMITH, P. A. The influence and impact of ageing and immunosenescence (ISC) on adaptive immunity during multiple sclerosis (MS) and the animal counterpart experimental autoimmune encephalomyelitis (EAE). **Ageing Research Reviews**, v. 41, p. 64–81, jan. 2018.

BROUX, B. et al. CX3CR1 drives cytotoxic CD4+CD28− T cells into the brain of multiple sclerosis patients. **Journal of Autoimmunity**, v. 38, n. 1, p. 10–19, fev. 2012.

BRUNNER-WEINZIERL, M. C.; RUDD, C. E. CTLA-4 and PD-1 Control of T-Cell Motility and Migration: Implications for Tumor Immunotherapy. **Frontiers in Immunology**, v. 9, 27 nov. 2018.

BUCHBINDER, E. I.; DESAI, A. CTLA-4 and PD-1 Pathways. **American Journal of Clinical Oncology**, v. 39, n. 1, p. 98–106, fev. 2016.

BUGBEE, E.; WANG, A. A.; GOMMERMAN, J. L. Under the influence: environmental factors as modulators of neuroinflammation through the IL-10/IL-10R axis. **Frontiers in Immunology**, v. 14, 3 ago. 2023.

BÜHRING, J. et al. Systematic Review of Studies on Telomere Length in Patients with Multiple Sclerosis. **Aging and disease**, v. 12, n. 5, p. 1272, 2021.

BURMAN, J. et al. Cerebrospinal fluid cytokines after autologous haematopoietic stem cell transplantation and intrathecal rituximab treatment for multiple sclerosis. **Brain Communications**, v. 5, n. 1, 29 dez. 2022.

CARBAJAL, K. S. et al. Th Cell Diversity in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. **The Journal of Immunology**, v. 195, n. 6, p. 2552–2559, 15 set. 2015.

CARMENA MORATALLA, A. et al. Specific alterations in NKG2D+ T lymphocytes in relapsing-remitting and progressive multiple sclerosis patients. **Multiple Sclerosis and Related Disorders**, v. 71, p. 104542, mar. 2023.

CARMO, L. DA SILVA. **Esclerose múltipla: tratamentos farmacológicos e perspectivas futuras**. sao paulo: USP, 2019.

CARSTENSEN, M. et al. Activated monocytes and markers of inflammation in newly diagnosed multiple sclerosis. **Immunology & Cell Biology**, v. 98, n. 7, p. 549–562, 5 ago. 2020.

CAWTHON, R. M. Telomere measurement by quantitative PCR. **Nucleic Acids Research**, v. 30, n. 10, p. 47e–447, 15 maio 2002.

CENCIONI, M. T. et al. B cells in multiple sclerosis — from targeted depletion to immune reconstitution therapies. **Nature Reviews Neurology**, v. 17, n. 7, p. 399–414, 1 jul. 2021.

CHUNG, J. B.; SILVERMAN, M.; MONROE, J. G. Transitional B cells: step by step towards immune competence. **Trends in Immunology**, v. 24, n. 6, p. 342–348, jun. 2003.

CIANO-PETERSEN, N. L. et al. Natalizumab-immunogenicity evaluation in patients with infusion related events or disease exacerbations. **Frontiers in Immunology**, v. 14, 22 ago. 2023.

COMPSTON, A.; COLES, A. Multiple sclerosis. **The Lancet**, v. 372, n. 9648, p. 1502–1517, out. 2008.

COULOUME, L. et al. Mass Cytometry Identifies Expansion of T-bet+ B Cells and CD206+ Monocytes in Early Multiple Sclerosis. **Frontiers in Immunology**, v. 12, 4 maio 2021.

DAVID, C. et al. Impact of NKG2D Signaling on Natural Killer and T-Cell Function in Cerebral Ischemia. **Journal of the American Heart Association**, v. 12, n. 12, 20 jun. 2023.

