UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM PSIQUIATRIA E CIÊNCIAS DO COMPORTAMENTO

TESE DE DOUTORADO

O PAPEL DA POLARIZAÇÃO DE MACRÓFAGOS NO TRANSTORNO BIPOLAR

Bruna Maria Ascoli

Orientadora: Profa. Dra. Adriane Ribeiro Rosa

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Aos meus maiores doutores da vida: Moacir Ascoli e Carmem Helena Ortolan Ascoli

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“If I can't feel, if I can't move, if I can't think, and I can't care, then what conceivable point is there in living?”

Kay Redfield Jamison

SUMÁRIO

Lista de abreviaturas

5-HT: Serotonina

BDNF: do inglês, *Brain derived neurotrophic factor*, Fator neurotrófico derivado do cérebro

BHE: Barreira hematoencefálica

DAMPs: do inglês, *Damage-associated molecular patterns*

EROs: Espécies reativas de oxigênio

IDO: Indoleamina 2,3-dioxigenase

IL: Interleucina

KYN: Quinurenina

LPS: Lipopolissacarídeo

MCH-II: Complexo principal de histocompatibilidade de classe II

NFκB: do inglês, *Nuclear factor κB*, Fator de transcrição nuclear kappa B

PCR: Proteína C-reativa

SNC: Sistema nervoso central

TB: Transtorno bipolar

TDO: Triptofano 2,3-dioxigenase

TLR: do inglês, *Toll-like receptor*, Receptor toll-like

TNF-α: do inglês, *Tumor necrosis factor-α,* Fator de necrose tumoral-α

RESUMO

A disfunção do sistema imune inato e a neuroinflamação tem sido cada vez mais reconhecidas como elementos importantes na fisiopatologia do transtorno bipolar (TB). Como componentes essenciais da imunidade inata, os macrófagos tem múltiplas funções tanto na inibição como na promoção da proliferação celular e na reparação tecidual, sendo a diversidade e a plasticidade características marcantes deste tipo celular. A polarização M1 clássica e a polarização alternativa M2 de macrófagos representam dois extremos de um estado dinâmico na mudança da ativação dos mesmos. Os macrófagos do tipo M1 sintetizam citocinas pró-inflamatórias que inibem a proliferação de células circundantes e danificam tecidos, enquanto os macrófagos do fenótipo M2 liberam citocinas antiinflamatórias que podem promover reparo tecidual. Um desequilíbrio da polarização M1-M2 dos macrófagos é frequentemente associado a várias doenças ou condições inflamatórias. O objetivo desta tese foi, além de revisar a importância da inflamação sistêmica na modulação da resposta inflamatória da microglia/macrófagos e consequentemente seu potencial envolvimento na fisiopatologia do TB, avaliar o perfil de polarização M1/M2 em cultura de macrófagos de sujeitos com TB comparados a indivíduos saudáveis. Monócitos foram isolados a partir de sangue periférico de dez sujeitos com TB e dez indivíduos saudáveis e diferenciados em macrófagos através da adição de fator estimulante de colônia de macrófagos (M-CSF) ao meio de cultura. Para induzir a polarização M1 ou M2, as culturas foram incubadas com IFN-y e LPS ou IL-4 respectivamente. Após a incubação, recolheram-se os sobrenadantes e mediram-se as citocinas (IL-1β, IL-6, IL-10 e TNF-α) por ensaio multiplex. A secreção das citocinas IL-1β, TNF-α e IL-6 características do protótipo M1 e citocinas IL-10 do protótipo M2 foram semelhantes entre os pacientes e os controles. Utilizou-se a razão TNF-α / IL-10 do fenótipo M1 para refletir o estado inflamatório dos participantes. Não foi observada diferença entre os grupos (p=0,627). Duas hipóteses diferentes poderiam explicar esses resultados: todos os pacientes incluídos neste estudo representam um estágio inicial da doença como evidenciado pela pontuação FAST total inferior a 11. De acordo com o modelo de estadiamento em TB, as alterações biológicas (incluindo a inflamação) parecem estar relacionadas com os episódios de humor e progressão

da doença. Juntamente com estudos anteriores, os nossos dados sugerem que os pacientes nos estágios iniciais ainda preservam a função do sistema imunológico sem apresentar um desequilíbrio a favor do perfil de macrófagos M1 como tem sido observado em pacientes no estágio tardio, destacando a relevância da intervenção precoce no TB. Ainda, estes pacientes estavam em tratamento com estabilizadores de humor e é plausível especular que esses fármacos exerçam efeitos sobre a polarização de macrófagos. Estudos futuros em pacientes *drug-free* são essenciais para avaliar esta questão. Em conclusão, nossos achados sugerem que os pacientes TB não apresentam desequilíbrio na polarização dos macrófagos em favor do fenótipo pró-inflamatório M1. O fato de todos estes pacientes estarem em estágios iniciais da doença reforça os efeitos protetores da intervenção precoce no TB na prevenção de alterações do sistema imune e, consequentemente, na progressão da doença.

Palavras-chave: transtorno bipolar, inflamação, polarização de macrófagos

ABSTRACT

Innate immune system dysfunction and neuroinflammation have been recognized as important elements in the pathophysiology of bipolar disorder (BD). As essential players of innate immunity, macrophages have multiple roles in inhibition and promotion of cell proliferation and tissue repair. The classical M1 polarization and the M2 alternative polarization of macrophages represent two extremes of a dynamic state in their change of activation. M1 macrophages synthesize proinflammatory cytokines that inhibit the proliferation of surrounding cells and damage tissues, whereas macrophages of the M2 phenotype release anti-inflammatory cytokines that may promote tissue repair. An imbalance of the M1-M2 polarization of macrophages is often associated with various diseases or inflammatory conditions. The aim of this thesis was to review the importance of systemic inflammation in modulating the inflammatory response of microglia/ macrophages and consequently their potential involvement in the pathophysiology of BD, and also evaluate the M1/M2 polarization profile in macrophages of patients with BD compared to healthy individuals. Blood monocytes were obtained from ten BD patients and ten healthy controls. These cells were activated/polarized into the M1 (IFNγ + LPS) or M2(IL-4) phenotype. Supernatants were collected and the cytokines (IL-1β, IL-6, IL-10 and TNF-α) were measured by multiplex assay. Secretion of the IL-1β, TNF-α, IL-6 and IL-10 were similar between patients and controls. The TNF-α/IL-10 ratio of the M1 phenotype was used to reflect the inflammatory state of the participants. There was no difference between groups ($p = 0.627$). Two hypotheses could explain these results: all patients included in this study represent an early stage of disease as evidenced by the FAST score below 11. According to the BD staging model, biological changes (including inflammation) appear to be related to mood episodes and disease progression. Together with previous studies, our data suggest that patients in early stages of BD still preserve immune system function without presenting an imbalance in favor of M1 macrophages as has been observed in late-stage patients, highlighting the relevance of early intervention. Moreover, these patients were under treatment with mood stabilizers and it is plausible to speculate that these drugs have effects on macrophage polarization. Future studies in drug-free patients are essential to assess this issue. In conclusion, our findings suggest that

BD patients do not present imbalance in macrophage polarization in favor of the M1 proinflammatory phenotype. The fact that all these patients are in the early stages of the disease reinforces the protective effects of early intervention in BD to prevent changes in the immune system and, consequently, prevent the progression of the disease.

Keywords: bipolar disorder, inflammation, macrophage polarization

1 INTRODUÇÃO

O transtorno bipolar (TB) é uma doença mental grave, associada a um elevado prejuízo cognitivo e funcional além de altos custos de cuidados em saúde e mortalidade prematura (1). A hipótese de que as alterações na plasticidade e na resiliência neuronal possam determinar o início de transtornos de humor tem ganhado impulso com base em achados recentes sobre as bases fisiopatológicas dessas doenças (2). A progressão da doença e, consequentemente, a ocorrência de múltiplos episódios leva ao prejuízo da homeostase com desencadeamento de um processo inflamatório – tanto em nível central quanto periférico – que associado ao aumento de fatores pró-inflamatórios e à diminuição de neurotrofinas, acomete os mecanismos de neuroproteção. Assim, há um aumento da vulnerabilidade individual ao estresse psicológico, à atrofia cerebral e ao déficit cognitivo (3).

Uma das hipóteses que tem emergido recentemente é a significativa contribuição de células do sistema imune e sua sinalização alterada, no balanço entre promoção e supressão da inflamação em indivíduos com transtornos psiquiátricos. Distúrbios imunológicos têm sido associados à progressão da doença evidenciada por múltiplos episódios, maior duração da doença e comorbidades médicas (4). A inflamação sistêmica está diretamente relacionada à ativação de macrófagos e ao aumento da produção de citocinas pró-inflamatórias no sistema nervoso central (SNC).

Embora muitos avanços tenham sido feitos nas últimas décadas, pouco se sabe sobre os mecanismos fisiopatológicos envolvidos na progressão do TB. A melhor compreensão de tais mecanismos, sem dúvida, ajudaria no desenvolvimento de novos alvos terapêuticos. Esse tratamento teria como objetivo prevenir recaídas, bem como, tratar indivíduos em estágios mais avançados do transtorno, que, em geral, respondem pobremente aos tratamentos disponíveis.

2 REFERENCIAL TEÓRICO

2.1 Transtorno Bipolar

O TB é uma doença psiquiátrica crônica, complexa e multifatorial, caracterizada pela ocorrência de episódios de humor chamados de mania e depressão, aliados a períodos de remissão de sintomas denominados eutimia (5). Segundo a Organização Mundial de Saúde (OMS) o TB é a sexta principal causa de incapacitação dentre todas as condições médicas (6). O TB é responsável por um quarto das ocorrências de suicídio, apresentando um risco aumentado em quinze vezes nos pacientes em relação à população em geral (6). Além disso, pacientes com esta doença sofrem com comorbidades psiquiátricas e médicas, trazendo prejuízos e custos significativos para o portador e para a sociedade (7).

De acordo com o Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-5), o TB é classificado em dois subtipos. O TB tipo I é caracterizado por um curso clínico no qual há a presença de um ou mais episódios de mania ou episódios mistos, intercalados frequentemente com episódios depressivos. Por outro lado, o TB tipo II possui como característica marcante a ocorrência de episódios depressivos maiores e episódios hipomaníacos (8).

Os episódios maníacos são caracterizados por períodos de humor elevado, euforia e/ou irritabilidade com duração de no mínimo sete dias, associado a três ou mais dos seguintes sintomas: distratibilidade, hiperatividade, agitação psicomotora, pensamentos acelerados, grandiosidade, agressividade, diminuição da necessidade de sono, taquilalia e impulsividade. Estes comportamentos excessivos são suficientemente graves para causar prejuízo acentuado ao funcionamento social ou profissional, podendo muitas vezes resultar em hospitalização com o objetivo de prevenir danos ao paciente ou a terceiros. A hipomania consiste em um estado de euforia, porém menos grave que a mania e em geral deve causar prejuízos funcionais de intensidade leve no âmbito familiar, social ou ocupacional. Também não apresenta sintomas psicóticos nem requer necessidade de internação, portanto diferindo dos sintomas normalmente apresentados na mania (9).

Em contrapartida, nos episódios depressivos é observado um período de pelo menos duas semanas na presença de cinco ou mais sintomas como: humor deprimido, alterações de sono e apetite, retardo psicomotor, baixa autoestima, redução da velocidade de fala e pensamento e ideação suicida. A gravidade dos sintomas de humor, tanto na mania/hipomania quanto na depressão, bem como a duração dos episódios, pode variar de maneira considerável conforme o paciente (9).

Além das alterações de humor bem estabelecidas, altas taxas de morbidade e mortalidade são observadas em indivíduos com TB. Entre as comorbidades psiquiátricas mais frequentemente observadas nos pacientes estão os transtornos de ansiedade e personalidade e o abuso de álcool e drogas (9,10). Em relação às comorbidades médicas, dados clínicos e epidemiológicos relatam que mais da metade dos indivíduos com TB estão acima do peso ou obesos e um terço sofre com a síndrome metabólica, associada ao desenvolvimento de doenças cardiovasculares, derrame e diabetes *mellitus* do tipo II (7). Pacientes com essas comorbidades apresentam pior prognóstico com respostas menos favoráveis ao tratamento e maiores taxas de desemprego, representando, portanto, maiores custos para a sociedade, quando comparados a pacientes sem comorbidades. Deste modo, estas alterações metabólicas tornam-se favoráveis à progressão da doença e devem contribuir para uma redução na expectativa de vida entre 9-20 anos (11). Embora os mecanismos fisiopatológicos subjacentes à ligação entre o TB e comorbidades médicas sejam provavelmente multifatoriais, a disfunção imunológica foi recentemente proposta como um nexo chave.

Com base nos recentes avanços na elucidação da fisiopatologia do TB, alguns pesquisadores tem sugerido que esta doença segue um curso progressivo desde a forma latente até as apresentações mais graves (12,13). De acordo com esses modelos a doença começa com um período de risco, evoluindo a partir do primeiro episódio de humor até a doença em estágio tardio, quando os sintomas são mais crônicos e resistentes ao tratamento. Esta progressão pode ser resultado de alterações cerebrais relacionadas à ação de mediadores neurotóxicos, redução do aporte de neurotrofinas e suporte neuroprotetor. De acordo com este conceito, pacientes bipolares em estágios mais avançados da doença tendem a ter um pior

prognóstico, com mais déficits cognitivos e marcado prejuízo psicossocial, além de exigir alternativas de tratamento mais dispendiosas (14).

O TB geralmente se manifesta entre o fim da adolescência e início da idade adulta, em indivíduos com idade entre 18 e 25 anos. Segundo dados do CDC, o TB possui uma prevalência mundial aproximada de 1-2%, sendo 0,9% no Brasil (15,16). Quanto à proporção de gênero, o TB tipo I possui razão igual entre homens e mulheres, enquanto que o TB tipo II parece acometer mais o sexo feminino. Além disso, as mulheres são mais suscetíveis a estados de ciclagem rápida e mistos, bem como possuem maior probabilidade de apresentar sintomas depressivos (9).

2.2 Inflamação no TB

Sem possuir etiologia única definida, o TB é considerado uma doença multifatorial. Desta forma, acredita-se que exista um conjunto de elementos capazes de promover e facilitar a progressão da doença. Atualmente, já foram descritos fatores como o estresse oxidativo e disfunção mitocondrial, prejuízo do aporte de neurotrofinas, alterações estruturais e funcionais no cérebro, além da contribuição de fatores genéticos (17–19). O TB é caracterizado pela progressão temporal de sintomas, isto é, aumento da frequência e severidade dos episódios de humor e concomitante diminuição da resposta ao tratamento. Além disso, estudos clínicos têm mostrado importantes deficiências cognitivas e funcionais em pacientes bipolares, mesmo entre aqueles que estão em tratamento (20,21). O termo “neuroprogressão” tem sido aplicado para se referir a esta progressão temporal com base na noção clínica de estadiamento que se aplica, por exemplo, na oncologia e na medicina interna. Este termo tem sido cada vez mais utilizado para definir a reorganização patológica do SNC que ocorre ao longo do curso de transtornos mentais graves, como resultado de diversos insultos, dentre eles, a inflamação (22).

A disfunção do sistema imune inato e a neuroinflamação têm sido cada vez mais reconhecidas como elementos importantes na fisiopatologia de numerosos distúrbios psiquiátricos, incluindo o TB (4,23). A inflamação parece ser um forte nexo entre o TB e a alta prevalência de comorbidades clínicas observada nestes pacientes. Nos últimos anos, demonstrou-se que a disfunção imune é parte integrante da doença cardiovascular, da obesidade e da resistência à insulina, uma vez que um estado inflamatório crônico de baixo grau também tem sido associado a essas doenças (25). O aumento dos níveis de citocinas pró-inflamatórias tem sido consistentemente demonstrado tanto para as doenças cardiovasculares como para a disfunção metabólica. Além disso, níveis dos marcadores inflamatórios como a proteína C-reativa (PCR) têm sido usados clinicamente como um indicador prognóstico forte para desfechos piores (25). Em nível molecular, a aterosclerose tem se mostrado um processo imunomediado. A obesidade e o diabetes *mellitus* tipo II aumentam a inflamação através do aumento dos níveis de adipocinas (citocinas pró-inflamatórias produzidas principalmente pelos tecidos adiposos), aumentando a

disfunção mitocondrial e aumentando a produção de espécies reativas de oxigênio, resultando em estresse oxidativo e resposta inflamatória (24).

Em vista do crescente número de evidências sugerindo a presença de uma inflamação crônica leve em indivíduos com TB, tanto na periferia quanto em nível central, é que este transtorno tem sido definido como uma doença inflamatória multisistêmica por alguns autores (4,25). Níveis elevados de citocinas pró-inflamatórias já foram relatados em sujeitos com TB, e estes achados já foram replicados em diversos estudos e metanálises. Por exemplo, Kim e colaboradores encontraram um aumento dos níveis séricos de interleucina-6 (IL-6) e do fator de necrose tumoral- α (TNF-α) em pacientes maníacos, quando comparados a um grupo controle de indivíduos saudáveis. Ainda, os níveis de IL-6 retornaram ao normal após seis semanas de tratamento (26). O'Brien e colaboradores também encontraram associação estatisticamente significativa entre os episódios de humor e TNF-α, mesmo em pacientes medicados (27).

Uma metanálise recente mostrou níveis séricos elevados de interleucinas (IL) IL-1β, IL-4, IL-10, TNF-α, receptor solúvel da IL-2 (sIL-2R), receptor solúvel da IL-6 (sIL-6R) e do receptor solúvel 1 do TNF-α (sTNFR1) em indivíduos com TB quando comparados a controles saudáveis (23). Resultados semelhantes foram publicados por outro grupo, que reportou a elevação de TNF-α, sIL-2R, sIL-6R, sTNFR1 e IL-4. No entanto, aumentos nos níveis de IL-1β e IL-10 não foram significativos (28).

De uma maneira geral, os episódios de mania e depressão têm sido caracterizados como estados pró-inflamatórios. Neste contexto, os mediadores da inflamação parecem atuar como agentes tóxicos, contribuindo para a as modificações que ocorrem nesse transtorno, tais como os prejuízos cognitivos e comorbidades clínicas. Os níveis de citocinas parecem variar também de acordo com fatores como estágio da doença e tratamento (29). Em um estudo de Kauer-Sant'Anna e colaboradores os níveis séricos de IL-10, uma citocina com propriedades antiinflamatórias, diminuíram ao longo da progressão da doença, enquanto as citocinas pró-inflamatórias IL-6 e TNF-α permaneceram elevadas tanto em pacientes nos estágios iniciais quanto em pacientes nos estágios tardios (30).

A PCR é uma proteína de fase aguda produzida em resposta a um estímulo inflamatório e é majoritariamente induzida pelas citocinas pró-inflamatórias IL-6 e IL-1β (31). Um aumento das concentrações de PCR já foi relatado em metanálises de estudos transversais em esquizofrenia e depressão (32,33). Recentemente, uma metanálise comparando 2.161 indivíduos com TB e 81.932 controles saudáveis mostrou que as concentrações de PCR estão moderadamente aumentadas em indivíduos com TB durante a depressão e eutimia e mais substancialmente aumentadas durante a mania. Além disso, após a resolução do espisódio maníaco, as concentrações de PCR foram moderadamente diminuídas e, após a resolução de um episódio depressivo, ligeiramente diminuídas Estes achados fornecem mais evidências da presença de inflamação no TB (32).

A morte celular também parece estar associada com o TB e, provavelmente, envolvida na sua fisiopatologia (34). Por exemplo, um trabalho recente mostrou um aumento de apoptose precoce em células mononucleares do sangue de pacientes com TB (35). Stertz et al. (2015) mostrou que existe um aumento de DAMPs (*damage-associated molecular patterns*) no soro de pacientes com TB (36). As DAMPs são constituintes celulares que podem ser identificadas pelo sistema imune inato, cuja liberação é deflagrada por processos de estresse, lesão ou morte celular. Elas incluem açúcares, metabólitos, lipídeos e ácidos nucléicos, tais como DNA e RNA, que podem ligar-se a receptores *toll-like* (TLRs), ativando vários mecanismos de sinalização que culminam em resposta inflamatória (37), como por exemplo, a ativação clássica de macrófagos (M1). O aumento dessas moléculas corrobora evidências de aumento da morte celular em pacientes com TB, proporcionando uma ligação entre a TB, ativação imune e toxicidade sistêmica.

Citocinas, proteínas de fase aguda e outros fatores químicos e celulares têm sido extensivamente estudados nos transtornos psiquiátricos. Alterações nestes biomarcadores, tais como um aumento nos mediadores inflamatórios periféricos e centrais, sugerem que a inflamação sistêmica persistente, embora em baixo grau, deve promover uma alteração na integridade do SNC. Na verdade, a disfunção crônica do sistema imune associada a outros fatores como estresse oxidativo e disfunção mitocondrial e alterações nos fatores neurotróficos deve ser a causa ou

consequência da neuroprogressão em doenças psiquiátricas crônicas, como por exemplo, o TB.

A barreira hematoencefálica (BHE) é um importante componente que conecta o SNC e os tecidos periféricos, além de funcionar como uma interface que limita e regula a troca de substâncias entre a periferia e o SNC. A disfunção da BHE tem sido descrita há muito tempo como um elemento-chave da progressão de várias doenças do SNC. Provavelmente isto esteja relacionado à exposição do micro-ambiente cerebral a substâncias potencialmente nocivas, podendo resultar em uma perda da homeostase, comprometimento da oferta de sinalização neuronal e morte celular (38).

Durante a inflamação crônica, o aumento da liberação de citocinas pró-inflamatórias aumenta a permeabilidade microvascular levando à migração de leucócitos para o parênquima cerebral. Essa infiltração desencadeia a liberação de mais citocinas que intensificam o rompimento da BHE e a inflamação do SNC através da ativação das células microgliais (39). A ativação da microglia e a subsequente liberação de mediadores inflamatórios pode modular a expressão de moléculas de adesão nas células endoteliais, o que estimula o recrutamento de células mielóides do sangue para o cérebro (40).

Considerando a inflamação como um elemento importante na neuroprogressão do TB, Patel e Frey sugeriram um modelo de disfunção da BHE no TB. Neste modelo, a perda persistente ou temporária da integridade da BHE é associada à diminuição da proteção do SNC, com aumento da permeabilidade de mediadores pró-inflamatórios do sangue periférico para o cérebro, provocando ativação microglial e promovendo dano (41).

2.3 Polarização de macrófagos/microglia no TB

O sistema imune inato é a primeira linha de defesa durante um processo de infecção e fornece uma rápida resposta a patógenos microbianos e injúria tecidual (42). O sistema imune adaptativo, por outro lado, é responsável pela eliminação de agentes nas fases posteriores da infecção (43). As células fagocíticas, tais como os macrófagos, representam um componente essencial do sistema imune inato, e prejuízos à atividade dessas células podem resultar em inflamação crônica (43,44).

A microglia, que é considerada o macrófago residente do parênquima cerebral, é responsável pela manutenção da homeostase no microambiente do SNC, tem demostrado um papel importante na etiologia dos transtornos psiquiátricos (45). A microglia responde a eventos patológicos no SNC, tais como, os desencadeados pelo TB, através de uma série de respostas celulares, como alterações morfológicas e funcionais, conhecidas como ativação microglial (46). Estas respostas incluem modificações na expressão e na síntese de receptores de membrana, e aumento na produção de citocinas e outros mediadores inflamatórios. Agudamente, essas respostas fazem parte de um mecanismo adaptativo que contribui para o manejo do estresse, limitando os danos teciduais e reestabelecendo a homeostase. Entretanto, quando cronicamente ativada, a microglia passa a produzir quantidades elevadas de fator de necrose TNF-α e IL-1β, levando a inflamação tecidual, apoptose neuronal e alteração na síntese de neurotransmissores (31,47–49).

A microglia, e da mesma forma os macrófagos, apresenta-se em diversos fenótipos de ativação – M0, M1 e M2. O fenótipo M0 é característico da microglia em um estado, equivocadamente, denominado de repouso. Este fenótipo é caracterizado principalmente pela constante vigilância do microambiente do SNC, além de estar relacionado à liberação do fator de crescimento semelhante à insulina 1 (IGF-1) e do BDNF – fatores neurotróficos muito importantes. Desta forma, pode-se considerar que a microglia não está em pleno repouso, e que M0 é um fenótipo protetor atenuado (50). Entretanto, quando alguma injúria ou patógeno atinge o SNC, a microglia é a primeira célula a efetuar uma resposta imune inata e promover uma ativação inflamatória fisiológica. O fenótipo que caracteriza este estado é o M1, ativado principalmente por lipopolissacarídeo (LPS), interferon γ (INF-γ) e TNF-α (50). A polarização microglial para M1 é caracterizada pela intensa produção de

espécies reativas de oxigênio e nitrogênio (EROs e ERNs) e secreção de citocinas pró-inflamatórias, as interleucinas (IL), tais como IL-1β, IL-2 e IL-6, e quimiocinas, como a proteína quimiotática de monócitos-1 (MCP-1, atualmente denominada CCL2) (51). Por isso, M1 é descrito como um fenótipo pró-inflamatório. A ativação M1 é caracterizada por um aumento na expressão de diversos receptores de membrana, como o complexo principal de histocompatibilidade de classe II (MHC-II), CD32, CD80, CD86 e CD68 (50). A inflamação no SNC, também denominada neuroinflamação, ocorre inicialmente como um mecanismo protetor responsivo ao dano. Entretanto, se este processo inflamatório for prolongado ou intenso, ele pode ser nocivo ao parênquima cerebral e causar morte celular (51).

Em condições normais, este evento patológico pode ser evitado pela polarização microglial para M2. Devido a sua plasticidade, a microglia é capaz de polarizar para este outro fenótipo ativado, considerado antiinflamatório (45). Também denominada como ativação alternativa, a polarização M2 possui subdivisões relacionadas com a função e tipo de moléculas (citocinas, quimiocinas e receptores) produzidas e expressas nessa situação. Quando ativada por IL-4 ou IL-13, a microglia desenvolve um fenótipo M2a, com potentes características anti-inflamatórias. Esse fenótipo tem como particularidade a *upregulation* da enzima arginase-1, inibição das isoformas do fator de transcrição nuclear kappa B (NFκB), aumento na expressão de CD163 e CD206 e produção de receptores de fagocitose (52,53). A polarização para M2b é a menos conhecida e ocorre frente à estimulação dos receptores TLRs induzida por complexos imunes ou LPS (45). A exposição a IL-10 e fator de crescimento transformante β (TFG-β), direciona a microglia para o fenótipo M2c (50,51). Este fenótipo exerce atividades relacionadas ao remodelamento tecidual e deposição de matriz extracelular após a atenuação do processo inflamatório (51).

Em um estudo qualitativo *post mortem*, Bayer et al. (1999) observou uma abundante ativação microglial (expressão de HLA-DR, um MHC-II) no córtex pré-frontal de pacientes com transtorno afetivo e esquizofrenia, em estágios mais avançados, quando comparados aos indivíduos controles (sem sinal de microgliose) (54). Este mesmo padrão de ativação foi observado por Radewicz et al. (2000) em pacientes esquizofrênicos crônicos (55). O perfil de ativação microglial, descrito nas

doenças psiquiátricas, foi relacionado com a ocorrência de suicídio por Steiner et al. (2008). Nesse estudo observou-se uma marcada microgliose no córtex pré-frontal e tálamo nos pacientes que cometeram suicídio (56).

Uma vez ativada, a microglia apresenta alterações fenotípicas como, por exemplo, o aumento da expressão de uma proteína de membrana de 18 kDa conhecida como proteína translocadora (TSPO). Essa proteína está localizada exclusivamente nas células da microglia e astrócitos do parênquima cerebral, e tem sido utilizada como um biomarcador de inflamação do SNC (57). Um único estudo em TB, que investigou neuroinflamação, através da marcação de TSPO, foi recentemente publicado por Haarman et al. (2014). Os autores demonstraram um aumento na captação dos raios gama da sonda [(11)C]-(R)-PK11195, utilizada para avaliar a ativação da microglia, no hipocampo de pacientes bipolares, quando comparados a indivíduos saudáveis (58).

A neuroinflamação está intimamente relacionada com o estresse oxidativo. Tanto a microglia quanto os astrócitos ativados podem produzir EROs que podem contribuir para dano neuronal. Devido ao alto consumo de oxigênio e à capacidade antioxidante relativamente baixa dessa estrutura, o SNC é mais vulnerável à lesão oxidativa do que outros tecidos (59). O aumento dos níveis oxidativos neuronais pode ter efeitos deletérios na transdução de sinal, plasticidade e resiliência celular, além de estarem relacionados à apoptose. Além disso, as citocinas são mediadores críticos do estresse oxidativo. As citocinas têm o potencial de alterar o equilíbrio redox, afetando o sistema antioxidante da glutationa, um dos principais responsáveis pelo equilíbrio redox, durante o estresse oxidativo (60,61).