DE MARTINIS, M. et al. Inflamm-ageing and lifelong antigenic load as major determinants of ageing rate and longevity. **FEBS letters**, v. 579, n. 10, p. 2035–2039, 11 abr. 2005.

DEMA, M. et al. Immunosenescence in multiple sclerosis: the identification of new therapeutic targets. **Autoimmunity Reviews**, v. 20, n. 9, p. 102893, set. 2021.

DEMIREL OZBEK, E. et al. Teriflunomide-Induced Palmoplantar Pustular Psoriasis: Case Report and Review of the Literature. **Cureus**, 2 ago. 2023.

DENDROU, C. A.; FUGGER, L.; FRIESE, M. A. Immunopathology of multiple sclerosis. **Nature Reviews Immunology**, v. 15, n. 9, p. 545–558, 7 set. 2015.

DISANTO, G. et al. The evidence for a role of B cells in multiple sclerosis. **Neurology**, v. 78, n. 11, p. 823–832, 13 mar. 2012.

DUSZCZYSZYN, D. et al. Thymic involution and proliferative T-cell responses in multiple sclerosis. **Journal of neuroimmunology**, v. 221, p. 73–80, 1 mar. 2010.

ESCHBORN, M. et al. Evaluation of Age-Dependent Immune Signatures in Patients With Multiple Sclerosis. **Neurology - Neuroimmunology Neuroinflammation**, v. 8, n. 6, p. e1094, 19 nov. 2021.

FILIPPI, M. et al. Multiple sclerosis. **Nature Reviews Disease Primers**, v. 4, n. 1, p. 43, 8 nov. 2018.

FOLEY, J. Recommendations for the selection, treatment, and management of patients utilizing natalizumab therapy for multiple sclerosis. **The American journal of managed care**, v. 16, n. 6 Suppl, p. S178-83, jun. 2010.

FRANCESCHI, C. et al. Inflamm-aging: An Evolutionary Perspective on Immunosenescence. **Annals of the New York Academy of Sciences**, v. 908, n. 1, p. 244–254, 25 jan. 2006.

GANJI, A. et al. Effects of interferon and glatiramer acetate on cytokine patterns in multiple sclerosis patients. **Cytokine**, v. 126, p. 154911, fev. 2020.

GILIO, L. et al. Fatigue in Multiple Sclerosis Is Associated with Reduced Expression of Interleukin-10 and Worse Prospective Disease Activity. **Biomedicines**, v. 10, n. 9, p. 2058, 23 ago. 2022.

GJELSTRUP, M. C. et al. Subsets of activated monocytes and markers of inflammation in incipient and progressed multiple sclerosis. **Immunology and Cell Biology**, v. 96, n. 2, p. 160–174, fev. 2018.

GLOBERSON, A.; EFFROS, R. B. Ageing of lymphocytes and lymphocytes in the aged. **Immunology Today**, v. 21, n. 10, p. 515–521, out. 2000.

GOLD, R. et al. Placebo-Controlled Phase 3 Study of Oral BG-12 for Relapsing Multiple Sclerosis. **New England Journal of Medicine**, v. 367, n. 12, p. 1098–1107, 20 set. 2012.

GORONZY, J. J.; WEYAND, C. M. Mechanisms underlying T cell ageing. **Nature Reviews Immunology**, v. 19, n. 9, p. 573–583, 11 set. 2019.

GREBENCIUCOVA, E.; BERGER, J. R. Immunosenescence: the Role of Aging in the Predisposition to Neuro-Infectious Complications Arising from the Treatment of Multiple Sclerosis. **Current Neurology and Neuroscience Reports**, v. 17, n. 8, p. 61, 1 ago. 2017.

GROH, V. et al. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. **Proceedings of the National Academy of Sciences**, v. 100, n. 16, p. 9452–9457, 5 ago. 2003.

GROSS, C. C. et al. Regulatory Functions of Natural Killer Cells in Multiple Sclerosis. **Frontiers in Immunology**, v. 7, 19 dez. 2016.