As citocinas pró-inflamatórias produzidas por células da microglia quando há alguma perturbação da homeostase do SNC parecem exercer efeitos prejudiciais ao processo de formação de novos neurônios no hipocampo. A IL-1β é uma das citocinas pró-inflamatórias com maior atividade de inibição da neurogênese. As células progenitoras da zona subgranular possuem receptores para IL-1β, que age inibindo a proliferação das mesmas (62). Em um estudo recente, Zunszain et al. mostrou que a inibição da neurogênese induzida pela IL-1β pode estar relacionada com a via da quinurenina (63). A via da quinurenina é uma das vias metabólicas do triptofano, através da qual ocorre formação de quinurenina (KYN) ao invés de

serotonina (5-HT), diminuindo a síntese deste neurotransmissor. Este desvio da síntese de 5-HT para KYN ocorre pela ativação das enzimas indoleamina 2,3-dioxigenase (IDO) e triptofano 2,3-dioxigenase (TDO) (64). Outras citocinas também estão envolvidas na regulação desta via, por exemplo, INF-γ e TNF-α estimulam a atividade da IDO, enquanto a citocina anti-inflamatória IL-4 inibe a mesma (65).

A regulação da via da quinurenina afeta diretamente a disponibilidade de 5-HT no SNC, e pode estar relacionada aos sinais clínicos observados em transtornos de humor. De fato, pacientes com TB apresentaram maiores níveis de KYN e da razão KYN/triptofano quando comparados a indivíduos saudáveis (66). Além disso, em um estudo *post mortem* foram observadas atividade aumentada da TDO e maiores concentrações de KYN no córtex cingulado anterior de sujeitos com TB em comparação a controles (67).

2.4 Monócitos no TB

Monócitos são células efetoras do sistema imune que circulam no sangue periférico e representam a primeira linha de defesa do organismo. Estas células possuem receptores de quimiocinas e receptores capazes de reconhecer patógenos que atuam mediando a migração das mesmas do sangue para os tecidos durante infecções. Os monócitos produzem citocinas pró-inflamatórias e podem se diferenciar em células dendríticas ou macrófagos em resposta a determinados fatores de crescimento (69).

Nas últimas décadas, duas classes de monócitos foram definidas através da expressão dos receptores CD14 e CD16. As diferenças não existem apenas quanto ao seu fenótipo, mas também em relação a sua função. Hoje em dia, sabemos que existem três classes de monócitos, são elas: a clássica (90%, CD14++ e CD16-), e os outros 10% são divididos entre a intermediária (CD14++ e CD16+) e não-clássica (CD14+ e CD16++) (68,69). Os monócitos clássicos e intermediários apresentam uma atividade fagocitária e inflamatória alta, enquanto os monócitos não-clássicos apresentam uma função de monitoramento celular. Além disso, os monócitos não-clássicos são menores produtores de TNF-α, IL-1β e IL-6 após estímulo com LPS (69). É importante salientar que em situações de estresse agudo ou crônico, e durante a inflamação, nota-se um aumento no número de células imunes, um aumento no recrutamento de monócitos clássicos e uma migração acentuada desses monócitos em direção aos tecidos. Esses monócitos apresentam um maior poder de infiltração nos tecidos inflamados além de se diferenciar em macrófagos M1 quando estimulados por TNF-α. Isto facilita o aumento no número de macrófagos residentes durante a inflamação (44). Os monócitos não-clássicos podem se diferenciar em M2 após serem estimulados por IL-4, assim como a microglia (68–70). Portanto, o uso de monócitos a partir de sangue periférico de indivíduos com TB poderia auxiliar no entendimento da resposta dos macrófagos M0 a insultos que levem à polarização à M1 ou M2.

Em um estudo recente conduzido por Barbosa et al. (2014), mostrou-se que indivíduos com TB possuem uma maior proporção de monócitos (CD14+) em sangue periférico, sugerindo que existe uma ativação do sistema fagocítico mononuclear nesses pacientes (71). De maneira semelhante a este achado, Knijff et

al. (2007) mostraram que monócitos de pacientes com TB apresentam uma resposta pró-inflamatória alterada após estímulo com LPS, incluindo maior produção de IL-6, quando comparados a controles saudáveis (72). Níveis aumentados de citocinas pró-inflamatórias circulantes já foram relatados em pacientes bipolares, particularmente o TNF-α e seus receptores, além de outras ILs, tais como IL-6, IL-2R e IL-1β (23). Assim como as citocinas pró-inflamatórias são produzidas principalmente por células ativadas do sistema imune, alterações em leucócitos circulantes poderiam indicar a existência de um desequilíbrio imunológico no TB.

Brambilla et al. (2014) mediram os níveis de mRNA de quimiocinas, receptores de quimiocinas, citocinas e marcadores de células T-regulatórias (Tregs) em células mononucleares de pacientes com TB (70). Os marcadores da ativação microglial clássica (M1) IL-6 e CCL-3 foram significativamente aumentados nos pacientes com TB quando comparados a controles saudáveis, enquanto os marcadores da ativação alternativa (M2) CCL-1, CCL22 e IL-10 estavam diminuídos (70). Drexhage et al., (2011) também mostrou que existe um aumento do estado pró-inflamatório em monócitos de pacientes com TB, sugerindo a existência de um desequilíbrio entre os monócitos clássicos e não-clássicos neste transtorno (73).

Os monócitos desempenham uma função fundamental na manutenção da homeostase, principalmente na remoção de células apoptóticas e compostos tóxicos. Além disso, também são responsáveis por sintetizar inúmeras moléculas efetoras envolvidas na defesa contra microorganismos e no processo inflamatório de diversas doenças. Entretanto, sua função predominante é servir como fonte de macrófagos teciduais em doenças como infecções, aterosclerose, Alzheimer e tumores, entre outras (69).

2.5 Modelos de cultura celular de macrófagos

Considerando a contribuição importante do sistema monocítico-macrofágico para a imunidade inata, diversos modelos de cultura celular têm sido desenvolvidos com o objetivo de aumentar o entendimento da participação deste sistema em inúmeras doenças, tais como os modelos murinos. No entanto, a principal limitação da utilização de células provenientes de ratos ou camundongos são as diferenças fenotípicas já descritas entre macrófagos murinos e macrófagos humanos, o que sugere que estas células desempenham funções distintas nestas espécies. Desta forma, a extrapolação de resultados obtidos em macrófagos murinos para aplicação em seres humanos é pouco fidedigna (74).

Como alternativa às células animais são utilizadas linhagens representativas de monócitos humanos, como HL-60, THP-1 e U937. Estas linhagens podem ser obtidas facilmente sem a necessidade de punção venosa ou biópsia, porém, não são fenotipicamente e funcionalmente idênticas às células primárias humanas. Cho et al. compararam a assinatura genética de macrófagos primários humanos com dados previamente publicados da linhagem THP-1 e mostraram que a correlação existente entre estes dois tipos celulares é significativa, porém moderada (75–77).

A cultura primária de macrófagos humanos parece ser, portanto, a opção que melhor reflete a heterogeneidade deste tipo celular. No entanto, o isolamento de macrófagos teciduais apresenta uma série de desvantagens, como por exemplo, o baixo rendimento celular. Além disso, a obtenção de macrófagos provenientes de tecido depende de procedimentos invasivos, como lavados ou biópsias, o que afeta a viabilidade celular (78). Desta forma, o modelo de macrófagos diferenciados *in vitro* a partir de monócitos obtidos de sangue periférico tem sido bastante utilizado e bem aceito como uma forma adequada de investigar a biologia deste tipo celular.

Neste modelo, os leucócitos do sangue periférico são subfracionados e os monócitos são separados com base na sua característica de aderência a substratos artificiais como o plástico, permanecendo aderidos à superfície de placas de cultura celular. Após o isolamento dos monócitos, segue-se a diferenciação destes em macrófagos através da adição de fatores de crescimento específicos ao meio de cultura e em seguida a indução da polarização aos fenótipos M1 e M2. Nestes

protocolos utilizam-se fatores como o fator estimulador de colônia de macrófagos (M-CSF) para a diferenciação de monócitos em macrófagos e posteriormente citocinas como IFN-γ e IL-4 para polarização ao perfil M1 e M2 respectivamente (78,79)

3 JUSTIFICATIVA

O TB segue um curso progressivo desde as formas mais leves até as apresentações mais graves, que se caracteriza por uma maior frequência de episódios de humor, tempo de doença e menor responsividade aos tratamentos disponíveis. Embora grandes avanços tenham sido feitos nas últimas décadas, os mecanismos envolvidos na fisiopatologia do TB não estão completamente elucidados. Uma das hipóteses que tem emergido recentemente é a significativa contribuição de células do sistema imune e sua sinalização alterada no balanço entre promoção e supressão da inflamação em indivíduos com transtornos psiquiátricos. De fato, o estresse psicológico crônico presente nesses indivíduos causa, nos monócitos do sangue periférico, uma expressão de citocinas e quimiocinas pró-inflamatórias aumentada. Além disso, a inflamação sistêmica está diretamente relacionada à ativação de macrófagos e ao aumento da produção de citocinas pró-inflamatórias no sistema nervoso central. Considerando que estados de polarização M1 e M2 dos macrófagos desempenham um papel significativo na mudança da resposta imune para uma resposta pró-inflamatória ou antiinflamatória, o presente projeto visa investigar se a fisiopatologia do TB está relacionada a um desequilíbrio em favor de um perfil pró-inflamatório (macrófagos com um predomínio do fenótipo pró-inflamatório ou M1).

4 OBJETIVOS

4.1 Objetivo geral

O objetivo geral desta tese foi analisar alterações nas células do sistema imune e sua sinalização em cultura de macrófagos de sujeitos com TB comparados a indivíduos saudáveis.

4.2 Objetivos específicos

4.2.1 Revisar a importância da inflamação sistêmica na modulação da resposta inflamatória da microglia e consequentemente seu potencial envolvimento na fisiopatologia do TB.

4.2.2 Avaliar o perfil de polarização M1/M2 em sujeitos com TB comparados com um grupo controle.

5 ASPECTOS ÉTICOS

Todos os participantes deste estudo foram capazes de entender os instrumentos de pesquisa, bem como compreender e assinar o Termo de consentimento livre e esclarecido (TCLE). O grupo de pesquisa garantiu a confidencialidade das informações e os participantes estavam livres para decidir em qualquer momento sobre sua descontinuidade, independente do seu atendimento no Programa de Atendimento do Transtorno de Humor Bipolar (PROTAHBI) do Hospital de Clínicas de Porto Alegre (HCPA). Os princípios bioéticos de autonomia, beneficência, não-maleficência, veracidade e confidencialidade foram seguidos. O presente estudo foi aprovado pelo Comitê de Ética em Pesquisa do HCPA.

6 Artigos

6.1 Artigo 1

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Carta de aceite:

From: <journal.assist@sydney.edu.au>
Date: 2016-03-09 20:31 GMT-03:00
Subject: ANZJP: Decision for Manuscript ANP-2015-00659.R1
To: adrianerrosa@gmail.com

Dear Dr. Rosa,

Manuscript No: ANP-2015-00659.R1
Title: The role of macrophage polarization on bipolar disorder: identifying new therapeutic targets

We are pleased to advise that your paper has been accepted for publication in the Australian and New Zealand Journal of Psychiatry (ANZJP) and we encourage you to promote your research by citing your work widely and informing colleagues of your forthcoming article.
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Yours sincerely,

Prof. G.S. Malhi
Editor – ANZJP

Versão do manuscrito aceita:

The role of macrophage polarization on bipolar disorder: identifying new therapeutic targets

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Abstract

Objective: Bipolar disorder is a chronic, severe and disabling disease; however its pathophysiology remains poorly understood. Recent evidence has suggested that inflammation and immune dysregulation play a significant role in the neuroprogression of bipolar disorder. This review is aimed to highlight the importance of systemic inflammation in modulating the inflammatory response of microglia and hence its potential involvement with bipolar disorder.

Method: This article presents a theoretical synthesis of the effects of systemic inflammation on the immune response of the central nervous system in bipolar disorder. The complex relationship between stress, pro-inflammatory cytokines and microglial dysfunction is summarized, emphasizing the role of the kynurenine pathway in this process, and consequently, their effects on neuronal plasticity.

Results: Bipolar patients demonstrate increased serum levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and lower hypothalamic-pituitary-adrenal axis sensitivity. This imbalance in the immune system promotes a change in blood brain barrier permeability, leading to an inflammatory signal spread in the central nervous system from the periphery, through macrophages activation (M1 polarization). Chronic microglial activation can result in neuronal apoptosis, neurogenesis inhibition, hippocampal volume reduction, lower neurotransmitters synthesis and cytotoxicity, by increasing glutamate production and kynurenine metabolism.

Conclusions: This review suggests that an imbalance between M1 and M2 polarization of microglia may contribute to the pathophysiology of bipolar disorder. Activated microglia release a large amount of damage signals that may affect neuroplasticity with a negative impact on mood symptoms and treatment response. Therefore, therapeutic strategies that normalize the imbalance between M1 and M2 microglial polarization states may provide a valuable therapeutic target for the treatment of these disorders.

Keywords

Bipolar disorder, macrophage, polarization, microglia, inflammation

Introduction

Bipolar disorder (BD) is a chronic, recurrent illness that represents a major public health concern and often shows incomplete recovery and increased mortality (Vieta et al., 2011). By 2020, BD is estimated to become the sixth leading cause of time lost due to disability or death among those aged 15 to 55 years (Gore et al., 2011). Most importantly, the disability related to BD is not only restricted to the symptomatic phases but also occurs during periods of remission (Rosa et al., 2014). Furthermore, poor functioning has been strongly associated with cognitive impairment in bipolar patients (Bonnín et al., 2014).

The natural history of BD progression involves relapses, persistent symptoms, comorbidities, cognitive impairment and neurobiological changes (Berk et al., 2011; Kapczinski et al., 2008). Post et al. (1992) suggested that multiple episodes lead to permanent alterations in neuronal activity, which may be transduced at the level of greater liability to relapse and poorer treatment response (Post et al., 1992). Therefore, episode frequency and severity, together with an augmented sensitivity to stress factors, may increase with the passing of time or with each new recurrence (Kapczinski et al., 2008; Post et al., 1992). However, little is known about the pathophysiological mechanisms involved in the neuroprogression of BD. Undoubtedly, a better understanding of these mechanisms might not only help to predict treatment response but also improve outcome measures, such as cognitive and psychosocial functioning.

Emerging evidence had shown that BD is accompanied by the activation of immune-inflammatory pathways as indicated by the increased levels of pro-inflammatory cytokines, positive acute-phase proteins, complement factors and increased levels of T cell-related activation markers (Dargél et al., 2015; Rege and Hodgkinson, 2013). Immune disturbances have been associated with progression of the disorder evidenced by multiple episodes, longer duration of illness and medical comorbidities (Stertz et al., 2015). Nonetheless, systemic inflammation is directly related to macrophage activation and increased production of pro-inflammatory cytokines in the central nervous system (CNS). Th1 cytokines activate microglial enzyme tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO),

shifting microglial kynurenine (KYN) catabolism towards quinolinic acid (QUIN). This imbalance between QUIN and kynurenic acid (KYNA) synthesis promotes microglial susceptibility to stress which may be related to recurrence of new mood episodes and poor treatment response (Dantzer et al., 2008). In addition, BD is associated with disturbances in circadian rhythm and melatonin synthesis, facts that are related to the strength of CNS inflammatory signal (Etain et al., 2012).

This review summarizes the importance of systemic inflammation in modulating the inflammatory response of microglia and hence its potential involvement in the neuroprogression of BD. The authors also discuss novel therapeutic strategies that emerge from this new research.

Functioning of the hypothalamus-pituitary-adrenal (HPA) axis

Emerging evidence has shown the presence of an inflammatory profile in bipolar patients across all phases of the illness (Tsai et al., 2014). Compared to control subjects, patients with BD show an increase in the plasma concentration of interleukins (IL) such as IL-1 receptor antagonist, soluble IL-2 receptor, soluble tumor necrosis factor receptor 1 and C-reactive protein (CRP) (Tsai et al., 2014). Furthermore, an increase in IL-2 production and secretion is linked to activation of T-lymphocytes, more specifically, activation of Th1 lymphocytes, that exhibit inflammatory activity. The Th1 lymphocytes may trigger activation of the microglia, to activate the central inflammatory response and perform phagocytosis (Cherry et al., 2014). Recently, one study investigated inflammatory profile and functioning of HPA axis in patients with mood disorders. Their results showed that depressed men with lifetime hypomanic episodes had higher levels of CRP and cortisol than those without (hypo)mania (Becking et al., 2015). Thus, it has been proposed that cytokines secreted in chronic inflammation, may cross the blood-brain barrier (BBB) and reach the cerebrospinal fluid (CSF), spreading the inflammatory signal to the different regions of the CNS, including the HPA axis (Miller et al., 2013).

Increased levels of inflammatory cytokines can act by stimulating the HPA axis, as part of the physiological response to stress. The activation of the HPA axis is critical in situations of acute inflammation, with the purpose to fight infection through increased production of cortisol in humans, and corticosterone in rodents (Wright et al., 2005). However, in cases of chronic inflammation, the continued production of cytokines causes a reduction in expression and sensitivity of glucocorticoid receptors (GR) in the hypothalamus and pituitary. The GR are responsible for maintaining physiological negative feedback, therefore, in conditions of chronic stress, it is noted a reduction in this regulation. Systemically, the reduction in GR sensitivity also inhibits anti-inflammatory response triggered by cortisol. Interestingly, the insensitivity of GR has been independently associated with BD (Fries et al., 2014). Also, the use of a GR antagonist (mifepristone RU-486) has been shown effective in improving cognitive function in bipolar patients (Young et al., 2004). Taken together, HPA axis dysfunctions and elevated levels of pro-inflammatory cytokines may affect neuroplasticity with a negative impact on mood symptoms and cognition. Indeed, the lower sensitivity of the HPA axis, as well as disturbances in the production of the

corticotrophin-releasing hormone, and adrenocorticotropic hormone have been related to a cortico-limbic dysfunction, with increased amygdala activity and less regulatory activity of hippocampus (HIP) in BD (Drevets et al., 2008). To understand how the HIP responds to glucocorticoids and how those changes are related to volume measures, Tata et al. (2006) carried out a study where male Sprague-Dawley rats were injected with corticosterone or vehicle for 60 days. They identified altered dendritic and glial processes as well as altered numbers and sizes of synapses on hippocampal CA3 (Tata et al., 2006). Other studies in rodents have shown that corticosterone administration decreases cell proliferation and survival in the dentate gyrus of HIP (Brummelte and Galea, 2010). Furthermore, it has been suggested that the inflammatory signal in the CNS, macrophages polarization, and high-mobility group box 1 (HMGB1) secretion may be influenced by the concentration of cortisol and increased expression of GR (Frank et al. 2015; Weber et al. 2015). Finally, cortisol and chronic inflammation may affect the function of the CNS, increasing the enzyme TDO and IDO activity, reducing serotonin (5-HT) synthesis (Maes et al., 2011).

Structure and functions of the BBB

The concept of BBB began at the end of XIX century with Paul Ehrlich reports that various dyes injected into the circulatory system were capable of staining all organs, but failed to stain the brain and spinal cord, leading to a hypothesis of the existence of two separated compartments (Patel and Frey, 2015). The BBB is composed of a monolayer of brain endothelial cells that form the walls of the capillaries, a basal membrane, pericytes, astrocyte end-feet, and perivascular macrophages. Endothelial junctions like tight junctions and adherens junctions control cell permeability, resulting in a sealed structure (Abbott et al., 2010). The BBB regulates the exchanges between the CNS and the periphery, protecting the brain from toxic substances in the blood, supplying brain tissues with nutrients, and filtering harmful compounds from the brain back to the bloodstream (Patel and Frey, 2015). Therefore, an intact and functional BBB is crucial to the maintenance of CNS homeostasis.

Besides the BBB, the CSF and meninges act as an anatomical protection to the CNS. This complex structure of layers surrounds the lymphatic system of CNS and avoids the entry of most stressors and pathogens into the brain. The meningeal lymphatic system located in the dura mater is responsible for draining the interstitial fluid, macromolecules and immune cells from the CNS to the lymph nodes (Louveau, Smirnov, et al., 2015). Interestingly, adaptive and innate immune cells have access and occupy meningeal spaces contributing to neuroimmune reactions (Louveau, Smirnov, et al., 2015). If a pathogen reaches the brain, a slow cascade of events occurs in order to remove the damaging antigen. First, the pathogenic antigen diffuses into the CSF and interstitial fluid via glymphatic system, through the vein walls and astrocytes endfeet (Louveau, Smirnov, et al., 2015). Consequently, those molecules are drained by the meningeal lymphatic vessels or by the lymphatic system of the nasal mucosa together with the immune cells and reach the cervical lymph nodes (Louveau, Harris, et al., 2015). At this point, this process should result in the onset of inflammatory response through the activation of adaptive immune cells, like T lymphocytes (Louveau, Harris, et al., 2015). This mechanism of clearance accounts to the immune privilege of the brain and is also important for the maintenance of homeostasis. However, if the peripheral inflammatory system is first

activated, the cleaning process occurs much more quickly once the pathogen reaches the brain (Louveau, Harris, et al., 2015)

During chronic inflammation, toxins like lipopolysaccharide (LPS) and other pathogens enhance the release of pro-inflammatory cytokines, HMGB1 and other components that may increase microvascular permeability and lead leukocytes migration to the brain parenchyma (Frank et al., 2015; Weber et al., 2015). This infiltration triggers cytokines release and matrix metalloproteinases (MMPs) activation intensifying BBB disruption and inflammation in the CNS, through glia activation (Sumi et al., 2010). In fact, microglia activation and subsequent release of inflammatory mediators modulate the expression of adhesion molecules on endothelial cells that stimulates migration and recruitment of myeloid-derived blood cells to the brain. Therefore, reactive oxygen species (ROS) and cytokines (e.g. tumor necrosis factor α – TNF-α – and IL-1β) produced by activated microglia may impair BBB by altering the expression of molecules associated with endothelial cell junctions, such as claudin-5 and occludin, that are essential for its integrity (da Fonseca et al., 2014).

Considering that inflammation plays a role in the neuroprogression of BD, as well as oxidative stress and microglial activation, Patel and Frey (2015) proposed a model of BBB dysfunction in BD. In this model, a persistent or transient loss of BBB integrity is associated with decreased CNS protection and increased permeability of pro-inflammatory mediators from peripheral blood into the brain, triggering microglial activation and promoting damage (Patel and Frey, 2015). In this regard, Zetterberg et al. (2014) assessed blood-CSF barrier function in 134 patients with BD and compared to 86 healthy controls showing a significant increase in CSF/serum albumin ratio in BD patients (Zetterberg et al., 2014). Furthermore, it has been reported that mood stabilizers used for the treatment of BD, like lithium and valproate can inhibit MMPs function and attenuate BBB dysfunction (Yu et al., 2013).

Furthermore, it has been suggested that BBB may be vulnerable to changes in the microbiota and dysfunctions on intestinal barrier may contribute to the pathophysiology of psychiatric disorders. The intestinal barrier has an important role in the immune system modulation as it regulates the flow of external molecules or pathogens, but the primary function of this barrier remains in the absorption of

nutrients, electrolytes and water from the diet (Kelly et al., 2015). The arrangement of the mucus layer, the microvilli epithelial cells layer and tight junctions contribute to intestinal functions and create a barrier responsible for its protection (Kelly et al., 2015). However, the intestinal permeability can be easily altered by factors such as the diet, stressful events, alcohol ingestion and microbiota alteration (Berk et al., 2013). When the intestinal barrier is disrupted, bacteria from the gut can translocate to the lymph nodes and system circulation (Kelly et al., 2015). The LPS of the enterobacteria actives the innate immune response, mainly through toll-like receptor (TLR) 4 activation, which triggers the release of pro-inflammatory cytokines and production oxidative stress compounds (Berk et al., 2013). Under stress conditions, this pro-inflammatory state could either contribute both to CNS immune activation, represented by TLR4 activation in the microglia cells and further increase the gut permeability (Berk et al., 2013; Kelly et al., 2015).

In this context, Maes et al. (2013) described higher levels of immunoglobulin (Ig) A and IgM, against LPS in chronically depressed patients. The increased bacterial translocation may be a consequence of secondary systemic inflammation and could act intensifying the primary inflammatory response in those, individuals or may be a primary trigger factor associated with onset of depressive symptoms (Maes et al., 2013). Finally, a dysfunctional intestinal barrier could permit a microbiota-driven pro-inflammatory state with implications for the brain development, function and behavior and thus may contribute to the mood dysregulation.

Macrophage/microglia polarization

Macrophages are responsible for innate immune response activation in the periphery while microglia is the tissue-resident macrophages of the CNS, responsible for homeostasis and synaptic modulation (Nakagawa and Chiba, 2015). Microglia can be activated by damage-associated molecular patterns (DAMPs) as well as pathogen-associated molecular patterns (PAMPs) molecules through its receptors, such as TLRs family (Stertz et al., 2015). This activation triggers the expression of nuclear factor kappa B (NFkB), mitogen-activated protein kinase (MAPK) and interferon regulatory factor (IRF) signaling pathways that are accompanied by increased expression of pro-inflammatory genes. When acutely activated, microglia is considered an innate and adaptive mechanism that diminishes tissue damage and restores homeostasis (Nakagawa and Chiba, 2015). However, if the activation process extends for a longer period, the microglia produces higher amounts of TNF-α, IL-1β, and HMGB1, leading to tissue inflammation, apoptosis and damaged neurotransmitter synthesis (Dantzer et al., 2008). It should be noted that HMGB1 can be released by other cell types (neurons and astrocytes) in stress situations, acting on TLRs and thereby mediating the inflammatory response and release of IL-1β by microglia (Frank et al., 2015). Furthermore, the release of pro-inflammatory cytokines promotes the BBB disruption – previously described – which could be an alternative for activated monocytes from peripheral blood to access the brain and exacerbate the neuroinflammation (da Fonseca et al., 2014).

Since microglia exhibits significant plasticity, it can polarize into different phenotypes, depending on the subsets of transcription factors and secreted proteins that will define effector functions, such as killing or repair. Under normal conditions, microglia is responsible for CNS surveillance and also for the release of important neurotrophic factors. (Franco and Fernández-Suárez, 2015). However, an inflammatory stimulus, such as LPS, interferon (IFN)-γ and TNF-α (Franco and Fernández-Suárez, 2015), a pro-inflammatory, namely M1 profile with killing activity is induced. As a consequence of M1 polarization, several reactive oxygen and nitrogen species, newly synthesized pro-inflammatory cytokines (IL-1β and IL-6), chemokines (monocyte chemoattractant protein 1, MCP-1) and co-stimulatory molecules and receptors (MHC-II, CD32, CD80, CD86 and CD68) are expressed or up-regulated (Cherry et al., 2014; Franco and Fernández-Suárez, 2015). For

example, certain IRFs such as IRF5 and IFR7, expressed under M1 polarization, activate the transcription of pro-inflammatory genes and suppress the expression of anti-inflammatory cytokines, such as IL-10 (Ferrante and Leibovich, 2012). After TLRs activation, those factors bind to an adaptor protein, the MyD88, leading the signaling cascade to the activation of NFkB and activator protein 1 (AP-1) and, consequently, the production of pro-inflammatory cytokines (Negishi et al., 2005).

At physiological conditions, a harmful event can be avoided by an M2 antagonist polarization, which is considered a resolving or repair anti-inflammatory phenotype (Nakagawa and Chiba, 2015). The M2 polarization is divided into three groups: M2a, M2b, and M2c. When M2 is activated by IL-4 and IL-13, the phenotype is defined as M2a and has potent anti-inflammatory properties. Particularly, this phenotype exhibits up-regulation of arginase, inhibition of NFκB isoforms, enhanced expression of CD163, CD206 and phagocytic receptors (Duluc et al., 2007). Although the consequences of M2b polarization are still unknown, this phenotype is activated under TLRs stimulation by LPS or immune complexes (Nakagawa and Chiba, 2015). M2c is activated by IL-10 and transforming growth factor (TGF)-β and its function is related to tissue remodeling and extracellular matrix deposition after inflammatory process attenuation (Cherry et al., 2014). Anti-inflammatory interleukins, such as IL-10, released by M2 phenotype, can also down-regulate M1 functions (Nakagawa and Chiba, 2015). IRFs like IRF4 may exert a negative control in the TLRs activation, by competing with IRF5 for MyD88 interaction, inducing the expression of M2 specific genes, such as arginase 1, and inhibiting the expression pro-inflammatory genes dependent of IRF5 (Ferrante and Leibovich, 2012; Negishi et al., 2005).

Furthermore, we should consider the current lack of knowledge about specific markers that distinguish resident microglia from circulating blood-derived macrophages in the human brain that represents a limitation for cell-specific targeting of microglia in BD. In this regard, Durafourt et al. (2012) compared polarization properties of human adult microglia and blood-derived macrophages *in vitro* showing a greater phagocytic activity in microglia than in macrophages. The expression of cell surface markers – like CD23, CD163, and CD206 – was only observed in M2 peripheral macrophages. Regarding M1 phenotype, microglia released higher levels of IL-10 than macrophages, while under M2 polarization both cells produced IL-10 equally (Durafourt et al., 2012). Other researchers have shown that M1 response in

peripheral macrophages may be activated by Th1 cells while the M1 response from microglia could be self-activate through autocrine and paracrine mechanisms (Cherry et al., 2014). As mentioned before, there is no validated method or marker for microglia and peripheral macrophage differentiation in the brain parenchyma, but it is considered that they may coexist in the CNS under some pathological situations (Franco and Fernández-Suárez, 2015).