GRUN, L. K. et al. TRF1 as a major contributor for telomeres' shortening in the context of obesity. **Free Radical Biology and Medicine**, v. 129, p. 286–295, dez. 2018.

HAEGERT, D. G. Multiple Sclerosis: A Disorder of Altered T-Cell Homeostasis. **Multiple Sclerosis International**, v. 2011, p. 1–6, 2011.

HARVEY, D. et al. Analysis of killer immunoglobulin-like receptor genes in ankylosing spondylitis. **Annals of the Rheumatic Diseases**, v. 68, n. 4, p. 595–598, abr. 2009.

HASCHKA, D. et al. Expansion of Neutrophils and Classical and Nonclassical Monocytes as a Hallmark in Relapsing-Remitting Multiple Sclerosis. **Frontiers in Immunology**, v. 11, 29 abr. 2020.

HECKER, M. et al. Transcriptome alterations in peripheral blood B cells of patients with multiple sclerosis receiving immune reconstitution therapy. **Journal of Neuroinflammation**, v. 20, n. 1, p. 181, 2 ago. 2023.

IANNETTA, M. et al. Natalizumab Affects T-Cell Phenotype in Multiple Sclerosis: Implications for JCV Reactivation. **PLOS ONE**, v. 11, n. 8, p. e0160277, 3 ago. 2016.

JACOBSEN, M. et al. Oligoclonal expansion of memory CD8+ T cells in cerebrospinal fluid from multiple sclerosis patients. **Brain : a journal of neurology**, v. 125, n. Pt 3, p. 538–550, 2002.

KASINDI, A. et al. Glatiramer Acetate Immunomodulation: Evidence of Neuroprotection and Cognitive Preservation. **Cells**, v. 11, n. 9, p. 1578, 7 maio 2022.

KAULFUSS, M. et al. The NK cell checkpoint NKG2A maintains expansion capacity of human NK cells. **Scientific Reports**, v. 13, n. 1, p. 10555, 29 jun. 2023.

KOHM, A. P. et al. Cutting Edge: CD4+CD25+ Regulatory T Cells Suppress Antigen-Specific Autoreactive Immune Responses and Central Nervous System Inflammation During Active Experimental Autoimmune Encephalomyelitis. **The Journal of Immunology**, v. 169, n. 9, p. 4712–4716, 1 nov. 2002.

KRYSKO, K. M. et al. Telomere Length Is Associated with Disability Progression in Multiple Sclerosis. **Annals of Neurology**, v. 86, n. 5, p. 671–682, 2 nov. 2019.

KWONG, B. et al. T-bet-dependent NKp46+ innate lymphoid cells regulate the onset of TH17-induced neuroinflammation. **Nature Immunology 2017 18:10**, v. 18, n. 10, p. 1117–1127, 14 ago. 2017.

LARONI, A. et al. Dysregulation of regulatory CD56bright NK cells/T cells interactions in multiple sclerosis. **Journal of Autoimmunity**, v. 72, p. 8–18, 1 ago. 2016.

LIU, P.-J. et al. Characterization of antigen-specific CD8+ memory T cell subsets in peripheral blood of patients with multiple sclerosis. **Frontiers in Immunology**, v. 14, 4 maio 2023.

LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. **Methods**, v. 25, n. 4, p. 402–408, dez. 2001.

LUBLIN, F. D. et al. Defining the clinical course of multiple sclerosis: The 2013 revisions. **Neurology**, v. 83, n. 3, p. 278, 15 jul. 2014.

LUDWIN, S. K. et al. Astrocytes in multiple sclerosis. **Multiple Sclerosis**, v. 22, n. 9, p. 1114–1124, 1 ago. 2016.

MA, Y. et al. Telomere length and multiple sclerosis: a Mendelian randomization study. **International Journal of Neuroscience**, p. 1–5, 13 jul. 2022.

MARIONI, R. E. et al. The epigenetic clock and telomere length are independently associated with chronological age and mortality. **International Journal of Epidemiology**, v. 45, n. 2, p. 424–432, 1 abr. 2016.