Microglial dysfunction in BD

More recently, microglial dysfunctions have been the subject of study in the different psychiatric diseases (Watkins et al., 2014). In a qualitative post-mortem research, Bayer et al. (1999) found increased microglial activation associated with HLA-DR expression – an MHC-II – in the prefrontal cortex (PFC) of affective disorder and schizophrenia patients at later stages, when compared to healthy individuals (Bayer et al., 1999). Interestingly, Steiner et al. (2008) found a marked microgliosis in the PFC and thalamus of patients who committed suicide, suggesting a strong association between microglial activation and suicide in psychiatric patients (Steiner et al., 2008). Rao et al. (2010) found a significant increase in c-Fos, inducible nitric oxide synthase mRNA, protein and mRNA levels of IL-1β and IL-1R, and components of the NFκB, in the post-mortem PFC from BD patients (Rao et al., 2010).

Importantly, despite suffering the influence of the inflammatory component, few *in vivo* studies have evaluated the activation of microglia in individuals with BD. Haarman et al. (2014) was the first report to demonstrate the activation of microglia in the HIP of bipolar patients compared with healthy individuals, through an increase in the uptake of gamma rays with the microglia-specific [(11) C]-(R)-PK11195 probe (Haarman et al., 2014). In CSF of euthymic patients, Jakobsson et al. (2015) showed higher levels of MCP-1 and chitinase-3-like protein 1 (YKL-40), a glial activation biomarker, compared to healthy subjects. Higher levels of soluble CD14 and YKL-40 were also observed in the serum of these patients (Jakobsson et al., 2015). Additionally, higher levels of YKL-40 and MCP-1 observed in the CSF of BD patients suggest an inflammatory state, which may explain, in part, poor executive function and outcome (Rolstad et al., 2015).

As *in vivo* experiments evaluating microglial dysfunctions in psychiatric disorders are scarce, preclinical studies are a useful strategy to investigate the relationship between immune system alterations and neurological diseases. Even though there is no ideal model of mood disorders, most of them present certain similarities regarding pathophysiology or etiology of the disorder and treatment response. For instance, systemic immune activation by LPS administration may promote neuroinflammation and depressive-like behavior in mice, and these effects seem to be attenuated by chronic antidepressant administration (O'Connor et al.,

2009). Using this model, Biesmans et al. (2013) observed a sickness behavior and mild depressive-like behavior associated with an increase in the IL-1β, IL-6, TNF-α and INF-γ levels in the serum and in the brain of treated animals. Also, in the LPS group, the authors observed increased glial activation expressed by glial fibrillary acidic protein and ionized calcium binding adaptor molecule (Iba)-1 immunoreactivity, when compared to vehicle-treated mice (Biesmans et al., 2013). Dong et al. (2014) showed that lithium supplementation inhibited microglial activation and pro-inflammatory cytokines production, after LPS stimulation, in primary culture of microglial cells. Accordingly to the authors, these effects of lithium may be mediated by the PI3K/Akt/FoxO1 pathway activation (Dong et al., 2014).

Chronic unpredictable mild stress (CUMS) is probably the most widely used animal model of depression. With this model, Pan et al. (2014) detected increased levels of IL-1β in the serum, CSF, and PFC of stressed animals. As expected, the expression of glial markers investigated in this study – such as CD11b for activated glia and Iba1 for activated microglia – were increased in the PFC of rats submitted to CUMS and these effects were reversed by the administration of antidepressants (Pan et al., 2014). Furthermore, the model of mania induced by d-amphetamine is well established and, besides the characteristic hyperlocomotion, there are evidence that treated animals show increased IL-4, IL-6, IL-10, TNF-α and carbonyl levels in the PFC, striatum and serum (Valvassori et al., 2015). Additionally, in the Valvassori's study, lithium administration reversed d-amphetamine hyperactivity and decreased pro-inflammatory cytokine levels.

N-acetylserotonin (NAS)/melatonin in BD

Sleep and biological rhythms are involved in the neurobiology of BD. Indeed, changes in the sleep–wake cycle, such as decreased need for sleep or insomnia/hypersomnia, are part of the diagnostic criteria for BD (Pinho et al., 2015). Sleep/wake disturbance has been observed during mood states and even in periods of remission. Sleep patterns and circadian rhythms are influenced by melatonin, a neurohormone synthesized primarily in the pineal gland during the dark phase of the night. The synthesis of melatonin involves the conversion of 5-HT to NAS by the arylalkylamine N-acetyltransferase (AANAT) followed by the conversion of NAS to melatonin by the acetylserotonin O-methyltransferase (ASMT) (Etain et al., 2012).

More recently, researchers have investigated the relationship between immune-pineal melatonin and the innate immune response. The pineal gland is a target for PAMPs (e.g., LPS) or pro-inflammatory cytokines (TNF) and these signals can suppress the nocturnal release of melatonin by the gland, promoting the migration of leukocytes to the site of the lesion. Moreover, mononuclear and polymorphonuclear leukocytes can also synthesize melatonin. According to Muxel et al. (2012) during inflammatory response macrophages induce the synthesis of melatonin, and that macrophage-synthesized melatonin may modulate the function of these professional phagocytes in an autocrine manner. In addition, the transcription factor NFkB seems to mediate PAMPs and pro-inflammatory cytokines-induced AANAT (the key enzyme involved in the synthesis of melatonin) in macrophages. These results indicate that the communication between pineal and extra-pineal sources of melatonin is dependent on NFkB nuclear translocation, which plays a significant role in the innate immune response, and that melatonin is part of this complex response.

Interestingly, Etain et al. (2012) assessed genetic and functional abnormalities of the melatonin biosynthesis showing that one frequent polymorphism located in the ASMT promoter, rs4446909, was associated with BD, with a lower mRNA level of ASMT and a lower enzymatic activity. These findings suggest that deleterious variants of ASMT might be associated with low 5-HT and NAS concentrations as well as melatonin level. Abnormalities of melatonin secretion may explain, in part, sleep/wake disturbance, abnormal actimetric parameters and circadian preference

evening observed in bipolar patients. Finally, sleep disturbance can also lead to changes in daily activities such as work, social activities and diet.

Nevertheless, Jang et al. (2010) proposed that the administration of NAS exerts antidepressant effect in mice during forced-swim test. It has been demonstrated that chronic treatment with fluoxetine increased the content of AANAT mRNA in the rat HIP, which suggests that NAS could be a mediator of the antidepressant action of drugs. The NAS, like brain-derived neurotrophic factor (BDNF), exerts its neuroprotective effect by the activation of tropomyosin receptor kinase B (TrkB) and this action seems to be independent of a neurotrophin or MT3 NAS receptor. Therefore, the NAS but not other 5-HT metabolites showed to have effects on synaptic plasticity, neurogenesis, and synaptogenesis.

Taken together, these data support the concept of immune-pineal axis might be relevant to a better understanding of disorders with melatonin rhythms dysfunctions, for example, BD as might open new horizons for the treatment of psychiatric patients.

Cytokines effects on neuronal plasticity

Neurogenesis is the process by which neurons are generated from neural stem cells (Kim et al., 2016). Substantial production of new neurons in the adult brain mainly occurs in two limited areas: the subgranular zone in the hippocampal dentate gyrus and in the subventricular zone in the lateral ventricle (Kim et al., 2016). Neurogenesis consists of multiple steps, including neuronal precursor cell (NPC) proliferation, differentiation, survival, migration, and integration into pre-existing hippocampal networks (DeCarolis and Eisch, 2010). Several studies have shown that adding new neurons into the preexisting neural circuitry is crucial to the maintenance of neuronal plasticity and cognition in patients with psychiatric diseases, in particular, BD (DeCarolis and Eisch, 2010).

Neuroimaging studies of BD have demonstrated abnormalities in neural circuits supporting emotion processing, emotion regulation, and reward processing (Phillips and Swartz, 2014). Those neuroanatomic abnormalities include ventricular enlargement, gray matter loss in the HIP and cerebellum, volume decreases in the PFC and variations in the size of the amygdala (Roda et al., 2015). Although these changes become more pronounced with repeated episodes and longer duration of disease (Kapczinski et al., 2009), they have been demonstrated in individuals at risk of developing the disease (Phillips and Swartz, 2014). Furthermore, the reduced volume of amygdala and HIP in adults with bipolar depression tends to be normalized with lithium therapy (Hallahan et al., 2011). The volume changes in brain regions involved in BD – mainly atrophy – are thought to be the result of a reduction in the number of neuronal and glial density as well as the size and shape of the cell body (Uranova et al., 2004).

Pro-inflammatory cytokines may exert harmful effects on adult neurogenesis in the HIP. These cytokines are produced by activated microglia when the homeostasis of the microenvironment is disturbed, resulting in neuroinflammation. IL-1β is one of the major pro-inflammatory cytokines inhibiting adult neurogenesis (Koo and Duman, 2008). Progenitor cells in the subgranular zone have IL-1β receptors, which decrease cell proliferation when stimulated (Koo and Duman, 2008). Impairment of IL-1β action prevents the attenuated rate of adult neurogenesis in response to stress (Koo and Duman, 2008). In a recent study, Zunszain et al. (2012) reported that IL-1β-induced impaired neurogenesis may be reversed by a kynurenine 3-monooxygenase (KMO)

inhibitor, which suggests the involvement of the kynurenine pathway (KYP) in the IL-1β regulation of hippocampal neurogenesis (Zunszain et al., 2012). Indeed, euthymic patients with BD showed an increase in blood KYN concentrations and KYN/tryptophan ratio (Reininghaus et al., 2014). Additionally, in patients with BD, higher levels of IL-1β were associated with dysfunction and suicide risk (Monfrim et al., 2014). Furthermore, when activated, microglia may promote expression of IFN-γ, which increases the activity of IDO followed by a rise in QUIN (Watkins et al., 2014), which causes excitotoxicity mediated by the N-methyl-D-aspartate (NMDA) receptor.

IL-6 and TNF-α are also pro-inflammatory cytokines associated with inhibition of adult neurogenesis. Monje et al. (2003) demonstrated that TNF-α and IL-6 decreased hippocampal neurogenesis in rats injected with LPS; such effects were completely blocked by systemic treatment with the nonsteroidal anti-inflammatory drug indomethacin (Monje et al., 2003). Another study from the same group showed that IL-6 induced depressive-like behaviors and impaired proliferation of hippocampal cells that may be mediated by NFκB signaling pathway (Monje et al., 2011). In addition, adult IL-6 knockout mice showed enhanced proliferation and survival of new neurons in the dentate gyrus and subventricular zone (Bowen et al., 2011). In the same line, Keohane et al. (2010) demonstrated that exposure of hippocampal NPCs to TNF-α during differentiation has a detrimental effect suggesting that TNF-α may also inhibit neurogenesis (Keohane et al., 2010).

5-HT and KYN metabolism

One of the main neurotransmitters affected by inflammation is the 5-HT. The decrease in bioavailability of tryptophan and increased in KYN formation lead to a reduction in the synthesis of 5-HT, which may be related to the classic depressive symptoms (Myint, 2012). It is important to remember the biochemical basis involved in the synthesis of these two compounds, to understand the influence of systemic inflammation on 5-HT metabolism and KYN in the CNS.

The deviation of 5-HT to KYN synthesis, through the action of IDO and TDO in the liver, astrocytes and microglia, has become an important etiologic factor for mood disorders (Watkins et al., 2014). In physiological situations, 1-5% of tryptophan is metabolized to 5-HT. Around 20-30% of the normal production of 5-HT takes place in the CNS, after the passage of systemic tryptophan through the BBB (Myint, 2012). An increase in KYN metabolism directly decreases the synthesis of 5-HT and melatonin. Melatonin formation is dependent on the metabolism of 5-HT to N-acetylserotonin. Melatonin secretion is bypassed in situations where observed an increase in the activity of IDO (Oxenkrug, 2013). Therefore, melatonin biosynthesis is essential for maintaining the circadian cycle in humans, and the reduction in melatonin concentration causes disturbances in sleep and circadian rhythms, which are common signs in patients with neuropsychiatric disorders.

It is known that under normal physiological conditions, tryptophan metabolism is primarily maintained by the liver function. However, in systemic or central inflammation, the activity of IDO is increased in extrahepatic tissues, including the CNS (Heyes et al., 1993). The activity of IDO is stimulated by IFN-γ and TNF-α and is inhibited by the anti-inflammatory cytokine IL-4 (Myint and Kim, 2003). Under these circumstances, the activity of the KMO is increased, leading to a greater formation of 3-hydroxykynurenine (3-HK) and QUIN compared to the KYNA formation (Munn et al., 1999). As previously mentioned, QUIN is an agonist of NMDA receptors while KYNA is an NMDA receptor antagonist and, therefore is protective against excitotoxicity of QUIN. This fine regulation of the KYN metabolism directly affects the availability of 5-HT in the CNS, and it may be related to the clinical signs observed in mood disorders.

It is well established that patients with mood disorders experienced increased pro-inflammatory cytokines such as IL-6, IL-2, TNF-α and IFN-γ (Myint, 2012). The

release of inflammatory markers correlates positively with the IDO activity and decreases the availability of 5-HT, as previously mentioned. In addition, an imbalance between QUIN/KYNA syntheses promotes microglial susceptibility to stress. The increased sensitivity of microglia appears to be related to the recurrence of mood episodes and treatment resistance (Myint and Kim, 2003). The relationship between KYN and tryptophan reinforces the relevance of this metabolic pathway in this pathological context. In fact, bipolar patients presented higher serum levels of KYN and KYN/tryptophan ratio compared to control subjects (Reininghaus et al., 2014). Furthermore, higher TDO activity and KYN concentrations were observed in the anterior cingulate cortex from patients with BD compared to controls (Miller et al., 2006). Another post-mortem study showed an increase in the gene expression of tryptophan hydroxylase, the enzyme responsible for the conversion of tryptophan to 5-hydroxytryptophan, in the dorsolateral PFC of patients with BD (De Luca et al., 2005).

As mentioned, microglia may promote the production of QUIN and 3-HK during inflammatory conditions; both mediators can induce neuronal death, especially, in the HIP and cortex (Chiarugi et al., 2001). In a morphometric magnetic resonance imaging study, a positive correlation between KYNA/QUIN ratio and the volume of gray matter in the HIP and amygdala of unmedicated patients with major depression was observed (Savitz et al., 2015). In BD, it has also been demonstrated a strong correlation between KYNA/3-HK and hippocampal volume, suggesting a relationship between the KYN pathway and the neuroprogression of BD (Savitz et al., 2015).

Emerging therapeutic strategies

New concepts of pathogenesis have shown that BD is associated with activation of immune-inflammatory pathways, which may lead to macrophage activation and pro-inflammatory cytokines production. These inflammatory signals increase BBB permeability allowing leukocytes migration through brain parenchyma with subsequent glial activation and damage augmentation. The microglia is mainly activated to M1 pro-inflammatory phenotype, which produces ROS – related to neuronal apoptosis – and more pro-inflammatory cytokines – responsible for neurogenesis impairment. Furthermore, pro-inflammatory cytokines stimulate HPA axis, decreasing GR expression and sensitivity and enhancing cortisol release. Both M1 activation and cortisol activate IDO/TDO leading to QUIN formation and reducing 5-HT synthesis. As an NMDA agonist, the QUIN stimulates glutamate release contributing to excitotoxicity, ROS production and, consequently, neuronal death (see Figure 1). Therefore, the search for new agents that can modulate the complex mechanisms above cited could represent a therapeutic opportunity for the treatment of these disorders.

IDO inhibitors - 1-methyl-D,L-tryptophan (1MT)

Emerging evidence suggests that pro-inflammatory cytokines may influence 5-HT signaling in the brain and periphery through the KYP. Cytokines, such as IL-2 and IL-6, released in the periphery from macrophages and IFN-γ from activated microglia may promote overstimulation of the KYP that effectively decreases overall 5-HT availability in the brain as well as alters neurotrophic factors and TNF-α. IDO activity is enhanced by IFN-γ, consequently decreasing 5-HT CNS levels and increasing neurotoxic metabolite QUIN. Overproduction of QUIN may decrease BDNF, activate pro-apoptotic cascades by TNF-α and increase glutamate and NMDA receptor-activated excitotoxicity (Watkins et al., 2014). Changes in synaptic resilience, connectivity of serotonergic neurons and programmed cell death may occur as a consequence of the activation of this mechanism.

The inhibition of IDO activity may protect against neurotoxicity mediated by NMDA receptors. This hypothesis was tested in a model of Huntington disease where a chronic increase in expression and activity of IDO was followed by the

production of neurotoxic metabolite 3-HK. In this model, IDO knockout mice exhibited lower sensitivity to QUIN-induced neurotoxicity in the striatum, when compared to control group. It suggests that IDO inhibitors may promote the best balance between QUIN and KYNA, protecting against excitotoxicity mediated by NMDA receptors (Mazarei et al., 2013).

In an LPS-induced-depressive-like behavior animal model, 1MT, an inhibitor of IDO, showed to reduce the immobility time in the forced swim test and tail suspension test. This behavioral improvement was correlated with a reduction in KYN/tryptophan ratio in the brain of mice (O'Connor et al., 2009). It is important to note that there are no clinical studies using 1MT in the psychiatric disorders.

In a chronic stress model, the co-treatment with allopurinol, an inhibitor of TDO activity, attenuated the immobility time in the forced swim test; this effect was correlated with reduced circulating KYN concentrations (Gibney et al., 2014). Furthermore, an increase in TDO activity was observed in post-mortem brain of patients with schizophrenia and BD and seems to be related to the increase in CNS metabolites of KYN (Miller et al., 2008). Recently, the benefits of allopurinol were also demonstrated in patients with BD (Jahangard et al., 2014).

Taken together, these evidence show that the M1 phenotype and inflammatory cytokines produced by these cells act on KYN metabolism, leading to an increase in toxic metabolites (QUIN) and, consequently, neurotoxicity. Although IDO inhibitors do not directly modify macrophage/microglia polarization towards M1 or M2 phenotype, these agents could modulate the KYP decreasing the production of toxic metabolites and promoting 5-HT secretion. Probably, this mechanism may explain, in part, the improvement of mood symptoms demonstrated in pre-clinical studies of depression.

Anti-TNF-α agents

TNF-α is an 185-aminoacid glycoprotein that was initially described for its ability to induce necrosis in certain tumors. TNF-α is mainly produced by monocytes and macrophages or microglia in the CNSIt is a potent inducer of the inflammatory response, a critical regulator of innate immunity, and plays an important role in the regulation of Th1 immune responses against pathogens (Karson et al., 2013; Miller et al., 2013). Given that BD is associated with a low degree of inflammatory state, it is

plausible to speculate that anti-TNF-α agents could modulate this process and be a useful therapeutic strategy for this illness. Şahin et al. (2015) showed that animals that were treated with the commercially available chimeric monoclonal antibody against TNF-α, infliximab, experienced lower cognitive impairment and higher BDNF levels compared to controls in a model of chronic stress (Şahin et al., 2015). Furthermore, chronic treatment with anti-TNF-α tends to decrease depressive-like behavior and anxiety symptoms in the same experimental model (Karson et al., 2013). Clinically, Raison et al. (2013) showed that depressed patients treated with infliximab plus antidepressants presented higher improvement in depressive symptoms, in particular in patients who had serum CRP levels higher than 5 mg/L, compared to placebo (Raison et al., 2013). This study was a first step towards individualizing treatment for patients with mood disorders by showing that individuals with a pro-inflammatory profile could benefit from therapy with biological agents.

Furthermore, Kroner et al. (2014) assessed the effects of TNF-α on macrophage polarization in a model of spinal cord injury. They showed that wild-type mice experienced greater M2/M1 ratio when compared to TNF knockout mice, as evidenced by a 2-fold increase in the M2 markers arginase-1 and CD206. It suggests that the expression of TNF may contribute to the predominantly M1 cytotoxic macrophages (Kroner et al., 2014).

To sum up, studies have demonstrated the influence of TNF-α on Th1 lymphocytes in promoting a release of pro-inflammatory cytokines or inducing the M1 polarization highlighting the potential use of anti- TNF-α in the modulating of peripheral or central immune system dysfunctions.

Glucagon- like peptide-1 receptor (GLP-1R) agonists

Glucagon-like peptide-1 (GLP-1) is a hormone released after food ingestion that facilitates insulin release from pancreatic cells (Hölscher, 2014). As GLP-1 has a short half-life, some commercially available analogs such as liraglutide and exenatide that are resistant to degradation by the dipeptidyl peptidase 4 were developed and had been used for the treatment of type 2 diabetes (Hölscher, 2014).

However, GLP-1R are not exclusive of the pancreas and have been observed in other tissues including the brain. GLP-1R agonists exert neuroprotective effects related to cell growth and repair, inhibition of apoptosis and reduction of inflammatory

responses (Kim et al., 2009; Isacson et al., 2011). For instance, astrocytes and microglia induce the GLP-1R expression and the treatment with GLP-1 agonists prevent the expression of inflammatory markers (NFκB and IL-1β) *in vitro* and *in vivo* (Hölscher, 2014).

The role of GLP-1 analogs has been suggested as a promissory therapeutic strategy for cognitive dysfunction and neurodegenerative disorders. For instance, Kim et al. (2009) found that exendin-4, an analog of GLP-1, prevented 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced microglial activation in the substantia nigra and striatum, in a rodent model of Parkinson's disease. In this study, exendin-4 suppressed the MPTP-induced expression of TNF-α and IL-1β. Additionally, dopaminergic neuron death in the substantia nigra was significantly reduced one week after MPTP administration in exendin-4-treated animals, emphasizing the protective effects of exendin-4 in the CNS.

Although the evidence assessing GLP-1R agonists in psychiatry are scarce, Isacson et al. (2011) showed that rats and mice treated with exendin-4 (during 1-2 weeks) had an improvement in anxiety and depressive symptoms when compared to controls and imipramine-treated animals. Additionally, they observed an increase in the hippocampal neurogenesis in those animals treated with exendin-4 chronically, which was correlated with the improvement during the behavior tasks. Further understanding of the distinct pathways involved in the neurogenesis may help reveal how GLP-1 analogs exert mood regulation.

.Agonists of the nicotinic alpha-7 receptor (α7 nAChRs)

Several lines of evidence have shown that cholinergic anti-inflammatory pathway may be directly involved in the pro-inflammatory cytokines release. The *α7 nAChRs* are expressed on the surface of macrophages as well as in neurons and microglial cells of the mammalian brain. In a mouse ischemic stroke and bone fracture model, Han et al., (2014) showed that the anti-inflammatory activity of α7 nAChRs agonists was mediated via normalization of imbalance microglia/macrophage polarization states. In particular, α7 nAChRs agonist treatment reduced microglia/macrophage activation, decreased M1 microglia/macrophages and increased M2 macrophages as well as increased anti-oxidant enzymes. Likewise, Li

et al. (2011) found that LPS-induced TNF-α release was inhibited by A-833834, a high affinity and selective α7 nAChR agonist, in both mouse peritoneal macrophages and human whole blood *in vitro*; such effects were attenuated by α7 nAChR antagonist. In addition, intraperitoneal administration of A-585539, another α7 nAChR agonist with limited brain penetration, decreased LPS-induced TNF-α release in mouse serum.

The use of α7 nAChRs agonists in psychiatry has recently gained attention as well as their use has shown positive effects on cognition in preclinical (McLean et al., 2012) and clinical studies (Olincy and Freedman, 2012). McLean et al. (2012) demonstrated that PNU-282987, a selective α7 nAChRs agonist, can reverse the cognitive deficit induced by phencyclidine in an animal model of schizophrenia. More recently, a double-blind, randomized, placebo-controlled study was conducted to test the efficacy of encenicline, a selective α7 nAChRs agonist, for the treatment of cognitive impairment in schizophrenia (Keefe et al., 2015). A decrease in mean Schizophrenia Cognition Rating Scale (SCoRS) total scores was observed over time, and there was a significant difference for encenicline 0.9 mg vs. placebo (Keefe et al., 2015).

As far as we know the role of α7 nAChRs agonists treatment was not investigated in BD, however the fact that these agents show to reduce inflammation and oxidative stress, which are often associated with the pathophysiology of illness progression, suggests that activation of α7 nAChRs could represent a therapeutic opportunity for the treatment of psychiatric patients.

Endocannabinoids

This system is an interesting possible target for modulating neuroinflammation due to the fact that the expression of the cannabinoid receptor type 2 (CB2) is low in resting microglia but is considerably high in activated microglia (Franco and Fernández-Suárez, 2015). In a model of traumatic brain injury, Tchantchou et al. (2014) showed that an inhibitor of anandamide (arachidonoyl ethanolamide) hydrolysis, an endogenous ligand to endocannabinoid, was capable of modulating microglial phenotype towards the M2 type, as evidenced by an increase in the arginase-1 activity (Tchantchou et al., 2014). Consistent with these results, in a

recent study Mecha et al. (2015) demonstrated that M2 polarization occurs upon exposure to anandamide and 2-arachidonoyl glycerol in microglial cultures (Mecha et al., 2015). Moreover, this study showed that an antagonist of cannabinoid receptor blocked M2 polarization, and that CB2 knockout mice have suppressed M2 polarization (Mecha et al., 2015). Give that the endocannabinoid machinery has an important role in the regulation of microglial activation; further studies should investigate the benefits of this pathway in psychiatric disorders.

Conclusions

This review provides a synthesis of the consequences of systemic inflammation in the immune response of the CNS in BD and discusses possible mechanisms involved in this process. M1 and M2 polarization states of macrophages play a significant role in shifting the immune response towards a pro-inflammatory or anti-inflammatory response. The biased pro-inflammatory milieu observed in BD patients may promote an increased permeability of the BBB, leading to a massive recruitment and infiltration of inflammatory markers from the periphery into the brain, triggering microglial activation and proliferation. Like M1 peripheral macrophages, M1 polarized microglia promotes the secretion of inflammatory cytokines, amplifying the inflammatory response, resulting in neural network dysfunctions with consequent impact on mood symptoms, cognition and treatment response. Consequently, new strategies that normalize the imbalance between M1 and M2 microglial polarization states may provide beneficial therapeutic opportunities for the treatment of BD.

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Conflict of interest

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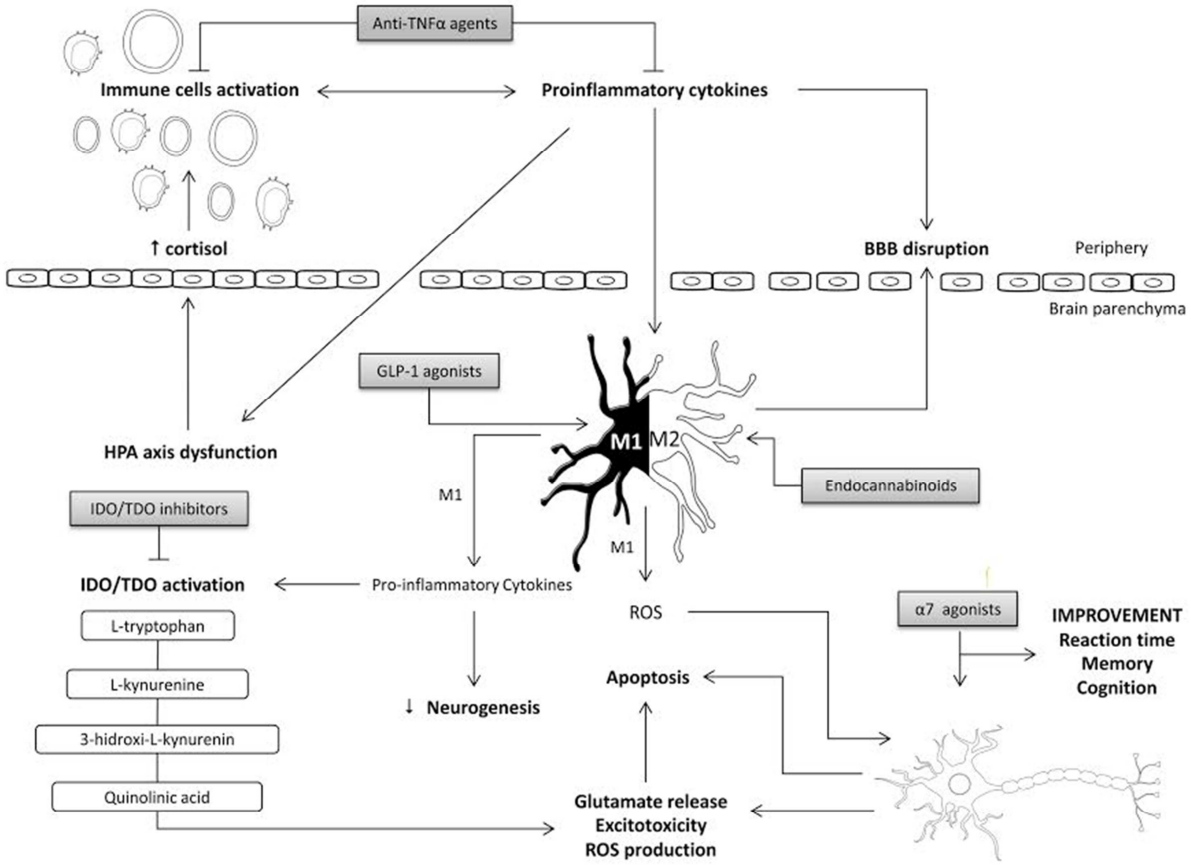

Figure 1. Activation of immune-inflammatory pathways in BD. Evidence suggests that BD is associated with inflammatory activation leading to macrophage activation and cytokines proinflammatory production. These inflammatory signals increase BBB permeability allowing leukocytes migration through brain parenchyma with subsequent glial activation and damage augmentation. The microglia is mainly activated to M1 pro-inflammatory phenotype which produces ROS – related to neuronal apoptosis – and more pro-inflammatory cytokines – responsible for neurogenesis impairment. Also, proinflammatory cytokines stimulate HPA axis, decreasing GR expression and sensitivity and enhancing cortisol release. Either M1 activation and cortisol activate IDO/TDO leading to QUIN formation and reducing 5-HT synthesis. As an NMDA agonist, the QUIN stimulates glutamate release contributing to excitotoxicity, ROS production and, therefore, neuronal death. Also, along the inflammatory pathway, the emerging therapeutic strategies are indicated accordingly to their site of action (gray boxes).