MARTIN, J. F. et al. An IL-2 Paradox: Blocking CD25 on T Cells Induces IL-2–Driven Activation of CD56bright NK Cells. **The Journal of Immunology**, v. 185, n. 2, p. 1311–1320, 15 jul. 2010.

MELAMUD, M. M. et al. Multiplex Analysis of Serum Cytokine Profiles in Systemic Lupus Erythematosus and Multiple Sclerosis. **International Journal of Molecular Sciences**, v. 23, n. 22, p. 13829, 10 nov. 2022.

MELLERGÅRD, J. et al. Natalizumab treatment in multiple sclerosis: marked decline of chemokines and cytokines in cerebrospinal fluid. **Multiple Sclerosis Journal**, v. 16, n. 2, p. 208–217, 9 fev. 2010.

MIMPEN, M. et al. Proportions of circulating transitional B cells associate with MRI activity in interferon beta-treated multiple sclerosis patients. **Journal of Neuroimmunology**, v. 358, p. 577664, set. 2021.

NAGPAL, R. et al. Gut microbiome and aging: Physiological and mechanistic insights. **Nutrition and Healthy Aging**, v. 4, n. 4, p. 267–285, 1 jan. 2018.

OREJA-GUEVARA, C. et al. TH1/TH2 Cytokine profile in relapsing-remitting multiple sclerosis patients treated with Glatiramer acetate or Natalizumab. **BMC Neurology**, v. 12, n. 1, p. 95, 18 dez. 2012.

OSTOLAZA IBÁÑEZ, A.; CORROZA LAVIÑETA, J.; AYUSO BLANCO, T. Immunosenescence: the role of age in multiple sclerosis. **Neurología (English Edition)**, mar. 2022.

PALMER, C. S. et al. Metabolically active CD4+ T cells expressing Glut1 and OX40 preferentially harbor HIV during in vitro infection. **FEBS Letters**, v. 591, n. 20, p. 3319–3332, 1 out. 2017.

RAMMOHAN, K. et al. The Development of Cladribine Tablets for the Treatment of Multiple Sclerosis: A Comprehensive Review. **Drugs**, v. 80, n. 18, p. 1901–1928, 1 dez. 2020.

RANGANATH, T. et al. Characterization of the Impact of Daclizumab Beta on Circulating Natural Killer Cells by Mass Cytometry. **Frontiers in Immunology**, v. 11, 24 abr. 2020.

RAWJI, K. S. et al. Immunosenescence of microglia and macrophages: impact on the ageing central nervous system. **Brain : a journal of neurology**, v. 139, n. Pt 3, p. 653–661, 1 mar. 2016.

REDER, A. T. et al. Monocyte activation in multiple sclerosis. **Multiple Sclerosis Journal**, v. 4, n. 3, p. 162–168, 2 jun. 1998.

RUCK, T. et al. CD4+NKG2D+ T Cells Exhibit Enhanced Migratory and Encephalitogenic Properties in Neuroinflammation. **PLoS ONE**, v. 8, n. 11, p. e81455, 25 nov. 2013.

SARETZKI, G. Telomeres, Telomerase and Ageing. **Subcellular Biochemistry**, v. 90, p. 221–308, 2018.

SCHWICHTENBERG, S. C. et al. Fingolimod Therapy in Multiple Sclerosis Leads to the Enrichment of a Subpopulation of Aged NK Cells. **Neurotherapeutics**, v. 18, n. 3, p. 1783–1797, 9 jul. 2021.

SHAW, A. C.; GOLDSTEIN, D. R.; MONTGOMERY, R. R. Age-dependent dysregulation of innate immunity. **Nature reviews. Immunology**, v. 13, n. 12, p. 875–887, dez. 2013.

SHU, M.-J.; LI, J.; ZHU, Y.-C. Genetically predicted telomere length and multiple sclerosis. **Multiple Sclerosis and Related Disorders**, v. 60, p. 103731, abr. 2022.