BBB: blood-brain barrier; HPÁ: hypothalamus-pituitary-adrenal; IDO: indoleamine 2,3-dioxygenase; TDO: tryptophan 2,3-dioxygenase; GLP-1: glucagon like peptide-1; ROS: reactive oxygen species.

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6.2 Artigo 2

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Dr. Alan Frazer

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Dear Editor

Please find enclosed the abstract of the manuscript "Unchanged immune system function in high functioning individuals with bipolar disorder" to be considered as a rapid communication for publication in the International Journal of Neuropsychopharmacology. I confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere.

In response to different microenvironments of the central nervous system, resting macrophages/microglia can be polarized into different phenotypes, proinflammatory (M1) or anti-inflammatory (M2), and perform different roles in different physiological or pathological conditions. The aim of this study was to investigate the role of macrophage polarization in the etiology of bipolar disorder. Monocytes purified from 10 patients with BD and 10 healthy individuals were polarized into M1 or M2 cells. Concentrations of secreted cytokines TNF-α, IL-1β, IL-6 and IL-10 were analyzed in macrophage culture supernatants. The secretion of prototype M1 cytokines and prototype M2 cytokines were similar between groups. TNF-α/IL-10 ratio from M1 phenotype was used as inflammatory state of participants and there was no difference between groups (p=0.627). Our data suggest that patients in the early stages still preserve their immune system function without presenting an imbalance in favor of the M1 profile. Also, all patients included were on treatment with mood stabilizing and it is plausible to speculate the effects of these drugs on macrophage polarization.

Yours sincerely,

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Versão submetida:

Unchanged immune system function in high functioning individuals with bipolar disorder

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Abstract

In response to different microenvironments of the central nervous system, resting macrophages/microglia can be polarized into different phenotypes, proinflammatory (M1) or anti-inflammatory (M2), and perform different roles in different physiological or pathological conditions. The aim of this study was to investigate the role of macrophage polarization in the etiology of bipolar disorder. Monocytes purified from 10 patients with BD and 10 healthy individuals were polarized into M1 or M2 cells. Concentrations of secreted cytokines TNF-α, IL-1β, IL-6 and IL-10 were analyzed in macrophage culture supernatants. The secretion of prototype M1 cytokines and prototype M2 cytokines were similar between groups. TNF-α/IL-10 ratio from M1 phenotype was used as inflammatory state of participants and there was no difference between groups (p=0.627). Our data suggest that patients in the early stages still preserve their immune system function without presenting an imbalance in favor of the M1 profile. Also, all patients included were on treatment with mood stabilizing and it is plausible to speculate the effects of these drugs on macrophage polarization.

Keywords: bipolar disorder, inflammation, macrophage polarization

Introduction

Macrophages, as well as their counterparts in the central nervous system, the microglia, are essential components of the innate immune system, involved in the initiation and progression of various inflammatory and autoimmune diseases including neuroinflammation and maintain homeostasis by acquiring different phenotypes according to microenvironment (Hammer et al. 2017). In the presence of an inflammatory stimulus, such as lipopolysaccharide (LPS), interferon (IFN)-γ and tumor necrosis factor (TNF-)α (Franco & Fernández-Suárez 2015) a complete different transcriptome program is developed and macrophages acquire a profile associated with enhanced killing activity, called M1 macrophages. M1 macrophages express, up-regulate and secrete large amounts of reactive oxygen and nitrogen species, cytokines (IL-1β and IL-6), chemokines and co-stimulatory molecules and receptors (Franco & Fernández-Suárez 2015). On the other hand, macrophages can also display opposite-abilities and acquire tissue and repair responses to promote tissue homeostasis through a resolving or repair anti-inflammatory phenotype (Nakagawa & Chiba 2015) called M2 phenotype. Although simplified and widely accepted, these two opposite phenotypes are not exclusive and different spectrums of intermediate phenotypes have been described for human macrophages (Xue et al. 2014).

Studies of macrophages *in vivo* in several pathologies like sepsis, cancer and metabolic disease, demonstrate the polarization and plasticity of these cells as important driving force in pathogenesis (Mantovani et al. 2013). Regarding psychiatric disorders, two post mortem studies have investigated microglia in patients with depression. Bayer et al. reported a stronger up-regulation of the MHC class II protein HLA-DR (marker for activated microglia) in hippocampus and prefrontal cortex of depression cases than in controls (Bayer et al. 1999). A second study found increased immunoreactivity of quinolinic acid in microglial cells of the anterior cingulate cortex from depression- and suicide cases (Steiner et al. 2011). Quinolinic acid is a metabolite that is formed following activation of indoleamine 2,3-dioxygenase (IDO). M1-polarized macrophage and microglial cells have a characteristic high enzymatic activity, suggesting that depressed patients have an upregulation of the M1-phenotype (Campbell et al. 2014).

In this context, we hypothesize that patients with bipolar disorder (BD) have an imbalance in favor of a proinflammatory profile (predominance of the proinflammatory phenotype or M1). Considering the similarity and potency of the interactions between macrophages and microglia, it is reasonable to speculate that correlations and interactions exist between the activation identified in microglia and peripheral macrophages in patients with psychiatric disorders. Also, in vivo studies regarding microglial cells in BD are scarce, therefore, the culture of macrophages may be a useful tool in the study of specific pathophysiological processes relevant to human disease. The primary aim was to investigate the role of macrophage polarization in the etiology of bipolar disorder. For that we investigated the potential effect of M1/M2 cytokine-dependent polarization on human monocytes isolated from peripheral venous blood of bipolar patients once they have become differentiated as macrophages.

Materials and Methods

Participants

Ten patients with high functionality patients with BD according to the Functioning Assessment Short Test (FAST<11) were recruited from Bipolar Disorders Program of Hospital de Clínicas de Porto Alegre. Inclusion criteria were euthymic subjects with BD type 1 according to the DSM-IV and aged between 18 and 60 years. The control group consisted of healthy volunteers who had no current or previous history as well as no first-degree family history of a major psychiatric disorder assessed by the non-patient version of the Structured Clinical Interview for DSM-IV (SCID). Exclusion criteria for both groups were a history of autoimmune diseases or a history of chronic infection/inflammatory disorders, as well as any severe systemic disease or use of immunosuppressive therapy. All patients and controls provided written informed consent. Procedures were approved by the Institutional Review Board of Hospital de Clínicas de Porto Alegre.

Monocyte isolation and macrophage differentiation

Peripheral blood (40 mL) obtained from patients and controls was purified by density gradient with histopaque®-1077 (d = 1.077, Sigma Aldrich) in the ratio of 1:1 and then centrifuged at 400xg for 30 minutes in BRAKE OFF module. PBMCs from the interphase were collected, washed with PBS (Sigma-Aldrich) and resuspended in RPMI-1640 media (Invitrogen) supplemented with 10% fetal bovine serum (Gibco), 200mg/mL glutamine (Invitrogen) and 100U/mL penicillin 100mg/mL streptomycin (Invitrogen). Monocytes were isolated from PBMC by cell culture plastic adherence as follows: $3\text{-}5\times10^6$ PBMC per well were seeded into 12-well cell culture plates, and allowed to adhere in a 5% CO_2 incubator at 37˚C for 2 hours. Non-adherent cells were removed. Adherent cells, mainly monocytes, were carefully washed twice with PBS and cultured for additional 7 days supplemented with macrophage colony-stimulating factor (M-CSF, 50 ng/mL) (Peprotech) (Becker et al. 2015).

To induce polarization, monocyte-derived M0 macrophages were polarized towards the M1 or M2 profile (Becker et al., 2015 protocol adapted from Solinas et al., 2010 and Ambarus et al., 2012). In this regard, M0 cultures were incubated for additional 18 hours with RPMI + FBS 10% supplemented with IFN-γ (20 ng/mL, Peprotech) and LPS (100 ng/mL, Sigma-Aldrich) or IL-4 (20 ng/mL, Peprotech), respectively. After incubation, supernatants were collected and cytokines (IL-1β, IL-6, IL-10 and TNF-α) were measured by multiplex assay using a Milliplex MAP Human High Sensitivity T Cell Panel Immunology Multiplex Assay Kit. Results are expressed as picograms per milliliter (pg/mL). The detection limits of the assays were 2,000.00 pg/mL for IL-1β; 750.00 pg/mL for IL-6; 6,000.00 pg/mL for IL-10 and 1,750.00 pg/mL for TNF-α.

Results are reported as means ±SD. Comparison between groups was performed with the parametric Student t test or non-parametric Mann-Whitney as appropriate; Chi-square was also used to detect differences on gender. $P<0.05$ denoted significant differences.

Results

Sample characteristics are shown in Table 1. Human monocytes purified from PBMCs of patients with BD and 10 healthy individuals were exposed to IFN-γ plus LPS or to IL-4 alone to induce their polarization into M1 or M2 cells, respectively, according to a published protocol (Becker et al. 2015). Concentrations of secreted cytokines TNF-alpha, IL-1β, IL-6 and IL-10 were analyzed in macrophage culture supernatants using Multiplex assay. As demonstrated in Table 1 the secretion of prototype M1 cytokines IL-1 β, TNF-alpha and IL-6, and prototype M2 cytokines IL-10 were similar between patients and controls. With regards to M0, patients had higher TNF-α and Il-6 levels than control group.

Finally, TNF-α/IL-10 ratio from M1 phenotype was used as inflammatory state of participants. As demonstrated in Figure 1, patients with BD and controls did not differ in terms of TNF-α/IL-10 ratio.

Among the bipolar patients, mood stabilizing agents were the most commonly prescribed agents. Seven patients were under treatment with valproate (VPA) while three patients were under treatment with lithium.

Table 1: Secretion of cytokines IL-1 β, TNF-α, IL-6, and IL-10 in macrophage culture. (Statistics: *T-test; **Mann-Whitney).

	Bipolar patients (n=10)		Control Group (n-10)	
	Mean	**SD**	**Mean**	**SD**
Age* (t=-1.306; p=0.208)	55.70	12.13	47.90	14.47
HAM-D*	3.00	1.94		
Log FAST* (F=1.811; p=0.072)	0.46	0.34		
YMRS*	1.2	1.99		
	Median	**IQ**		
Hospitalization**	1.5	(0.75-3.00)		
		n		**n**
Sex (x=0.267, p=1)				
Female		8		7
Male		2		3
Medication in use				
Lithium		3		
Valproate		7		

	M0			**M1**			**M2**		
IL-1β**	**MED (IQ)**		**n**	**MED (IQ)**		**n**	**MED (IQ)**		**n**
Control	0.28 (0.26-0.42)		9	8.42 (2.35-9.85)		9	0.41 (0.31-0.44)		9
BD	0.28 (0.27-0.44)		9	5.37 (1.92-8.80)		9	0.38 (0.28-0.66)		10
	p= 0.756			p=0.508			p=0.774		
Log TNF-α*	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Control	0.82	0.78	9	3.59	0.06	9	2.00	0.66	9
BD	1.45	0.36	9	3.50	0.15	10	1.85	0.44	10
	p=0.040			p=0.139			p=0.562		
Log IL-10*	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Control	0.86	0.63	8	2.60	0.68	9	1.33	0.88	9
BD	0.74	0.89	9	2.35	1.36	10	1.26	0.65	10
	p=0.769			p=0.617			p=0.659		
Log IL-6*	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**
Control	0.25	0.99	9	3.29	0.13	10	1.33	0.88	9
BD	1.20	0.29	9	3.19	0.27	10	1.26	0.65	9
	p=0.021			p=0.294			p=0.853		

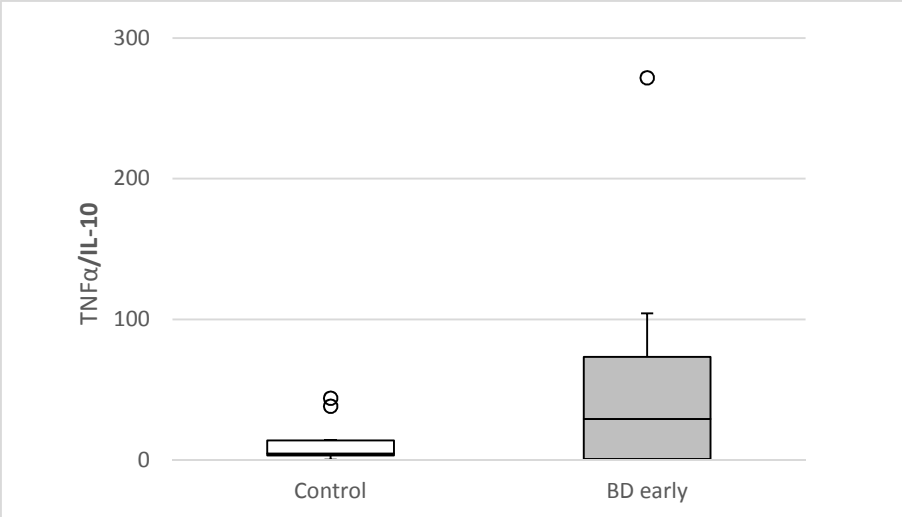

Figure 1: TNF-α/IL-10 ratio of BD patients and controls (Mann-Whitney, p=0. 627)

Discussion

To our knowledge, the present study is the first to assess the role of macrophage polarization in the etiology of BD. M1 and M2 macrophages produce distinct patterns of inflammatory cytokines, where M1 are a major source of TNF-alpha and IL-1 β, and M2 produce IL-10. Our results showed that the secretion of prototype M1 cytokines IL-1 β, TNF-alpha and IL-6, and prototype M2 cytokines IL-10 did not differ between BD patients and healthy individuals. Furthermore, patients with BD showed no difference in the TNF-α / IL-10 ratio when compared to healthy controls, suggesting that there is an unchanged immune system function in this population. Two different hypotheses could possibly explain these results: Firstly, all patients included in this study may represent an early stage of illness as evidenced by total FAST score lower than 11. According to the staging model in BD, patients in the early stages of illness experience greater cognitive and functional outcome than those in the late stages (Rosa et al. 2014). Indeed, biological changes (including inflammation) seem to be related to the mood episodes and progression of the illness (Berk et al. 2011). Kauer-Sant'anna showed increased TNF-α and IL-10 serum levels in BD patients compared to controls; such alterations were also related to the chronicity. More recently, Wollenhaupt et al., 2016 showed that serum of patients at a late stage of BD induce a significant reduction of neurite density and a decrease in the cell viability compared to control group (Wollenhaupt-Aguiar et al. 2016)

suggesting that the serum from BD patients may contain certain chemicals that could be toxic and alter neural cells. Furthermore, the role of cytokines on neural plasticity has been documented in several preclinical studies. The administration of IL-1β, for instance, seems to suppress cell proliferation within the hippocampal dentate gyrus subregion in a model of chronic stress while the blockade of IL-1β signaling was protective against antineurogenic effects and depressive-like behaviors (Koo & Duman 2009). Together with previous studies, our data suggest that patients in the early stages (e.g., high functioning) still preserve their immune system function without presenting an imbalance in favor of the M1 macrophage profile.

Second, our patients were on treatment with mood stabilizing (lithium or valproate) and it is plausible to speculate the effects of these drugs on macrophage polarization. Lithium, for instance, may inhibit microglial activation and cytokine/chemokine secretion in a neonatal hypoxic-ischemic brain injury rat model (Li et al. 2011). In addition, the anti-inflammatory properties of lithium have been shown in *in vitro* studies. Monocytes stimulated by LPS from non-lithium-treated bipolar patients were characterized by an abnormal IL-1β/IL-6 production ratio (e.g., low IL-1β and high IL-6 production) while lithium treatment seems to restore this ratio. Leu et al. compared the immuno-modulation activities of VPA and lithium on the differentiation and functions of dendritic cells (DC), which are part of the mononuclear phagocyte system as well as macrophages. Upon stimulation of immature DC with LPS, VPA, and lithium both reduced the secretion of IL-6 and TNF-α. However, only lithium significantly increased the production of IL-10. Treatment with VPA resulted in a reduced capacity of LPS-stimulated DC to promote the differentiation of T helper 17 cells that are critical in the promotion of inflammatory responses (Leu et al. 2017). VPA also showed to modulate immune response in vitro by significantly inhibiting LPS-induced production of TNF-α and IL-6 by THP-1 cells (Ichiyama et al. 2000). In view of these evidences the effects of mood stabilizers on the polarization of macrophages should be interpreted as limitation of the present research. Future studies in drug-free patients are required to better evaluate this issue in this model.

Conclusions

In conclusion, our findings suggest that patients at early stages of the illness (e.g. those with high functionality) have no imbalance in macrophage polarization towards proinflammatory phenotype M1. It reinforces the protective effects of early intervention in BD in preventing immune system alterations and consequently the progression of the illness.

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7 CONSIDERAÇÕES FINAIS

Com base nos dados apresentados, nossos resultados sugerem:

1. Indivíduos com TB demonstram níveis séricos aumentados de citocinas pró-inflamatórias (IL-1β, IL-6 e TNF-α) e menor sensibilidade do eixo hipotálamo-hipófise-adrenal. Este desequilíbrio no sistema imune promove uma alteração na permeabilidade da barreira hematoencefálica, que pode levar a um sinal inflamatório disseminado no sistema nervoso central a partir da periferia, através da ativação de macrófagos (polarização M1). A ativação microglial crônica pode resultar em apoptose neuronal, inibição da neurogênese, redução do volume do hipocampo, síntese de neurotransmissores menores e citotoxicidade, aumentando a produção de glutamato eo metabolismo da quinurenina. Os estados de polarização M1 e M2 dos macrófagos/microglia desempenham um papel significativo na mudança da resposta imune para uma resposta pró-inflamatória ou anti-inflamatória.

2. Indivíduos com TB em estágios iniciais da doença preservam o sistema imunológico, não apresentando desequilíbrio a favor do perfil pró-inflamatório M1, sendo comparáveis, neste sentido, a controles saudáveis.

O modelo utilizado neste trabalho constitui uma forma de cultura de macrófagos primários humanos e permite o estudo de macrófagos derivados de monócitos humanos *in vitro* sob várias condições (74). Uma vantagem clara deste modelo *in vitro* é que não se baseia em linhagens celulares imortalizadas como THP-1 ou outras (47). Linhagens de células podem ser facilmente obtidas e contornam a necessidade de extrair sangue, no entanto, elas não são fenotipicamente e funcionalmente idênticas às células primárias.

Evidências disponíveis em relação à inflamação periférica no TB mostraram que os biomarcadores podem diferir entre estágios iniciais e tardios da doença, em paralelo com alterações estruturais e neurocognitivas relacionadas ao estágio. Quando os pacientes em estágio inicial e tardio com TB foram comparados, IL-6 e TNF-α foram elevados em ambos os grupos, enquanto os níveis de IL-10 foram maiores nos estágios iniciais. No entanto, o TNF-α foi mais elevado nos estágios tardios do que no início (12). Uma falha do sistema imune para regular e

contrabalançar uma resposta inflamatória na fase tardia da doença pode explicar a elevação contínua das citocinas (30). Desta forma, é plausível pensar que nos estágios mais tardios deste transtorno, os pacientes não sejam capazes preservar o equilíbrio da resposta imune entre M1 e M2.

Com relação às limitações, o n pequeno e o fato de todos os pacientes estarem em tratamento farmacológico podem ter inteferido nos resultados. Tanto o lítio quanto o valproato já mostraram, *in vitro* e *in vivo,* efeitos antiinflamatórios e de atenuação da ativação da microglia.

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9 ANEXOS

Outros trabalhos produzidos no período que compreendeu este doutorado:

9.1 Anexo 1:

Trabalhos publicados:

1. RANZOLIN, ALINE; DUARTE, ANGELA LUZIA BRANCO PINTO; BREDEMEIER, MARKUS; DA COSTA NETO, CLÁUDIO ANTÔNIO; ASCOLI, BRUNA MARIA; WOLLENHAUPT-AGUIAR, BIANCA; KAPCZINSKI, FLÁVIO; XAVIER, RICARDO MACHADO. Evaluation of cytokines, oxidative stress markers and brain-derived neurotrophic factor in patients with fibromyalgia - A controlled cross-sectional study. Cytokine, v. 84, p. 25-28, 2016.

2. COLPO, GABRIELA D. ; ASCOLI, BRUNA M. ; WOLLENHAUPT-AGUIAR, BIANCA ; PFAFFENSELLER, BIANCA ; SILVA, EMILY G. ; CIRNE-LIMA, ELIZABETH O. ; QUEVEDO, JOÃO ; KAPCZINSKI, FLÁVIO ; ROSA, ADRIANE R. . Mesenchymal stem cells for the treatment of neurodegenerative and psychiatric disorders. Anais da Academia Brasileira de Ciências (Online), v. 87, p. 00-00, 2015.

3. BRISTOT, G.; ASCOLI, BRUNA MARIA; GUBERT, C.; PANIZZUTTI, B.S.; KAPCZINSK, F. ROSA, A. R.. Progesterone and its metabolites as therapeutic targets in psychiatric disorders. Expert Opinion on Therapeutic Targets, v. 18, p. 679-690, 2014.

4. RODRIGUES, A.; ROSA, A. R.; KUNZ, M.; Ascoli, Bruna; KAPCZINSK, F. Bipolar disorder: staging and neuroprogression. Psychiatria Polska, v. 48, p. 231-243, 2014.

9.2 Anexo 2:

Artigo aceito para publicação na *Trends in Psychiatry and Psychotherapy:*

Carta de aceite:

14-Feb-2017

Dear Dr. Rosa:

It is a pleasure to accept your manuscript entitled "Cell therapy in the treatment of bipolar mania in an animal model: a proof of concept study." in its current form for publication in the Trends in Psychiatry and Psychotherapy.

Thank you for your fine contribution. On behalf of the Editors of the Trends in Psychiatry and Psychotherapy, we look forward to your continued contributions to the Journal.

Sincerely,

Dr. Benicio Frey
Associate Editor, Trends in Psychiatry and Psychotherapy
benicio.frey@gmail.comsychotherapy

Cell therapy in the treatment of bipolar mania in an animal model: a proof of concept study.

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Abstract:

Introduction: The rationale of mesenchymal stem cells (MSCs) as a novel therapeutic approach in certain neurodegenerative diseases is based on their ability to promote neurogenesis. Hippocampal atrophy has been related to bipolar disorder (BD) in preclinical, imaging and postmortem studies. Therefore, the development of new strategies to stimulate neurogenesis process in BD is crucial.

Objectives: Our aim was to investigate the behavioral and neurochemical changes induced by transplantation of MSCs in the model of mania-like induced by Lisdexamfetamine dimesylate (LDX).

Methods: Wistar rats (n=65) received one oral daily dose of LDX (10mg/kg) or saline for 14 days. On the 8th day of treatment, the animals additionally received intrahippocampal saline (1µL) or MSC (1µL containing 25.000 cells) or lithium (47,5mg/kg) as an internal experimental control. Two hours after the last administration the behavioral and neurochemical analysis were performed.

Results: As expected, LDX-treated rats had increased locomotor activity compared to saline-saline rats ($p=0.004$) and lithium reversed LDX-related hyperactive behavior ($p<0.001$). In contrast, the administration of MSCs did not change hyperlocomotion, indicating no effects of this treatment on LDX-treated rats ($p=0.979$). Furthermore, we did not find differences between groups on BDNF levels ($p>0.05$) in hippocampus of rats.

Conclusion: Event though these results suggest that one dose of MSCs were not helpful to treat hyperactivity induced by LDX and neither BDNF secretion, we can not rule out the possible therapeutic effects of MSCs. Further research is required to determine direct LDX effects in brain structures as well as in other pathophysiological targets related to BD (e.g., inflammatory markers and neurotrophic factors).

Keywords: mesenchymal stem cells, cellular therapy, bipolar disorder, mania, neurogenesis, hippocampus.

9.3 Anexo 3

Capítulo “36 – Farmacologia do Transtorno Bipolar” da obra Farmacologia Clínica, 1ed (de Rosane Gomez e Iraci Lucena da Silva Torres), que está no prelo, e que tem lançamento previsto para julho de 2017.

Autores: Bruna Maria Ascoli e Luíza Géa

c0180

Farmacologia do Transtorno Bipolar 36

p0010 O transtorno bipolar (TB) é doença mental incapacitante, complexa e multifatorial, que se caracteriza pela ocorrência de episódios maníacos e depressivos alternados por períodos de remissão[1]. Os sintomas são graves e associados a baixo desempenho em tarefas no trabalho ou escola, dificuldade de relacionamentos e, em casos mais graves, suicídio. O TB é responsável por um quarto dos casos de suicídio, aumentando em 15 vezes esse risco em relação à população em geral[2]. Além disso, pacientes com TB sofrem com comorbidades psiquiátricas e médicas.

p0015 Episódios depressivos e maníacos possuem diferentes períodos de duração. Episódios depressivos podem durar 14 dias ou mais, os maníacos 7 dias ou mais e os hipomaníacos 4 dias ou mais[3]. Dessa forma, o TB pode ser classificado em tipo I (TB-I) ou tipo II (TB-II). O TB-I caracteriza-se por um ou mais episódios maníacos ou mistos, podendo ocorrer episódio depressivo – o que não definirá o diagnóstico[3]. Já o TB-II caracteriza-se por um ou mais episódios depressivos, associados ao menos por um episódio hipomaníaco[2].

p0020 O TB acomete mais frequentemente indivíduos no final da adolescência e no início da vida adulta, com idade entre 18 e 25 anos. Segundo dados do Centro de Controle e Prevenção de Doenças (CDC), o TB possui prevalência mundial aproximada de 1%-2%, sendo esta de 0,9% no Brasil[4,5]. Quanto à proporção de gênero, o TB-I possui razão igual entre homens e mulheres, já o TB-II parece acometer mais o sexo feminino[2]. Além disso, as mulheres são mais suscetíveis a estados de ciclagem rápida e mistos, bem como possuem maior probabilidade de apresentar sintomas depressivos[2].

st0010 ## TRATAMENTO DO TB

e de pessoas do seu entorno. A Tabela 36-2 mostra fármacos recomendados para o tratamento da mania segundo diretrizes de tratamento atuais. Apesar dos episódios de hipo/mania serem condição *sine qua non* para o diagnóstico do TB, o curso natural da doença tem sido dominado pela presença de sintomas depressivos. Em estudo clássico publicado por Judd et al. (2002), pacientes com TB-I permaneciam a maior parte do tempo com sintomas depressivos em comparação com os sintomas de mania[6]. A persistência de sintomas depressivos, ainda que sub-sindrômicos, tem impacto importante sobre o prognóstico da doença e tem sido fortemente associada a maior prejuízo cognitivo e psicossocial, assim como recorrência de novos episódios[7,8]. Isto enfatiza a necessidade de erradicação desses sintomas, com o intuito de obter plena recuperação sintomática bem como aumentar a funcionalidade desses indivíduos[7,8].

p0035 Embora o tratamento da depressão bipolar represente um desafio, a maioria dos estudos envolvendo o TB tem sido conduzida na fase maníaca, o que limita o número de fármacos disponíveis para esta fase da doença. Além disso, a depressão bipolar é frequentemente confundida com a unipolar, levando a tratamentos inadequados e piora do prognóstico.

p0040 O objetivo principal deste capítulo é revisar o tratamento da depressão bipolar, descrevendo os fármacos recomendados como primeira linha de tratamento na maioria das diretrizes internacionais, assim como nos Protocolos Clínicos e Diretrizes Terapêuticas (PCDT) para o TB, recentemente aprovados no Brasil (ver Figura 36-1).

st0015 ### ESTABILIZADORES DE HUMOR

p0045 O conceito de “estabilizador de humor” é usado para