SIEWERT, K. et al. Unbiased identification of target antigens of CD8+ T cells with combinatorial libraries coding for short peptides. **Nature medicine**, v. 18, n. 5, p. 824–828, maio 2012.

SOTO-HEREDERO, G. et al. Extremely Differentiated T Cell Subsets Contribute to Tissue Deterioration During Aging. **Annual Review of Immunology**, v. 41, n. 1, p. 181–205, 26 abr. 2023.

STEPHAN, D. et al. TWEAK and TNFα, Both TNF Ligand Family Members and Multiple Sclerosis-Related Cytokines, Induce Distinct Gene Response in Human Brain Microvascular Endothelial Cells. **Genes**, v. 13, n. 10, p. 1714, 24 set. 2022.

STRIOGA, M.; PASUKONIENE, V.; CHARACIEJUS, D. CD8+ CD28− and CD8+ CD57+ T cells and their role in health and disease. **Immunology**, v. 134, n. 1, p. 17–32, set. 2011.

SY, M. et al. N-acetylglucosamine inhibits inflammation and neurodegeneration markers in multiple sclerosis: a mechanistic trial. **Journal of Neuroinflammation**, v. 20, n. 1, p. 209, 13 set. 2023.

TALBOT, J. et al. Relationship between cerebrospinal fluid biomarkers of inflammation and tissue damage in primary progressive multiple sclerosis. **Multiple Sclerosis and Related Disorders**, v. 68, p. 104209, dez. 2022.

TOMAS-OJER, P. et al. Characterization of Antigen-Induced CD4+ T-Cell Senescence in Multiple Sclerosis. **Frontiers in Neurology**, v. 13, 3 fev. 2022.

VAN COILLIE, S.; WIERNICKI, B.; XU, J. Molecular and Cellular Functions of CTLA-4. Em: [s.l: s.n.]. p. 7–32.

VANDEBERGH, M.; DUBOIS, B.; GORIS, A. Effects of Vitamin D and Body Mass Index on Disease Risk and Relapse Hazard in Multiple Sclerosis. **Neurology - Neuroimmunology Neuroinflammation**, v. 9, n. 3, p. e1165, 7 maio 2022.

VON ZGLINICKI, T. Oxidative stress shortens telomeres. **Trends in Biochemical Sciences**, v. 27, n. 7, p. 339–344, 1 jul. 2002.

WEKERLE, H. B cells in multiple sclerosis. **https://doi.org/10.1080/08916934.2017.1281914**, v. 50, n. 1, p. 57–60, 2 jan. 2017.

WINGER, R. C.; ZAMVIL, S. S. Antibodies in multiple sclerosis oligoclonal bands target debris. **Proceedings of the National Academy of Sciences of the United States of America**, v. 113, n. 28, p. 7696–7698, 12 jul. 2016.

WISGALLA, A. et al. Alterations of NK Cell Phenotype During Pregnancy in Multiple Sclerosis. **Frontiers in Immunology**, v. 13, 4 jul. 2022.

ZANGHÌ, A. et al. Editorial: Immunosenescence and multiple sclerosis: Prognostic and therapeutic implications. **Frontiers in Neurology**, v. 13, 30 ago. 2022.

ZHENG, M. et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. **Cellular & Molecular Immunology**, v. 17, n. 5, p. 533–535, 19 maio 2020.

ZUROFF, L. et al. Immune aging in multiple sclerosis is characterized by abnormal CD4 T cell activation and increased frequencies of cytotoxic CD4 T cells with advancing age. **eBioMedicine**, v. 82, p. 104179, ago. 2022.

Pontifícia Universidade Católica do Rio Grande do Sul
Pró-Reitoria de Pesquisa e Pós-Graduação
Av. Ipiranga, 6681 – Prédio 1 – Térreo
Porto Alegre – RS – Brasil
Fone: (51) 3320-3513
E-mail: propesq@pucrs.br
Site: www.pucrs.br